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Synergistic Effect of Microorganisms and Charcoal on the Removal of BTEX and TPH from Crude Oil Contaminated Soil

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ABSTRACT

This study investigates the synergistic effect of microbial consortia and activated charcoal on the remediation of crude oil-contaminated soil in the Niger Delta region of Nigeria. Soil samples were treated over nine weeks using *Aspergillus niger*, *Pseudomonas aeruginosa*, *Sargassum filipendula*, activated charcoal, and their combinations. Key physicochemical parameters including pH, temperature, organic matter (OM), and total organic carbon (TOC) were monitored. The combined treatment of microorganisms and charcoal (S6) achieved the highest total petroleum hydrocarbon (TPH) removal efficiency (91.45%), outperforming individual treatments. BTEX compounds (benzene, toluene, ethylbenzene, and xylene isomers) showed substantial removal, with final degradation efficiencies ranging from 95.5% to 100% based on preliminary spectrophotometric data. However, due to limitations in the analytical method used (UV-Vis at 600 nm), these BTEX results are considered indicative and require validation through standard chromatographic techniques. The findings suggest that activated charcoal enhances microbial degradation by adsorbing toxic intermediates and providing a surface for microbial colonization. This integrated approach offers a cost-effective, scalable, and environmentally sustainable strategy for remediating oil-polluted soils, particularly in ecologically vulnerable regions such as the Niger Delta.

HIGHLIGHTS

- Synergy of microbes and charcoal boosted soil pollutant removal.
- Achieved 91.45% TPH removal, outperforming single treatments.
- Near-neutral pH supported microbial growth and remediation efficiency.
- Eco-friendly, low-cost method offers scalable soil recovery solutions.

1. INTRODUCTION

Crude oil is a complex mixture of several hydrocarbons, consisting of both low and high molecular weights. This mixture includes fully saturated hydrocarbons, branching hydrocarbons, unsaturated hydrocarbons, cyclic hydrocarbons (composed of carbon atoms only and with other atoms), and aromatic compounds (Kuppusamy et al., 2020; El Sabagh et al., 2019). Petrol-derived components such as total petroleum hydrocarbons (TPH), are among the most common environmental pollutants, along with benzene, toluene, ethylbenzene, and three isomers of xylene (ortho-, meta-, and para-) (collectively known as BTEX).

Crude oil and its derivatives commonly contain mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene, and various isomers of xylene, all of which are major soil and groundwater contaminants. Accidental spills of diesel fuel or gasoline during transportation, as well as leaks from underground storage tanks and pipelines, release BTEX compounds into the environment (Khodaei et al., 2017). BTEX compounds are volatile aromatic hydrocarbons known to cause health problems such as irritation, headaches,

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liver and kidney damage, as well as cancer (Gunasinghe et al., 2021).

The Niger Delta region of Nigeria has suffered extensive environmental degradation due to oil pollution and gas flaring, affecting both terrestrial and aquatic ecosystems. The consequences include a decline in the soil productivity, which negatively impacts both biodiversity and the economic well-being of local communities. This degradation contributes to high rates of poverty and unemployment (Akpan et al., 2015; Ikhumetse et al., 2022). In Nigeria, oil spill incidents often experience delayed responses and limited remediation efforts, particularly in the Niger Delta region, where logistical, regulatory, and infrastructural challenges hinder timely environmental restoration.

Bioremediation refers to the use of biological mechanisms to degrade, decompose, convert, or effectively eliminate pollutants or substances that reduce the quality of soil and water (Nnaemeka and Iyiegbu, 2015). Bioremediation involves the application of microorganisms or their products (bioaugmentation), nutrients (biostimulation), and plants (phytoremediation) to restore environments contaminated with crude oil (Nnaemeka and Iyiegbu, 2015). The use of organic waste materials is increasingly replacing the utilization of chemical fertilizer due to its inherent advantages. Although numerous methods have been proposed for remediating petroleum contaminated soils, there remains a need for cost-effective and environmentally sustainable techniques that utilize locally available hydrocarbon-degrading agents. Bioremediation (a biological treatment) has proven to be both eco-friendly and economically viable (El Sabagh et al., 2019), compared to physical and chemical methods such as solidification/stabilization, thermal desorption, and burning. The novelty of this study lies in its examination of the synergetic effect achieved by the combination of microorganisms with activated charcoal for the bioremediation of crude oil pollution in soil. While previous studies have focused on either microbial treatments or the adsorptive capacity of charcoal individually, this study uniquely integrates both strategies to enhance the recovery of TPH and BTEX components. This synergy is expected because activated charcoal can adsorb toxic intermediates that inhibit microbial activity and simultaneously provide a porous surface that enhances microbial colonization and biofilm formation, thereby accelerating hydrocarbon degradation. This innovative approach offers a low-cost, eco-friendly and scalable solution for the remediation of oil polluted soils, particularly regions like the Niger-Delta. The study also presents new data on the microbial activity and adsorption processes, promoting the use of bioremediation.

