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Revolutionizing Soil Remediation: Harnessing the Potential of Chicken Manure Digestates for Petroleum Hydrocarbon Contamination

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Abstract

This study aimed to assess the effectiveness of chicken manure digestates (CMD) in bioremediating soils contaminated with hydrocarbons. The experiment involved three levels of nutrient stimulation using CMD (0%, 10%, and 20%) and two levels of petroleum hydrocarbon-polluted soils (5% and 10% concentration). The nutrient and microbiological composition of the locally sourced contaminated soil was analyzed. Total petroleum hydrocarbon (TPH) concentrations were measured at regular intervals (0, 14, 28, 56, 84, 168, and 336 days) before and after the treatment using gas chromatography with flame ionization detection (GC-FID) following standard protocols. The study revealed that CMD exhibited significant potential as a source of hydrocarbon-utilizing microbes, with total hydrocarbon-utilizing bacteria (THUB) and total hydrocarbon-utilizing fungi (THUF) reaching values of 1.6×10^4 and 1.3×10^4 colony-forming units per gram (cfu/g), respectively. These findings suggest that CMD can serve as an effective inoculant for bioremediation of hydrocarbon-contaminated soils and related biodegradable contaminants. Comparatively, the 20% CMD treatment exhibited 52% and 35% remediation rates for the respective pollution levels, while the 10% CMD treatment showed superior TPH degradation at day 56, with removal rates of 59% and 39% for the 5% and 10% polluted soils, respectively. However, over longer cleanup durations (e.g., day 168), higher TPH removal rates of 83% and 66% were observed for the aforementioned samples. Notably, the 20% CMD stimulation demonstrated better long-term bioremediation performance, especially for high levels of hydrocarbon pollution, while the 10% CMD stimulation proved more effective for short-term remediation. Overall, this study highlights the efficacy of CMD as an organic stimulant for the removal of organic contaminants from soils, particularly in bioremediation applications.

Keywords Chicken manure digestates · Bioremediation · Petroleum hydrocarbons · Polluted soils

1 Introduction

The introduction of pollutants into the environment, causing detrimental effects, is referred to as pollution [1]. Pollution can be caused by various substances (solid, liquid, or gas) or forms of energy (such as radioactivity, heat, sound, or light). These pollutants can originate from natural sources or external factors impacting the environment [2]. While natural disasters can contribute to environmental contamination, the term "pollution" commonly implies that the toxins stem from human activities or artificial sources. Pollution can be classified as either point source or nonpoint source pollution [3, 4]. Notable forms of pollution encompass thermal pollution, visual pollution, noise pollution, litter, plastic pollution, radioactive soil contamination, air pollution, and light pollution [5–7].

Bioremediation technology has gained extensive use in the remediation of various environmental contaminants,

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such as heavy metals and petroleum products [8–11]. Its environmental compatibility, cost-effectiveness, and friendly nature have contributed to its widespread application [9, 12–15]. Bioremediation heavily relies on environmental microorganisms [11, 15–18]. To maximize the habitat and potential of these microbes during the bioremediation process, it is crucial to eliminate factors that hinder their development, diversity, and activities. This has led scientists to explore diverse approaches to enhance bioremediation techniques. One notable advancement in recent years is remediation by enhanced natural attenuation (RENA) [11, 16–19].

RENA encompasses three primary methods: biostimulation, which involves providing sufficient nutrients, particularly nitrogen (N) and phosphorus (P); bioaugmentation, which entails introducing microbial inoculants to enhance population and microbial diversity in polluted environments; and biofacilitation (e.g., land farming), which aims to improve soil physicochemical conditions, microbial accessibility to pollutants, and oxygen supply to microbes in the environment [8, 9, 11, 15, 19–25]. Among these, land farming, involving soil excavation and spreading to enhance oxidative potential of pollutants, remains a traditional RENA practice [26]. Land farming offers economic and environmentally friendly benefits [26–28]. Therefore, an advanced approach for the remediation of hydrocarbon contaminants would involve integrating land farming with nutrient stimulation, microbial inoculation, or both. Conventionally, land farming in petroleum-polluted environments has been enhanced using inorganic fertilizers and composted animal and poultry manure [9, 15, 22, 29–32]. However, there is limited research on the utilization of organic biodigestates, specifically those derived from chicken layer droppings, for the remediation of petroleum-contaminated soil.

