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Bioremediation Efficacy and Total Petroleum Hydrocarbon Reduction in Crude Oil Contaminated Soil Using Cow Dung

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ABSTRACT: Crude oil contamination poses significant ecological and agricultural challenges, particularly in oil-producing regions such as Nigeria's Niger Delta. The persistence of toxic petroleum hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), adversely affects soil health, plant growth, and food security. Rich in essential nutrients, cow dung enhances the growth and activity of oil-degrading microorganisms such as *Marinobacter spp.* and *Pseudomonas spp.*, promoting the breakdown of these hydrocarbons. Hence, the objective of this paper was to evaluate the bioremediation efficacy and total petroleum hydrocarbon (TPH) reduction in crude oil contaminated soil using cow dung using appropriate standard methods. Data obtained indicates a reduction range of between 54.48 - 60.19% of total petroleum hydrocarbon (TPH) content in the crude oil contaminated soil after bioremediating with the cow dung. This result is attributed to increased nutrient availability and improved oxygen diffusion associated with optimal amendment rate. In comparison, natural attenuation in untreated soil achieved only a 17.22% reduction. This approach aligns with Sustainable Development Goals (SDG 12 and SDG 15) which is to promote responsible waste utilization and ecosystem restoration. The findings underscore cow dung's viability as an eco-friendly and cost-effective strategy for mitigating the environmental impacts of crude oil pollution.

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Crude oil, an unrefined fossil fuel, plays a significant role in global energy production but it is also a major environmental contaminant. Petroleum hydrocarbons pose serious ecological threats and health risks when released during oil extraction, transportation, refining, and accidental spills (Omokaro 1998; Wokem and Madufuro 2020). These pollutants contaminate water bodies and severely degrade soil leading to loss of soil fertility, reduced agricultural output, and forced migration of farming communities, especially in oil-producing regions such as Nigeria's Niger Delta (Ikeh and Yemi-Jonathan 2020). Complex aromatic hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs), benzene derivatives, and cycloalkanes, are particularly toxic and resist microbial degradation (Adams *et al.*, 2014; Rehmann *et al.*, 1998). This resistance contributes to long-term environmental toxicity especially in oil-producing regions such as Nigeria’s Niger Delta. The presence of these harmful compounds affects plant growth, soil health, and ultimately food security (Agarry *et al.*, 2012).

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Soil contamination is caused by industrial activities, agricultural chemicals, and improper disposal of waste from human activities. Physical, chemical, photodegradation, and biological amendments have been developed to remediate contaminated soil, but the latter offers the best environmentally friendly method because it uses indigenous microorganisms in the environment to break down the hydrocarbons and heavy metals found in this soil into harmless substances (Fadina *et al*., 2019). The use of organic waste products, such as cow dung, to enhance bioremediation is gaining attention as a sustainable and cost-effective approach. Bioremediation refers to the natural ability of certain microorganisms or organic amendments to degrade pollutants (hydrocarbons) into less harmful compounds (Oludele *et al.,* 2021; Bidwell *et al.,* 2002). Hence, it has emerged as an effective and environmentally friendly alternative method to address these issues of crude oil contaminations. Cow dung is rich in essential nutrients such as nitrogen and phosphorus, which promote the growth of oil-degrading microorganisms (bacteria and fungi) and contain enzymes that assist in breaking down complex hydrocarbons (Osazee *et al.,* 2015; Okolo *et al.,* 2005; Ijah *et al.,* 2008). The application of cow dung to oil-contaminated soils accelerates the microbial degradation of crude oil, helping to restore soil health and support ecosystem recovery (Okafor and Onyido 2022; Haftka *et al.,* 2015). Bioremediation of crude oil-contaminated soil using cow dung restores ecosystems affected by oil spills and utilizes agricultural waste in a productive manner, underscoring its potential as a sustainable solution for environmental restoration. Two (2) of the United Nation's Sustainability Goals (SDG12 and SDG15) emphasizes on responsible consumption and conservation. Categorically, the SDG12 advocates responsible consumption and production by reducing the environmental impact on industrial activities, SDG15 promotes life on land, by conserving and restoring terrestrial ecosystems. Consequently, this study aligns with these two UN Sustainability goals with the objective of evaluating the bioremediation efficacy and total petroleum hydrocarbon (TPH) reduction in crude oil contaminated soil using cow dung

MATERIALS AND METHODS

Materials: The materials used in this work include n-hexane, four plastic containers of equal sizes, white soil, crude oil, fresh cow dung, Agilent GC Equipment, weighing balance.