2. METHODOLOGY

2.1 Chemicals and media

Chemicals: The BTEX hydrocarbons used in this work comprised of a mixture of benzene (99.9% purity, M & B, England), toluene (99.5% purity, BDH, England), ethylbenzene (99% purity, JHD, China) and xylene isomers (99% purity, JHD, China). The extraction solvent, dichloromethane (DCM) is a product of Merck and 98% purity. It was further distilled to obtain higher purity (99.9%) meeting analytical standards. BTEX degradation was assessed using microbial isolates obtained from treated soil samples. The isolates were cultured in liquid media supplemented with BTEX compounds, and degradation was quantified via UV spectrophotometry. This assay was conducted to assess the intrinsic BTEX-degrading capacity of microbes enriched in each soil treatment.

2.2 Sampling site

The site chosen for the experiment was located at the Port Harcourt Refinery Depot area in Alesa-Eleme, Rivers State, Nigeria (Figure 1). Situated within the Niger Delta region, this area has been heavily impacted by oil-related activities, including extraction, refining, and transportation, leading to frequent occurrences of oil spills. These spillages have caused substantial environmental degradation in the Alesa-Eleme area. Soil samples were collected in the vicinity of the Port Harcourt Refining Company Limited (PHRC), a subsidiary of the Nigerian National Petroleum Corporation (NNPC) Port Harcourt (Lat: 4°49'.0012" N and Long: 7°2'0.9996" E). The sample areas were selected because of their proximity to where crude oil products are refined, stored, and dispensed into tanker trucks for distribution.

2.3 Collection of microbes

In the Microbiology Laboratory at the University of Port Harcourt, concentrated suspension of three different microorganisms—fungi (*Aspergillus niger*), bacteria (*Pseudomonas aeruginosa*), and algae (*Sargassum filipendula*)—were prepared. Each strain was propagated under optimal growth conditions and prepared as a concentrated suspension with the following specifications: Bacteria and fungi: 1.0×10^{8}

colony-forming units per milliliter (CFU/mL) Algae: 1.0×10^{6} cells/mL (estimated via hemocytometer count). For each treatment, 0.5 mL of the respective suspension was applied to 250 g of contaminated soil, resulting in an effective dose of 5.0×10^{7} CFU for *P. aeruginosa*, *A. niger* and 5.0×10^{5} cells for *S. filipendula*. To preserve the viability and integrity of the microorganisms for future use, the bottles were promptly stored at 4°C. Refrigeration serves as a preservation method to ensure the stability and longevity of the microorganisms by slowing down their metabolic activities and preventing their proliferation before they are utilized for specific laboratory experiments, studies, or other scientific investigations. This controlled storage condition ensured the stability and characteristics properties of the microorganisms until they were required for further research or analysis in the laboratory setting.