Furthermore, it has been observed that the bioremediation process becomes less effective when soil pollution levels exceed 5% hydrocarbons [Mmom and Deekor, 2010]. Hence, this study aims to develop an improved method of nutrient stimulation for bioremediation, particularly in soils with higher levels of hydrocarbon contamination. The objectives include assessing: (1) the nutritional value of locally produced chicken manure digestates; (2) the potential for microbial inoculation using locally sourced bio-digested chicken droppings; and (3) the effectiveness and efficiency of locally produced chicken manure digestates in remediating petroleum hydrocarbon pollutants. Two levels of soil contamination, 5% and 10% hydrocarbons, were investigated in this experiment, with the total petroleum hydrocarbons (TPH) removal serving as the primary index. The findings will contribute to the existing knowledge on the application of various composts for the bioremediation of organic and inorganic soil contaminants.

2 Materials and Methods

BTEX, naphthalene, and other analytical reagents were ordered from Sigma-Aldrich in the UK by Pyrex-IG Scientific Company in Benin City, Nigeria. For the study, only analytical-grade chemicals and tools were employed.

2.1 Materials Sourcing and Processing

This investigation was completed in a screen house environment with typical day and night temperatures of 38.5 °C, 4 °C and 26.5 °C 1.5 °C, respectively, at the Department of Chemistry, University of Benin, in Benin City. Using a spade, large amounts of soil samples were taken from the University oil palm estate in Igue, Edo State, Nigeria, at depths between 0 and 30 cm. The dirt was ground and sieved through a 2 mm mesh after being dried by air. Diesel was obtained from a Total PLC filling station in Benin City, Nigeria, and weathered for three weeks by stirring it every day for roughly five minutes [14]. Digested chicken manure was produced locally. After roughly two hours of dumping, the droppings from chicken layers (from a battery cage system) were gathered from the dump site at Sorghai Delta Farm PLC, Amuokpe, Delta State, Nigeria, and transported in a plastic container to the screen house. By removing big stuff, such as broken egg shells, feathers, twigs, and wooden shavings, etc., at the greenhouse, the droppings were as uniformly mixed as possible. The digestates were created by combining the harmonized droppings with tap water at a ratio of 1:2.5, and they were mixed twice a week (In the first two weeks and then one per week) until the digestion process, which took eight weeks to complete. In this context, digestates refer to the organic mixture that is produced by combining harmonized droppings (in this case, chicken droppings) with tap water at a specific ratio of 1:2.5. The mixture is then subjected to a digestion process that spans a period of eight weeks. During this time, the digestates are regularly mixed twice a week in the first two weeks and then once a week thereafter. The digestion process involves the breakdown and decomposition of organic materials present in the droppings, resulting in a nutrient-rich and biologically active product known as digestates.

2.2 Soil Spiking

The spiking mixture, referred to as HCM, was created by dissolving the diesel, BTEX, and naphthalene in petroleum spirit. Following successive soil spiking with the HCM, samples were set up in a Randomized Complete Block Design (RCBD), a technique that was adapted from [14]. Table 1 shows the overall concentration of pollutants used,

Table 1 Sequential spiking of soils with 5% HCM (Adopted from [14])

Remediation indices (mg/kg)	Conc. (mg) and % of intended HCM added to the soils			
	Days of spiking			
	1 (10%)	7 (20%)	14 (30%)	21 (40%)
Diesel (DROs)	4000	8000	12,000	16,000
Benzene	200	400	600	800
Toluene	200	400	600	800
Ethyl Benzene	200	400	600	800
Xylene	200	400	600	800
Naphthalene	200	400	600	800
Sub-total	5000	10,000	15,000	20,000
Gross total	50,000			

which amounts to 5% pollution and is made up of 4, 0.8 and 0.2% of diesel, BTEX and naphthalene, respectively. 10% of the anticipated HCM was also injected into the soil on the first day. At days 7, 14, and 21, this was raised to 20, 30, and 40% of the targeted pollutant values, respectively. In comparison to the values used for the 5% pollution, the concentrations for the samples with 10% HCM contamination were twice. As a result, for the 5 and 10% HCM pollution levels, respectively, the overall pollution level was 50,000 mg and 100,000 mg/kg soil. The samples were left undisturbed after the final spiking for four weeks to allow for stability before the nutrients were stimulated [14]. During the soil spiking process to draw hydrocarbon-using microbes to the environment, the greenhouse floor was spiked with 5 L of diesel [33].