Sample Collection and Treatment: The crude oil sample used in the study was collected from an oil pit located at Okpai, Ndokwa East, Delta State with coordinates; latitude: 5.72^{o} N and Longitude: 6.36^{o} E. Fresh Cow dung was collected using a sterile plastic container from a cow grazing farm in Garki Emene, Enugu, Enugu State with coordinates; Latitude: 6^{o} 28' N and Longitude: 7^{o} 35'E. Bulk white soil was collected from a building site in Chukwuemeka Odumegwu Ojukwu University, Uli campus in Ihiala, Anambra State with coordinates; Latitude 5.78° N and Longitude 6.82° E. The collected soil was sieved and a portion was used to determine the physicochemical parameters of the soil.

Experimental Design

Soil Simulation with Crude Oil and Cow Dung Treatment 5000 g of soil free from impurities and contain low organic matter were weighed into a clean plastic container and 750 ml of crude oil was added and mixed manually until a homogeneous mixture was obtained without water. The mixture was allowed to stand for two weeks (14 days) with intermittent tilling of the soil with fork at after every 48 hours. 10g of the soil sample was collected with the aid of a spoon after the 14 days and weighed using a weighing balance, poured into a 1L glass bottle and sealed with aluminum foil. The bottle was allowed to stay for another 12 hours before taken for GC-MS analysis to determine initial Total Petroleum Hydrocarbon (TPH) (Ayotamuno *et al.,* 2006).

500 g of the simulated contaminated soil were each weighed into three clean 1000 ml plastic containers labeled 500CD, 1000CD, and Control, respectively. 500 g and 1000 g of fresh cow dung was added to the containers labeled 500CD and 1000CD while no cow dung was added to the container labeled Control. The entire mixture was stirred thoroughly until a homogeneous mixture was obtained. The three containers were left for another 14 days, with tilling in an open air for proper aeration at every 48 hours.

Soxhlet Extraction of the Oil after Contamination and Bioremediation The crude oil was extracted from the three containers using soxhlet extraction technique (Sutar *et al.,* 2011). The simulated soil samples were extracted with N-hexane and refluxed for 5 hours at 60℃. The crude oil and N-hexane mixture were further subjected to hot water bath at 50 ℃ to get the crude oil. The extent of the degradation of each sample was performed using GC-MS analytical tool using the crude oil extracted.

RESULTS AND DISCUSSION

Table 1 depicts the result of the soil sample profiles before the simulating with crude oil for ex-situ bioremediation of the oil-contaminated soil. The pH

(7.02) supports microbial growth, which aligns with optimal pH (6.5 - 8.5) for growth of oil-degrading microorganisms (Varjani and Upasani 2019). Moderate acidity (3.4 cmol/kg) enhance microbial activity as acidic soil promotes the growth of microorganisms (Mishra and Singh 2015). High sand content (96.22 %) facilitates oxygen availability and oil mobility, promoting aerobic biodegradation (Das and Chandran 2011). Low moisture content (3.46 %) limit microbial activity (Namkoong *et al.,* 2002), and low organic carbon (0.18 %) can adversely affect microbial growth and nutrient cycling (Chaineau *et al.,* 2005).

Table 1: Physiochemical parameters of the uncontaminated soil

Parameters	Values
Moisture content (%)	3.46
pH	7.02
Organic carbon (%)	0.18
Soil Acidity (cmol/kg)	3.40
% Silt	2.01
% Clay	1.77
% Sand	96.22

The result of the soil conditions outlined in Table 1 appears to be moderately favourable for crude oil bioremediation. Hence, the soil requires optimization with organic amendments and cow dung was used after simulating the soil samples with crude oil to breakdown the carbon long chains effectively. The addition of cow dung provides essential nutrients for microbial growth, enhancing biodegradation rates (Kartikey *et al.,* 2016).