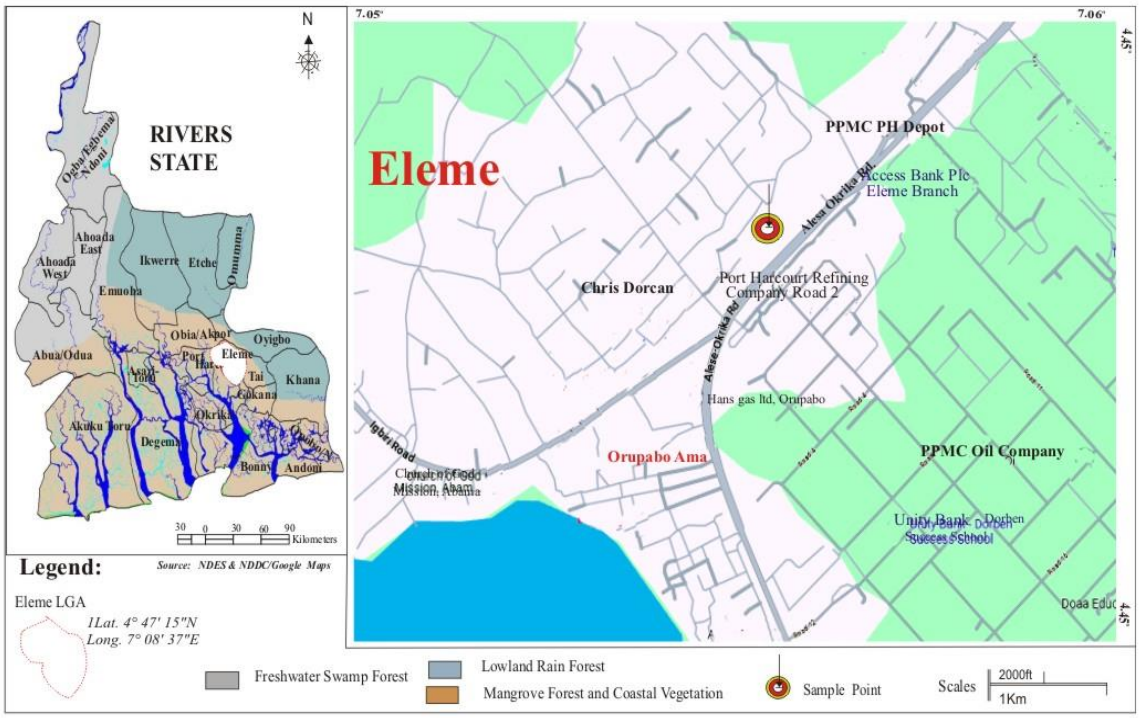

Figure 1. Map of sampling site

2.4 Sample collection and preparation

A stratified sampling approach was used in this study. The sampling field was divided into quadrants, and a total of fifteen (15) samples were taken by the proportionality rule, meaning that more samples were gathered from regions that contained a high concentration of contaminants. A composite sampling technique, which involves the blending of sampling units to generate a single sample, was utilized in conjunction with the stratified sampling approach. Oil-contaminated soil samples were collected using a sterile spatula, and the top two centimeters of each soil core were taken for analysis. To prevent cross-contamination, all samples were stored in clean, appropriate PVC bags, which were then sealed inside tin cans and sampling bottles. The experimental setup and corresponding samples groups are shown in Table 1.

2.5 Experimental design

The contaminated soil samples, contaminated with pollutants, were treated by combining them with specific biodegrading agents outlined in Table 1. This treatment aimed to facilitate the breakdown and remediation of pollutants present in the soil. To enhance aeration within the field cell environment, the soil samples were intermittently mixed by tilling. This process improved oxygen circulation, aiding the activities of microorganisms responsible for breaking down the contaminants in the soil. The experimental setup was allowed to settle for five-day intervals, after which samples were systematically collected for analysis. The primary focus of the analysis was to measure the remaining levels of both the TPH and BTEX within the treated soil samples. All experiments were conducted in triplicate, ensuring the reliability and consistency of the results. Furthermore, a control

sample was maintained at each stage of the experiment, providing a baseline for comparison with the treated samples. This approach enabled a comprehensive assessment of the efficacy of the biodegrading agents in reducing the levels of hydrocarbon contaminants in the soil. The structured experimental design enabled a comprehensive evaluation of the effectiveness of the biodegrading agents in reducing hydrocarbon contaminant levels in the soil. The systematic sampling and analysis at regular intervals, in triplicate, ensured a thorough and reliable assessment of the remediation process.

Table 1. Experimental sample set

Experimental set	Test experiment
S1	Polluted soil sample (250 g) + bacteria culture (0.5 mL)
S2	Polluted soil sample (250 g) + fungal culture (0.5 mL)
S3	Polluted soil sample (250 g) + algae culture (0.5 mL)
S4	Polluted soil sample (250 g) + charcoal (2.5 g)
S5	Polluted soil sample (250 g) + microbial consortium (0.5 mL)
S6	Polluted soil sample (250 g) + charcoal (2.5 g) + microbial consortium (0.5 mL) microbes (0.5 mL)
C1 (Control)	Unpolluted soil sample (250 g), no amendment

2.6 Soil pH

Fifty milliliters (50 mL) of distilled water was added to 20 g soil samples in a clean beaker. The mixture was stirred for 10 min, left to settle and stirred again for 2 min. The pH of the supernatant solution was measured using an Orion Research pH meter model 407 A.