2.3 Nutrient Stimulation

The bioremediation of the hydrocarbon-polluted soils utilized the CLD as a source of nutrients. According to a method adapted from [14], appropriate amounts of the fertilizers (as described below) were added to the samples on a weekly basis for the first four weeks, and they were correctly mixed using a plastic turner to resemble landfarming. Regarding the degree of contamination, three levels of nutrient stimulation—0 (Control) 10 and 20% were used. 5 g of CLD was added weekly up until the fourth week for the 10% treatment (for 5% HCM contaminated samples), and 10 g of the manures was added weekly for the same duration for the 20% treatments. As a result, for the 10% and 20% treatments, respectively, the total amount of manures added to the samples was 20 and 40 g/kg of soil. The same nutrient stimulation method was applied for the samples that were 10% HCM contaminated, but the concentration of the digestates used was doubled. That instance, for the 10% and 20% treatments, the total concentration of the digestates used in this example was 40 and 80 g/kg soil, respectively.

2.4 Sampling and Chemical Analyses

On several specific days (Day 1, 14, 28, 56, 84, 168, and 136), samples weighing approximately 50 g were collected. These samples were carefully stored and kept safe until they could be analyzed at the Earth Quest International Laboratory in Warri, Nigeria. Using widely accepted methods, the physical and chemical properties of the soil and digestates were determined [34]. To study hydrocarbons, a technique called GC-FID was used, following the guidelines provided in the USEPA method 8015B [35]. The levels of sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) were analyzed using flame photometers for K and Na, and atomic absorption spectroscopy (AAS) for Ca and Mg. The concentration of ammonium nitrogen ($NH4^+$-N) in the digestates was measured using the APHA method [36]. The APHA (American Public Health Association) method typically involves the use of a colorimetric technique called Nesslerization to determine the concentration of ammonium nitrogen. This method relies on the formation of a yellow–brown color complex when ammonium ions react with a reagent called Nessler's reagent. The intensity of the color is proportional to the concentration of ammonium nitrogen present in the sample. The absorbance or color intensity is usually measured using a spectrophotometer at a specific wavelength, and the concentration of ammonium nitrogen is determined by comparing the absorbance values to a calibration curve or standard solutions of known ammonium nitrogen concentrations. APHA suggests employing a technique called direct Nesslerization cum colorimetry for this measurement.

The concentration of NH_4^+–N was calculated as:

$$NH_4^+ - N = \left(\frac{mg}{kg = C \times \frac{v}{w}} \right) \quad (1)$$

In the formula, the final volume of the digestate in liters is represented by "v", the weight of the digestate utilized in kilograms is represented by "w", and the NH_4^+-N concentration in the sample in milligrams per liter (as determined by the calibration curve regression equation) is represented by "C". The measurement of nitrate nitrogen (NO_3–N) was performed using a colorimetric approach, similar to the method used for NH_4^+-N, with the only difference being the use of 1 M K_2SO_4 solutions instead of KCl solutions for sample extractions [34, 37]. The total nitrate–N in the samples was calculated following the procedure described in a previous study [34]:

$$NO_3^- - N = C \times \frac{v}{w} \quad (2)$$

where v = the final volume of the digestate (litre), w is the weight of manure digestate utilized, and C is the concentration of NO_3-N in the sample in mg/l (as determined from the calibration curve regression equation) (kg). Ammonium-N and nitrate–N were added to determine total nutritional nitrogen (TNN). The Olsen approach [38], was used to measure the amount of bio-soluble phosphorus in the digestate.