Table 2: Summary of Concentrations of Samples for Total Petroleum Hydrocarbon (TPH) in ppm and Percentage Reduction of TPH

Samples	TPH (ppm)	% Reduced
A	106.72	-
500CD	42.182	60.49
1000CD	48.599	54.48
Control	88.378	17.22

In Figs. 1- 4, the GC profile results revealed that C_1-C_7 straight chain hydrocarbon fractions were non-existent in all the ex-situ crude oil contaminated soil but the heavier carbon chains C_8-C_{40} were detected in all the samples. However, the two different ratios of cow dung were able to reduce the C_8-C_{40} hydrocarbon fractions reasonably at different rates within two weeks of study (Table 2). Fig. 1 shows the graphical representation of the various carbon chains identified in the original soil sample and their concentration in parts per million (ppm) as a function of time. Data analysis of the sample shows 33 different carbon-chains (i.e., C_{8}-$_{40}$) including Pristine and Phytane compounds were identified. The Total Petroleum Hydrocarbon (TPH), cumulative of $C_{8\text{-}40}$ indicated by their concentration in the extract is approximately 106.762ppm (Tables 2 and 3).

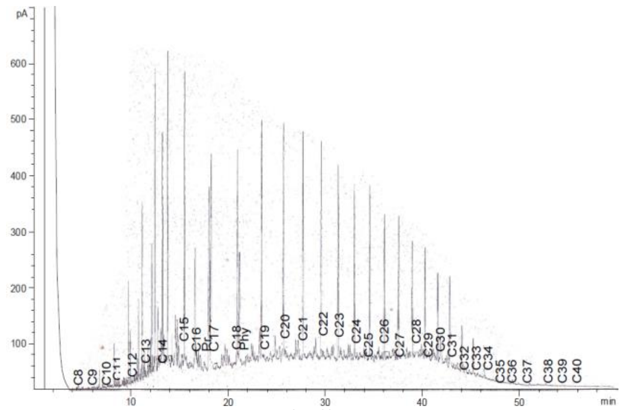

Fig. 1: Gas Chromatogram of different hydrocarbon chains identified in Original samples as plots of their different area peaks against time

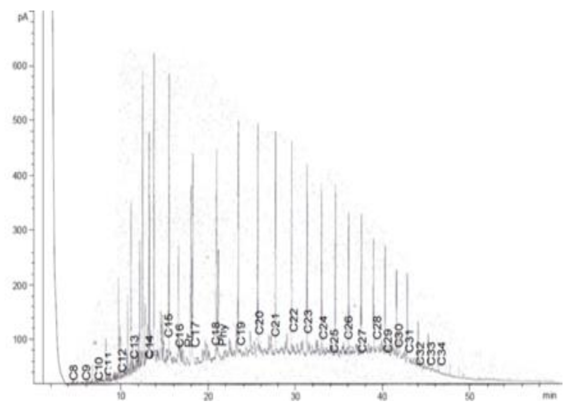

Fig. 2: Gas Chromatogram of different hydrocarbon chains identified in 500CD samples as plots of their different area peaks against time

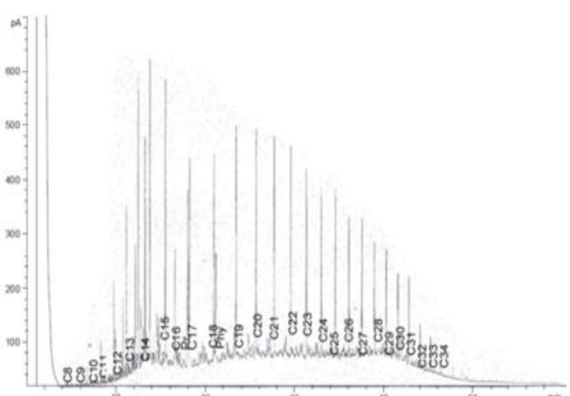

Fig. 3: Gas Chromatogram of different hydrocarbon chains identified in 1000CD samples as plots of their different area peaks against time