2.7 Determination of temperature

The soil sample was prepared in the soil-to-water ratio mix of 1:1 by combining soil and distilled water. The mixture was allowed to settle before pH measurement using a calibrated pH meter. The temperature of the sample was measured using liquid in glass thermometer.

2.8 Electrical conductivity

The electrical conductivity of the soil was determined by measuring the conductivity of the filtrate obtained from the water extract, using the conductivity meter. The water extract was obtained from the soil sample through filtration, separating the liquid portion from the solid components. The conductivity meter, designed to measure for aqueous solutions, was then used to assess the ability of the filtrate to conduct electricity, and the measured value was recorded.

2.9 Determination of total petroleum hydrocarbon (TPH)

The testing for this analysis followed the ASTM D3921 standard method .Soil samples weighing 10 g each were carefully measured and prepared for analysis. These samples were then placed into a specialized apparatus known as a Soxhlet extractor, which facilitates the extraction of target compounds from solid samples. Within the Soxhlet extractor, the soil samples were combined with anhydrous sodium sulfate. This addition served the purpose of absorbing any moisture present in the samples, ensuring a dry environment for the extraction process. The elimination of moisture is critical for achieving optimal extraction efficiency. For the extraction of total crude oil hydrocarbons, a solvent known as methylene chloride, also referred to as dichloromethane, was employed. A volume of 200 mL of methylene chloride was used due to its strong solvating ability and selectivity for hydrocarbon compounds. During extraction process, the solvent continuously circulated through the soil sample in the Soxhlet apparatus, dissolving and extracting hydrocarbons from the soil matrix. This process was maintained for a sufficient duration to ensure thorough extraction and concentration of the target compounds. After the extraction process, the resulting solvent extract containing the hydrocarbons was collected and prepared for further analysis, as per the specified ASTM method. This method provides a consistent and reliable procedure for the quantitative recovery and analysis of total petroleum hydrocarbons (TPH) from soil samples, ensuring accuracy and reproducibility of results.

2.10 BTEX degradation experiment by microorganisms

For the microbial degradation of BTEX, the pH was adjusted to pH 6 and supplemented with 1% v/v

BTEX as the sole carbon source. An aliquot (1.0 mL) of 96 h prepared spore suspensions (1.0×10^6 cell/mL) was inoculated into each treatment flask. The control was left uninoculated and sterilized to account for abiotic losses such as volatilization. Experiments were carried out in triplicates at room temperature on a rotary shaker (Griffin Mechanical Shaker-Gallenkamp, England) (180 rpm) for 25 days (Kamal et al., 2017). An aliquot (5 mL) samples were taken aseptically at 5-day intervals. The residual BTEX compounds were extracted using 5 mL dichloromethane (DCM) and centrifuged (Griffin-Gallenkamp, England) (5,000 rpm) for 5 min. In this study, the BTEX degradation experiment was designed to access the microbial breakdown of benzene, toluene, ethylbenzene and xylene isomers using absorbance measurements at 600 nm. However, 600 nm is not suitable for the quantitative determination of BTEX compounds, which typically exhibit absorbance maxima in the ultraviolet (UV) range of 254-260 nm. Therefore, the use of UVis reading were used only to monitor the microbial response to BTEX exposure, rather than to quantify residual hydrocarbon concentrations. Accordingly, all BTEX data presented in this study are preliminary and indicative of microbial activity rather than chemical quantification. Further studies will adopt Gas chromatography coupled with Flame Ionization Detection (GC-FID) or Mass Chromatography to accurately measure individual BTEX compound and validate degradation efficiency.

3. RESULTS AND DISCUSSION

3.1 Remediation techniques and physical properties of contaminated soil

The physicochemical properties of polluted soil treated or remediated with bacteria (S1), fungi (S2), algae (S3), charcoal (S4), a mixture of micro-organisms (fungi, bacteria, and algae) (S5), and a combination of the microbial consortium with charcoal (S6) are shown in Table 2. The physic-chemical properties considered were soil pH, temperature, organic matter content and total organic carbon (TOC). These parameters serve as important indicators of soil health and microbial activity during the bioremediation process.