2.5 Microbial Count

The microbial inoculation potential of the nutrient supplements was evaluated using standard techniques and procedures. Bacterial populations were assessed using bacteriological agar, while fungal populations were assessed using Rose Bengal agar [39]. To test the hydrocarbon-degrading bacteria, diesel gasoline was used as a carbon source on solid noble agar plates [40]. A soil suspension was created by combining 0.50 g of manure with 9.50 ml of distilled water. Serial dilution was performed 10 times, and the number of colonies was counted to determine the colony forming units (CFU) in each sample. The samples were cultivated for 8 days at 27 oC in triplicate before counting the CFU [41].

2.6 Statistical Analysis

The data was further analyzed using statistical techniques such as statistical averaging, analysis of variance (ANOVA), and regression analysis. SPSS statistical software was used for these analyses. The relationship between the different nutrient stimulations and TPH breakdown was also evaluated using post hoc interpretation with the harmonic mean.

3 Result and Discussion

3.1 The Physicochemical Characters of the Soil Used for the Study

Table 2 displays the physicochemical characteristics of the soil used in this study. The analysis revealed that the soil had a slightly acidic nature, with a pH of 5.51 ± 0.03. This acidity is commonly observed in agricultural soils within the Niger Delta region. A similar pH range of 4.20–5.90 was reported by [42] for oil palm plantations at the Nigeria Institute of Oil Palm Research (NIFOR) in Benin City, Edo State, which is geophysically similar to the source of the soils used in this study. Additionally, an oil palm field in Eko-Iyobhebhe, Irrua, Edo State exhibited a similar pH value of 5.03 [43]. However, a pH of 5.26 was previously recorded for the University of Benin Research farms and surrounding areas [44]. It is important to note that soil pH can vary significantly across different regions and is influenced by both inorganic and organic components. The pH of tropical agricultural soils, particularly in Southern Nigeria, typically ranges from approximately 5.00 to 6.80, as reported by [45]. Soil pH plays a crucial role in the chemistry, biochemistry, nutrient availability, and microbial life within the soil [46, 47]. The impact of soil pH on microbial development and biodiversity can hinder or reduce the bioremediation of oil pollutants in soils. For optimal soil microbial growth and performance, a slightly alkaline or less acidic pH is recommended [48, 49]. According to [49], the ideal pH range for hydrocarbon breakdown is 6.50–8.00. Similarly, microbial optimal growth is suggested to occur within a pH range from neutral to 8.50 [48]. The pH of the soil used in this study falls below the recommended range for microbial activity and biodegradation to occur at their best. However, the nutrient supplements employed in the study had a basic character and a pH of 7.58 (Table 3), indicating that they would have a liming effect on the soils to which they were applied for remedial improvement [20].

Table 2 Physicochemical Properties of Soil used in this study

Parameters	Units	Quantification	Remarks
pH		5.51 ± 0.03	Acidic soil
EC	µS/cm	315 ± 25	–
Sand	%	82.96 ± 08	High sand content
Silt	%	4.60 ± 0.90	Low content
Clay	%	11.67 ± 1.01	Low content
WRC	%	18.88 ± 1.88	Low WRC
OMC	%	4.16 ± 1.11	–
Total P	%	0.16 ± 0.16	–
Total N	%	0.33 ± 0.11	–
TOC	%	3.32 ± 0.21	–
K	mg/kg	354.52 ± 21.50	–
Na	mg/kg	227.14 ± 18.01	–
Ca	mg/kg	75.56 ± 5.15	–
Mg	mg/kg	17.53 ± 0.57	–

The soil used in this study was classified as loamy sand, with approximately 83% sand, 5% silt, and 12% clay content, which is consistent with the typical textural characteristics of agricultural soils in the Nigerian rainforest [50]. The concentration of soil organic matter (SOM) was measured to be 5.76%, which is relatively higher compared to the values of 1.18% and 1.80% reported by [42, 43] for similar oil palm estate soils. This higher SOM concentration can be attributed to the frequent clearance, absence of burning practices, and the use of organic fertilizers in the area, which contribute to the accumulation of organic matter. Similarly, the levels of macronutrients such as nitrogen (N), phosphorus (P), and base elements were also found to be elevated

Table 3 Selected physicochemical and microbial properties of the nutrient supplements used in this study