Figs. 2 and 3 are the GC-MS results spectrum showing the different identified carbon chains present in 500CD and 1000CD respectively, with concentration. The graphs showed that the addition of cow dung as an amendment significantly enhanced bioremediation efficiency which is consistent with previous studies demonstrating the effectiveness of organic amendments in enhancing bioremediation (Das and Chandran 2011; Mishra and Singh 2015). The nutrient-rich properties of cow dung, particularly carbon, nitrogen, and phosphorus, support microbial growth and activity, leading to increased biodegradation of petroleum hydrocarbons (Chaineau *et al.*, 2005).

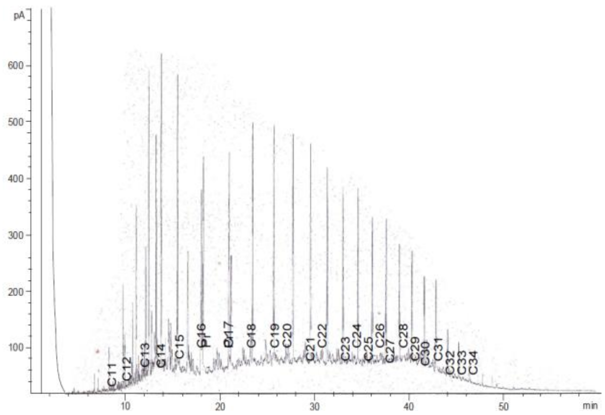

Fig. 4: Gas Chromatogram of different hydrocarbon chains identified in the Control sample as plot of their different area peaks against time

Fig. 4 portrays the graph of the peak area against time for the Control sample and it shows reduced concentrations of the identified carbon chain when compared with the original sample as detailed in Table 3. The TPH of the Control sample at the time of this analysis using GC equipment was 88.378 ppm and had 17.22 % decrease in TPH. It is worthy to note that control sample at the time of this analysis still contained much of the initial carbon-chained compounds found in the original sample, however, $C_{8-10,\ 33-40}$ compounds were noticeably absent.

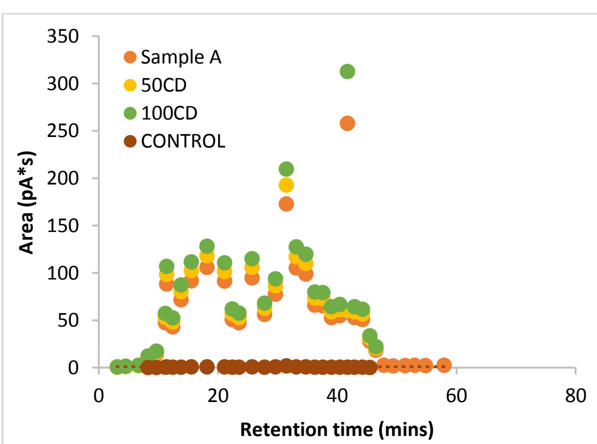

Fig. 5: Comparison of average peak area (pA) of the different hydrocarbon chains identified plotted against time (mins) for the three samples.

The study's findings indicate that cow dung amendments are effective in remediating crude oil-contaminated soil. Specifically, the results show that both 500CD (500g cow dung) and 1000CD (1000g cow dung) amendments significantly reduced TPH concentrations (Adebusoye *et al.*, 2007; Akinde and Obire 2008; Umanu *et al.*, 2013). However, the 500CD amendment outperformed the 1000CD amendment, achieving a 60.49 % TPH reduction compared to 54.48 % for 1000CD (Table 2). This greater reduction of TPH with the 500g cow dung amendment compared to 1000g may result from better microbial balance, optimal oxygen diffusion, and a more favorable C:N:P ratio in the smaller amendment. Excessive organic material can create anaerobic conditions and nutrient imbalances, limiting microbial efficiency. Studies by Joo *et al.*, 2008; Ayotamuno *et al.*, 2006) have shown that excessive amendment rates can lead to reduced biodegradation efficiency due to microbial saturation, poor aeration, and nutrient competition. The optimal amendment rate may not be directly correlated with the extent of carbon chain reduction.