Table 2. Effect of remediation techniques on physical properties of contaminated soil

	S1		S2		S3		S4		S5		S6	
	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9
pH	8.09	6.23	8.17	6.11	5.77	6.11	8.23	6.08	5.76	5.99	5.72	5.77
T ^oC	29.4	22.05	29.3	26.01	26.5	23.05	29.3	26.01	26.5	23.05	26.5	26.98
%OM	3.63	6.57	4.370	6.67	6.52	5.60	6.52	5.55	11.83	6.99	13.38	8.81
TOC	2.11	3.81	2.54	3.87	3.78	3.25	3.78	3.22	6.86	4.06	7.76	5.11

The pH of the contaminated soil after treatment with S1-S6 techniques was estimated at the range of 5.72-8.09, showing slight reductions in the value of the pH compared to the initial values. The pH values of soils treated with the mixture of microorganisms (*Aspergillus niger*, *Pseudomonas aeruginosa*, and *Sargassum filipendula*), and with the synergistic combination of microorganisms and charcoal were within the range of 5.76-5.99, indicating a slightly acidic condition when compared to soils treated with single microorganism. Neina (2019) reported that the pH within the proximity of neutrality aids the survival of bacteria, fungi, and algae, and as well supports the performance in remediating contaminated soil samples. The temperatures of the treated crude oil polluted soils ranged between 22.05^{o}C and 24.40^{o}C. For each of the techniques used, the temperature at week 1 was higher than that of the week 9. This temperature range also implies that the microorganisms used as remediation agents remained active and effective under the experimental conditions (Iranzo et al., 2001).

For the S1 and S2 techniques, the organic matter content increased progressively with time, indicating that both the *Aspergillus niger* and *Pseudomonas aeruginosa* were capable of decomposing the crude oil hydrocarbons. This biodegradation activity contributed to the accumulation of organic matter in the treated soils, as the microorganisms converted complex petroleum compounds into simpler organic substance (Hamdi et al., 2007; Jabeen et al., 2009).

The organic matter of the contaminated soil treated with S3, S4, S5, and S6 techniques contributed to a decrease in organic matter in the crude oil-polluted soil. The presence of algae and charcoal in the S3 and S4 likely contributed to the reduction in organic

matter, as these agents can decompose or adsorb organic compounds in crude oil-polluted soil. This also implies that the synergistic combinations of microorganisms with algae and charcoal (S5 and S6) may have enhanced the degradation or adsorption processes, leading to a further decline in organic matter. Similarly, Masciandaro et al. (2013) also observed a reduction in organic matter via a synergic approach, which involves organic matter, microorganisms, and plants in soil bioremediation. Macci et al. (2012) investigated the bioremediation of polluted soil using a combination of plants, earthworms, and organic matter and found a reduction in organic matter. Figure 2 illustrates the variation of organic matter for wk1 and wk9 using various treatment methods.

The total organic carbon (TOC) of the crude oil-polluted soil treated with different remediation techniques was recorded as S1 (2.11% and 3.81%), S2 (2.54% and 3.87%), S3 (3.78% and 3.25%), and S4 (3.78% and 3.22%) for week 1 and week 9. The S5 synergic approach showed a TOC reduction from 6.86% and 4.06% for week1 and week9, respectively, while the S6 technique treated soil with an estimated TOC removal of 7.76% and 5.11% for week1 and week 9, respectively (Figure 3).

The total organic carbon (TOC) showed a notable increase in S1 and S2 treatments by the end of the experiment. These findings are consistent with the results of Tanee and Kinako (2008) and Tanee and Abert (2011), who demonstrated that microbial activity can facilitate the decomposition of hydrocarbons, resulting in an elevation of organic carbon content in the soil. This phenomenon can be attributed to the inherently high carbon content present in crude oil, as documented by Speight (2014).