Parameters	pH	EC (µS/cm)	TOC (%)	TNN (mg/kg)	AP (mg/kg)	THB (CFU)	THF (CFU)	THUB (CFU)	THUF (CFU)
Quantity	7.58 ± 0.3	814.78 ± 77	5.02 ± 0.04	1421.59 ± 63	957.73 ± 47	1.5×10^4	1.4×10^4	1.6×10^4	1.3×10^4

TNN total nutrient nitrogen (NO_3^-–N^+ NH_4^+–N), *THB* total heterotrophic bacteria, *THF* total heterotrophic fungi, *HUB* hydrocarbons utilizing bacteria, *HUF* hydrocarbons utilizing fungi

compared to previous studies [43]. On the other hand, the water retention capacity (WRC) of the soil was determined to be 18.88%, falling within the range reported for loamy sand soils by [51]. This soil was specifically selected for the study due to its representation of the typical characteristics of agricultural soils in Nigeria's Niger Delta, where oil pollution has been a significant issue.

3.2 Physicochemical and Microbial Properties of the Digestates

In traditional agricultural practices, composting is commonly carried out by combining or separately composting green waste, food waste, and livestock droppings. In Nigeria, livestock dung and droppings are typically stored on the farm or in the yard for composting purposes, after which they are utilized as organic fertilizers. The specific methods of composting vary and depend on the available space. Some farmers store these organic materials in perforated bags, while others directly apply them to their farms. The nutrient content of these composted materials is influenced by the composting process employed [52, 53]. In this study, the droppings from chicken layers were subjected to a two-month digestion process before being utilized. Table 3 provides the physicochemical and microbiological characteristics of the resulting digestates.

The analysis conducted on the digestates (CLD manures) revealed that they had total nutritional nitrogen levels of approximately 1422 mg/l and accessible phosphorus levels of 957.73 mg/l. Furthermore, the pH of the organic manure was measured at 7.58, indicating an alkaline nature. This pH value falls within the recommended range of 6.50 to 8.00, which is considered optimal for microbial growth and bioremediation activities, thus making the alkaline character of the digestate beneficial for bioremediation purposes [48, 54]. Additionally, the digestate exhibited a relatively high phosphorus content, which reduces the likelihood of causing eutrophication and makes it more environmentally friendly.

3.3 Inoculant Status of Digested Chick Layers Droppings

Microorganisms, particularly bacteria and fungi, serve as the primary catalysts for the biodegradation of soil toxins and pollutants [39]. Heterotrophic bacteria and fungi have the ability to consume and oxidize various organic carbon sources, with a preference for non-hydrocarbons that can be easily oxidized. This means that they would only turn to hydrocarbons as a food source if there are no other available organic carbon sources or if they are insufficient. Conversely, hydrocarbon-utilizing bacteria and fungi have a strong affinity for hydrocarbons and are often attracted to soil contaminants that contain hydrocarbons [19, 55]. These hydrocarbon-utilizing microorganisms play a crucial role in breaking down such pollutants in hydrocarbon-contaminated soils. Therefore, in bioaugmentation studies, hydrocarbon-utilizing microorganisms are introduced into the environment to enhance their population for optimal bioremediation [56]. Table 4 provides an overview of the microbiological status of the soils and the nutrient supplementation prior to the various bioremediation treatments. The results present the existing population of total heterotrophic microorganisms as well as the levels of hydrocarbon-utilizing bacteria (THUB) and hydrocarbon-utilizing fungi (THUF) following nutrient supplementation.

The findings revealed that the digestate exhibited a high microbial population, with THB and THF values of 1.5×10^4 and 1.4×10^4, respectively. As previously mentioned, these hydrocarbon-utilizing bacteria (HUB) and fungi (HUF) are key players in soil bioremediation, particularly in the degradation of hydrocarbons. Considering the digestate's role as a source of nutrient stimulation, it is expected to possess strong bioremediation capabilities. Furthermore, the total organic carbon (TOC) content of the digestate is not excessively high, suggesting that the activity of heterotrophic bacteria and fungi in hydrocarbon breakdown would be significant. Previous studies have also reported the presence of HUB and HUF in similar organic waste materials [31]. These results imply that digested chicken layers dung