Furthermore, the optimal carbon-to-nitrogen (C/N) ratio for microbial growth and biodegradation may be achieved at lower cow dung amounts. According to Wang *et al.*, 2019, the ideal C/N ratio for bioremediation ranges from 10:1 to 20:1. Exceeding this ratio can lead to reduced biodegradation efficiency. In terms of cost-effectiveness, using lower cow dung amounts can reduce costs while maintaining bioremediation efficiency. A study by Romantschuk *et al*., 2023 found that optimizing amendment rates can lead to significant cost savings without compromising remediation outcomes. However, GC-MS analysis revealed a decrease in carbon chain lengths for both amendments, indicating effective biodegradation (Loibner *et al.*, 2020). The breakdown of complex hydrocarbons into simpler compounds is a critical step in bioremediation.

The Control sample showed a 17.22 % reduction in Total Petroleum Hydrocarbons (TPH), indicating natural attenuation processes (Wokem and Madufuro 2020). Natural attenuation relies on indigenous microorganisms to degrade hydrocarbons, but its effectiveness is often limited (Mishra *et al.*, 2015). Both cow dung amendments significantly enhanced remediation efficiency, with 60.49% (500CD) and 54.48% (1000CD) reductions when compared with the natural attenuation processes. This is consistent with previous studies demonstrating the effectiveness of organic amendments in enhancing bioremediation such as Adams *et al.*, (2014) where total petroleum hydrocarbon reduced up to 81 % by the metabolic activities of cow dung microorganisms.

Observing the summarized data in Fig. 2 and Table 3, the Gas Chromatography-Mass Spectrometry (GC-MS) results reveal an apparent discrepancy between the peak areas and concentrations of carbon chains in 500CD and 1000CD compared to control sample. Specifically, the peak areas for carbon chains in 500CD and 1000CD are higher than control sample, despite their significantly lower concentrations (ppm). This observation can be attributed to the complex processes occurring during bioremediation (Wang *et al.,* 2019; Cajthaml *et al.,* 2002). Biodegradation of hydrocarbons by microorganisms and compound transformation may play a vital role in this discrepancy. Microorganisms break down complex hydrocarbons into simpler compounds such as short-chain hydrocarbons, alcohols, and fatty acids during bioremediation, which are volatile and may exhibit higher detector response factors, contributing to increased peak areas (Baldwin *et al.,* 2019; Zhang *et al.,* 2015). Studies have shown that biodegradation can alter the carbon chain distribution, leading to an increase in peak areas for specific carbon chains (Joo *et al.,* 2008). Wang *et al.,* (2019) observed that biodegradation of petroleum hydrocarbons resulted in the formation of new compounds with higher detector response factors.

Additionally, detector response and sensitivity variations may contribute to the observed discrepancy as GC-MS detectors respond differently to various compounds, and biodegradation products may have higher detector response factors (Cajthaml *et al.,* 2002). Therefore, one may say that apparent discrepancy between peak areas and concentrations in 500CD and 1000CD can be attributed to biodegradation-induced transformations, changes in carbon chain distribution, and detector response variations.

Conclusion: The study confirms that lower cow dung amendments effectively remediate crude oil-contaminated soils due to the optimal microbial balance, oxygen diffusion, and favorable carbon-to-nitrogen ratio. The study also highlights that biodegradation leads to the breakdown of complex hydrocarbons into simpler, more volatile compounds with higher detector response, contributing to the increased peak areas observed in GC-MS analysis. Thus, cow dung amendments present a promising and efficient strategy for bioremediation of hydrocarbon-contaminated soils.

*Declaration of Conflict of Interest***:** The authors declare no conflict of interest (if none).

*Data Availability Statement***:** Data are available upon request from the first author or corresponding author or any of the other authors

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