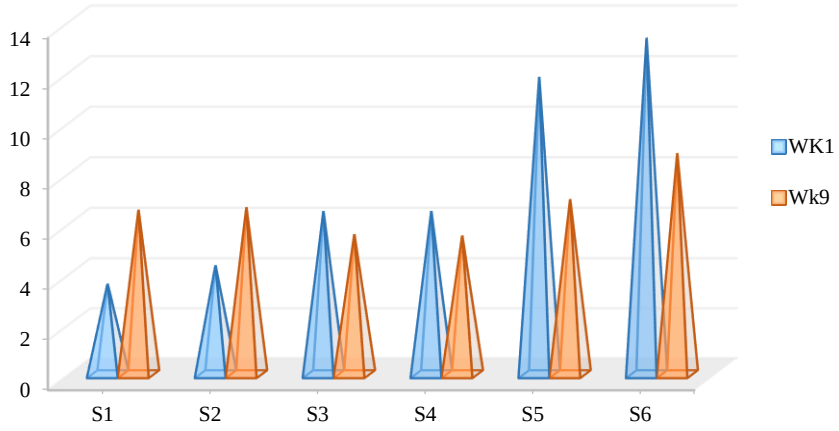

Figure 2. Variation of (a) organic matter for wk1 and wk9 at various techniques

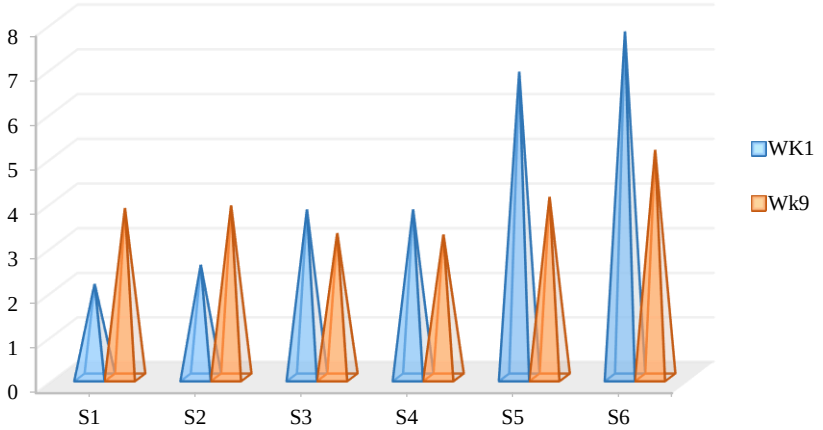

Figure 3. Variation of TOC for wk1 and wk9 at various techniques

In Contrast, when comparing week 1 to week 9, the S3, S4, S5, and S6 techniques helped to lower the TOC value of the crude oil-polluted soil. The algae and charcoal used in the S3 and S4 treatments, respectively, decreased the TOC in the crude oil soil. The observed decrease in TOC under the mixed microbial consortium (S5) contrasts with the increases recorded in the individual bacterial (S1) and fungal (S2) treatments. This may be attributed to synergistic microbial interactions that enhance the breakdown of complex hydrocarbons and accelerate mineralization of organic matter into CO_2. In mixed cultures, cooperative enzymatic activity and complementary metabolic pathways promote more complete degradation, thereby reducing residual organic carbon. Conversely, single-species treatments may promote microbial proliferation and partial transformation of hydrocarbons, contributing to TOC accumulation. Similar patterns have been reported in co-culture bioremediation systems where enhanced degradation correlates with reduced TOC levels (Li et al., 2021). Microorganisms may have converted the hydrocarbon in crude oil into other products, or charcoal may have adsorbed it. It also means that the algae and charcoal used in synergic techniques (S5 and S6) might be what lowers the TOC in soil that has been polluted by crude oil (Tanee and Kinako, 2008; Adams et al., 2017). The decrease in the TOC property for the synergy-treated soil was high when compared with the S3 and S4 techniques, which could also be linked to the interactive effects of the remediating agents on the polluted soil (Hamdi et al., 2007; Owhoeke et al., 2023; Agarry, 2018).

3.2 Analysis of TPH in polluted soil treated with different remediating techniques

The percentage removal of TPH from crude oil contaminated soil treated with S1-6 techniques is shown in Table 3.

Table 3. Percentage removal of TPH from contaminated soil

Periods	S1	S2	S3	S4	S5	S6
WK1	15.75	15.32	15.37	12.21	13.07	8.29
WK2	23.79	25.42	16.00	15.03	16.59	12.22
WK3	30.87	29.58	32.15	15.53	32.31	25.00
WK4	41.60	37.65	32.53	17.09	39.63	35.58
WK5	48.00	45.21	39.15	20.66	50.93	50.11
WK6	47.79	53.35	46.09	31.39	56.98	60.56
WK7	83.96	65.61	56.39	37.32	59.71	72.64
WK8	83.96	72.08	61.84	47.19	67.77	82.90
WK9	88.92	84.94	67.09	56.84	79.86	91.45

The percentage removal of TPH from the contaminated soil from week 1 to week. 9, using the different remediation techniques were S1 (15.75% and 88.92%), S2 (15.32% and 84.94%), S3 (15.37% and 67.09%), and S4 (12.22% and 54.84%), S5 (13.07% and 79.86%) and S6 (8.29% and 91.45%). The S4 technique which involved the use of charcoal had the lowest percentage of TPH removed, while S6 technique which combined the microorganisms and charcoal had the highest percentage of TPH removed at week 9 of the experiment.