Table 4 Microbial Counts of soils and the nutrients supplements prior to treatments

Samples	THB	THUB	THF	THUF
Unpolluted soil (UPS)	7.0×10^3	3.0×10^3	1.2×10^4	5.0×10^3
HC Polluted soil (PSC)	1.4×10^4	7.0×10^3	1.5×10^4	1.2×10^4
Layers manure Digestates	1.5×10^4	1.6×10^4	1.4×10^4	1.3×10^4

THB total heterotrophic bacteria, *THF* total heterotrophic fungi, *ND* not detected

Table 5 Selected hydrocarbons conc. mg/kg) of samples at day 1

Samples	Remediation indices		
	TDROs	TPAHS	TPH
PSC5	25302 ± 244	2398.20 ± 209	34003 ± 1501
CLD510	24507 ± 241	2457.58 ± 243	33389 ± 1560
CLD520	25317 ± 257	1952.28 ± 179	34295 ± 844

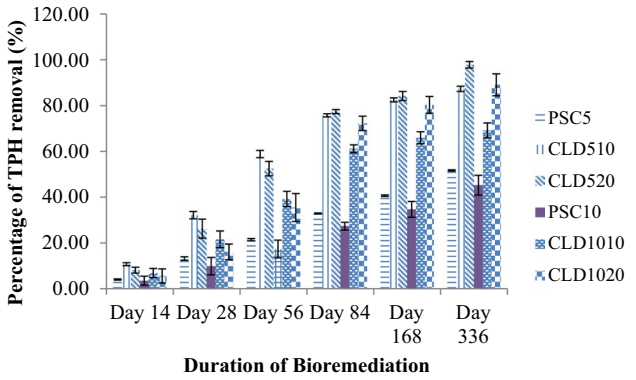

Fig. 1 Percentage removal of TPH at different periods of remediation

could serve as an enhanced source of nutrients and microbial inoculation in the landfarming of petroleum hydrocarbon-contaminated soils.

3.4 Bioremediation of Petroleum Hydrocarbon Polluted Soils

The base concentration of total hydrocarbons (TPH) after stabilization and before nutrient addition for remediation is shown in Table 5.

Analyzing the total petroleum hydrocarbons (TPH) is a common approach to assess the extent of bioremediation in petroleum-contaminated environments. TPH primarily consists of aliphatic, cyclic, and aromatic hydrocarbons, including DROs, BTEX, and PAHs (as shown in Table 5). The table presents the initial concentrations of TPH in the samples, while Fig. 1 illustrates the percentage of TPH removed during the remediation process. The results revealed that the early degradation of TPH in the 5% HCM-contaminated samples was minimal during the initial stages of the experiment. At day 14, the removal values ranged from approximately 4.07% (in PCS5) to 8% (in CLD520), and by day 28, they increased to around 13% (in PCS5) to 32% (in CLD510). Similarly, the 10% HCM-contaminated samples exhibited low levels of remediation, with values ranging from approximately 6% in PCS10 to about 7% in CLD510 at day 14, and from approximately 10% in PCS10 to approximately 22% in CLD1010 at day 28. These initial low values of contaminant remediation can be attributed to the acclimation period required by microbes to adapt to the treatments applied to the samples, especially when the use of inorganic fertilizers or easily oxidized carbon sources is involved, as discussed by [57, 58].

The percentage of TPH removal increased with the duration of the remediation process. In the case of the 10% CLD-treated samples, approximately 59% and 87% of TPH were degraded at days 56 and 336, respectively. Similarly, the 20% CLD treatments resulted in the elimination of around 52% and 97% of TPH on the same mentioned dates. Until day 56, the use of 10% CLD proved to be more efficient in remedying the 5% HCM-polluted samples. For the 10% HCM-contaminated samples, the application of 10% CLD treatment resulted in TPH degradation of approximately 39% and 69% at days 56 and 336, respectively, while the 20% CLD treatments led to TPH elimination percentages of 72% and 89% at days 84 and 336, respectively. Despite the higher level of HCM pollution, the 10% CLD treatment performed better than the 20% treatments at day 56. However, as the bioremediation period extended, the 20% manure digestate treatments exhibited higher TPH degradation. For instance, samples CLD520 degraded approximately 77% and 84% of TPH at days 84 and 168, respectively, compared to samples CLD510, which degraded about 76% and 82% of TPH at those respective times (refer to Fig. 1).