The percentage removal of TPH increased from week 1 to week 9 which implies the ability of the microorganisms to metabolize the hydrocarbons in the polluted soil. Previous studies have demonstrated the effectiveness of charcoal in removing TPH from contaminated soil owing to its adsorptive properties (Semenyuk et al., 2014; Arroyo et al., 2019; Adebayo et al., 2023). Similarly, research involving microbial remediation has shown significant TPH removal efficiencies. For instance, Suja et al. (2014) documented a 79% reduction in TPH levels in crude oil contaminated soil using microorganisms, while Almansoory et al. (2019) highlighted the hydrocarbon-degrading potential of specific bacterial strains.

The mixture of microorganisms and the combination of charcoal with microorganisms both resulted in an increased percentage of TPH removal from crude oil-polluted soil. By week 9, the charcoal and microorganism amendment showed a higher percentage of TPH removal than the microorganism mixture. This findings suggests that the synergistic interaction between microorganisms and charcoal was more effective than microbial activity alone in

degrading hydrocarbons (Tanee and Kinako, 2008; Orie et al., 2015; Rong et al., 2021). Parhamfar et al. (2020) also reported a high percentage TPH removal through the synergistic use of indigenous bacteria isolates. Likewise, the results align with Zuzolo et al. (2021), who reported an 89% TPH reduction using a plant-bacteria-mycorrhiza synergy.

Figure 4 illustrates the variation of TPH for week 1 and week 9 at different techniques of remediation.

3.3 Analysis of BTEX in crude oil polluted soil treated with Remediation techniques

The analysis of BTEX compounds in crude oil polluted soil treated with different remediating techniques, such as bacteria(S1), fungi (S2), algae(S3), Charcoal (S4), mixture of microorganism (fungi, bacterial, and algae) (S5), and the mixture of microorganism with charcoal (S6) culture are shown in Table 4.

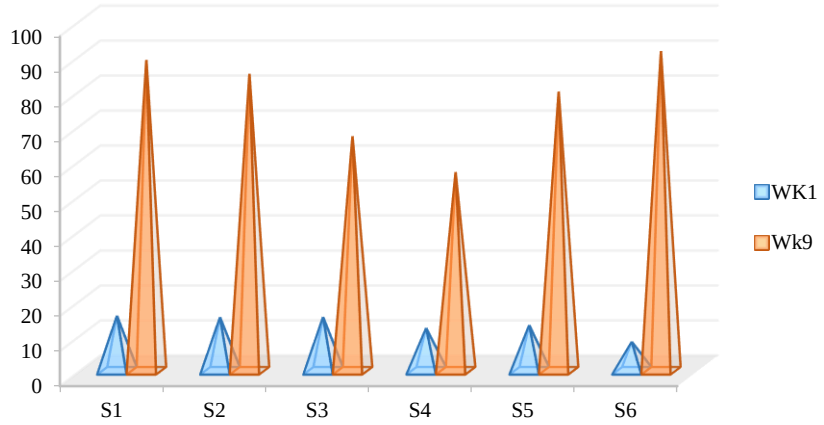

Figure 4. Variation of TPH for wk1 and wk9 at various techniques

Table 4. Percentage removal of BTEX from contaminated soil for week1 and week 9

	S1		S2		S3		S4		S5		S6	
Weeks	WK1	W9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9
Benzene	45.93	100	97.37	100	61.72	97.37	87.56	99.98	68.18	100	41.15	100
Toluene	18.07	100	86.48	100	82.90	100	86.48	100	100	100	83.88	100
Ethylbenzene	60.59	87.90	3.38	100	41.09	98.18	77.50	98.44	70.09	100	72.56	100
O-xylene	79.05	100	25.34	99.29	1.42	100	20.17	100	72.33	99.68	80.61	100
M-xylene	65.43	100	70.71	100	79.44	98.42	72.49	98.77	8.58	100	0.59	99.99
P-xylene	53.16	99.96	3.68	99.99	1.04	95.54	4.76	77.62	74.16	95.63	79.48	99.52

The percentage removal of benzene from soil treated with S1-S6 remediation techniques at week 1 and week 9 is presented in Table 4. The estimated benzene removal efficiencies for week 1 and week 9 were as follows: S1 (45.93% and 100%), S2 (97.37% and 100%), S3 (61.72% and 97.37%), and S4 (87.56% and 100%). The synergistic treatments also demonstrated notable benzene removal, with S5 (61.18% and 100%) and S6 (41.15% and 100%) showing complete degradation by the week 9.