The remediation of the 10% HCM-polluted samples followed a similar trend. The percentages of TPH elimination in all samples were generally low up to day 56 when compared to the levels degraded at days 84 or 168. Figure 2 provides an overview of the percentage of TPH eliminated during the remediation intervals. The results revealed that larger amounts of TPH were degraded between day 28 and day 56 in the CLD510 and CLD520 samples of the 5% HCM-polluted samples. However, between days 56 and 84, a greater TPH elimination was observed in the 10% HCM-contaminated samples, specifically in the CLD1010 and CLD1020 samples. For instance, during this period, approximately 22% and 37% of TPH were degraded in the CLD1010 and CLD1020 samples, respectively, compared to 18% and 19% in the CLD1010 and CLD1020 samples between days 28 and 56. This observation aligns with the findings from a study conducted in 2005, which reported higher levels of TPH degradation at the midpoint of bioremediation of crude oil-polluted soils using chicken droppings and rubber processing sludge as stimulants. The researchers attributed this increase to the adaptation of soil microbes to the environment, availability of sufficient nutrients, and an increase in microbial variety and population, thereby enhancing oil degradation. The decrease in TPH breakdown in the CLD1010 and CLD1020 samples may be expected as the nutritional content of the soil depletes along with the reduced level of HCM contamination. Adequate nutrients, favorable climatic conditions, and sufficient acclimation time for soil microbes

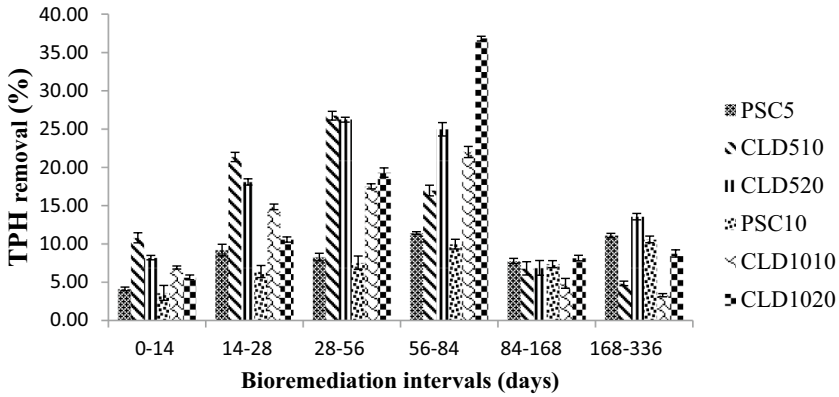

Fig. 2 Percentage amount of TPH removed at the remediation intervals

Table 6 Post Hoc interpretation of TPH percentage removal from 5% HCM polluted soils at day 84

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC5	3	32.8700			
CLD510	3				75.8100
CLD520	3				77.4400
Sig		1.000	0.774	0.862	0.994

Means for groups in homogeneous subsets are displayed. Harmonic Mean Sample Size = 3.000

Table 7 Post Hoc interpretation of TPH percentage removal from 5% HCM polluted soils at day 168

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC5	3	40.6000			
CLD510	3			72.5800	
CLD520	3				84.2700
Sig		0.124	0.642	0.943	1.000

Means for groups in homogeneous subsets are displayed. Harmonic Mean Sample Size = 3.000

Table 8 Post Hoc interpretation of TPH percentage removal from 5% HCM polluted soils at day 336

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC5	3	51.6700			
CLD510	3	57.3700	57.3700		
CLD520	3				87.8100
Sig		0.117	0.405	0.053	0.082

Means for groups in homogeneous subsets are displayed. Harmonic Mean Sample Size = 3.000

in oil-polluted areas have been emphasized for optimal biodegradation of soil pollutants. It is valuable to assess the amount of these hydrocarbons that were eliminated from the treatments in comparison to the control samples.

The results indicated that, at day 84, there was no statistically significant distinction between the utilization of 10% and 20% CLD stimulation (the two treatment levels), although there was a numerical difference, with CLD520 demonstrating greater TPH degradation. However, both levels of CLD treatments exhibited a significant difference compared to the control treatment, as shown in Table 6. However, at day 168, there were significant differences in TPH elimination among the control treatment (PSC5) and the CLD-treated samples, as well as between the two treatment levels, as depicted in Table 7.

At the end of the remediation period (i.e., day 336), significant differences in TPH degradation were observed between the CLD510 and CLD520 treatments, as well as compared to the control treatment. The CLD520 treatment exhibited a higher elimination of TPH compared to the CLD510 treatment. This enhanced effectiveness of CLD520 may be attributed to its higher nutrient content, in contrast to the reduced nutrient level of CLD510, as indicated in Table 8.

During the initial phases of bioremediation, there were no significant differences observed between the application of 10 or 20% nutrient stimulation in the 10% HCM-polluted soils, compared to the 5% HCM-polluted soils. Both CLD1010 and CLD1020 treatments exhibited significant differences when compared to the control samples, but no significant differences were observed between the two treatments in terms of TPH elimination (as of day 84), as shown in Table 9. However, by day 168, there were significant differences observed between the control and all treatment groups (Table 10).

Similarly, at day 336, significant differences in TPH degradation were observed between the CLD-stimulated samples and the control, as well as between CLD1010 and CLD1020 treatments, as indicated in Table 11. This

Table 9 Post Hoc interpretation of TPH percentage removal from 10% HCM polluted soils at day 84

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC	3	27.3300			
CLD10	3				69.1800
CLD20	3				72.2700
Sig		1.000	0.975	0.990	0.899

Means for groups in homogeneous subsets are displayed. Harmonic Mean Sample Size = 3.000

Table 10 Post Hoc interpretation of TPH percentage removal from 10% HCM polluted soils at day 168

Treatments	N	Subset for alpha = 0.05		
		1	2	3
PSC10	3	34.6500		
CLD1010	3		65.9800	
CLD1020	3			80.3800
Sig		0.059	0.997	0.065

Means for groups in homogeneous subsets are displayed. Harmonic Mean Sample Size = 3.000

Table 11 Post Hoc interpretation of TPH percentage removal from 10% HCM polluted soils at day 336

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC10	3	45.2100			
CLD1010	3		61.2300	61.2300	
CLD1020	3				79.0900
Sig		0.130	0.966	0.057	0.605

Means for groups in homogeneous subsets are displayed. Harmonic Mean Sample Size = 3.000

observation was further supported by the percentage of TPH degraded in the three treatments (PSC10, CLD1010, and CLD1020) during days 84 to 336, as depicted in Fig. 1.

4 Conclusion

Efforts are continually being made to develop bioremediation methods that are efficient, environmentally friendly, cost-effective, and require less technical expertise for the decontamination of petroleum-contaminated soils and waters. This study aimed to contribute to the improvement of bioremediation techniques for petroleum hydrocarbon-polluted soils. The findings of this research demonstrated that locally produced chicken manure digestates could serve as a valuable source of hydrocarbon-utilizing microorganisms and be utilized as both a nutrient supplement and a microbial inoculant for bioremediation purposes in petroleum hydrocarbon-polluted soils. Previous studies have highlighted the effectiveness of organic compounds as inoculants and nutrient sources. In our study, the digestion of chicken dung resulted in the removal of up to 10% of total petroleum hydrocarbons from petroleum-contaminated soil. Previous research has suggested that the efficacy of bioremediation is limited, especially in the short term, when petroleum soil pollution levels exceed 5%. However, our study demonstrated that organic manure digestates can effectively remediate higher levels of petroleum soil pollution beyond 5%. Furthermore, the degree of treatment with organic digestates was found to be influenced by the level of pollution, the duration of remediation, and the application rate of the manure. While a 20% organic stimulation is optimal for petroleum soil contamination levels above 5% and intended for remediation periods of 84 days or longer, a 10% treatment could be sufficient for a 56-day remediation period, or repeated application of the specified quantity when the remediation extends beyond 56 days.

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Declarations

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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