There were notable increases in the percentage of benzene removal across the different remediation techniques applied on the contaminated soil. Complete benzene removal from the crude oil polluted soil was observed using the following treatments S1 (week 8), S2 (week 7), S5 (week 8) and S6 (week9). For S3 and S4 the benzene removal efficiencies reached 97.37% and 99.98% by the week 9.

The findings of Aburto-Medina and Ball (2015) are consistent with the present study, as they documented the impact of microorganisms on the anaerobic degradation of benzene. Similarly, in a study conducted by Soares et al. (2010), benzene was successful eliminated from the polluted soil through the combined application of soil vapor extraction and bioremediation techniques. The research findings were further supported by Wolicka et al. (2009), who demonstrated the effective removal of benzene at high concentrations from petroleum-contaminated soil

contaminated through the application of aerobic microorganism's bioremediation.

The chart for weekly analysis of benzene removal is illustrated in Figure 5.

The estimated toluene removal percentages at weeks 1 and week 9 were as follows: S1 (18.07% and 100%), S2 (86.48% and 100%), S3 (82.90% and100%), and S4 (86.48% and 100%) (see Table 4). The synergistic treatments showed the following removal efficiencies; S5 (10% and 100%) and S6 (83.88% and 100%) at weeks 1 and 9, respectively.

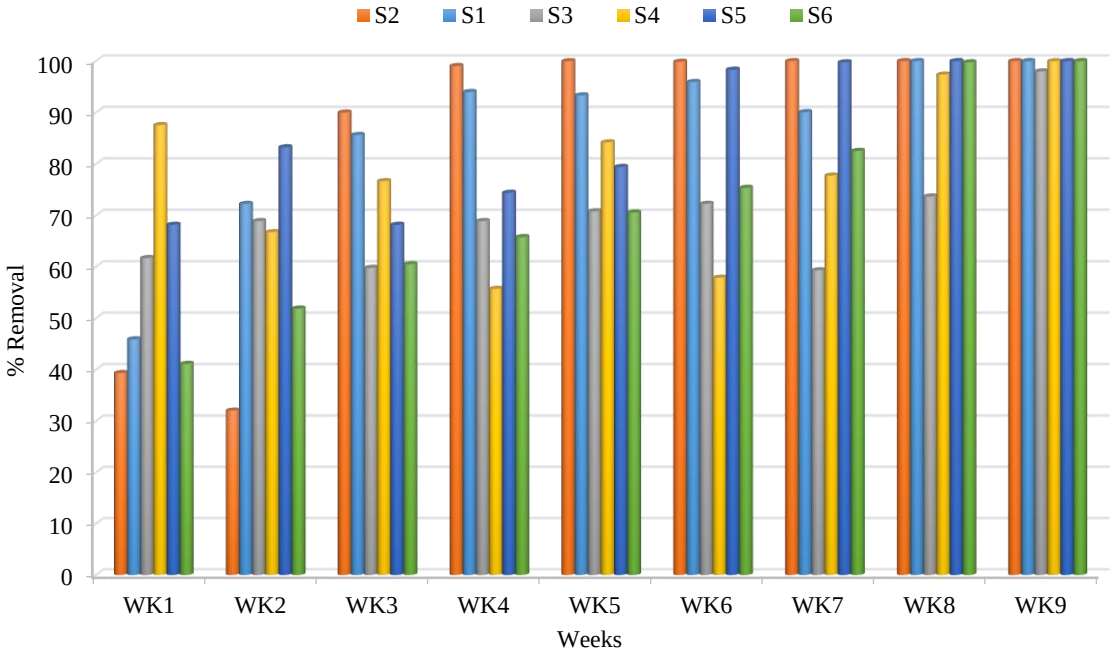

Figure 5. Benzene removal from week 1 to week 9 at various techniques

An overall increase in the toluene removal was observed across all remediation techniques from week 1 to week 9. During week 7, the removal of toluene was achieved by S1, S3, and S6. Complete toluene removal was achieved by week 7 for S1, S3, and S6; by week 6 for S4 and S5; and by week 5 for S2, demonstrating the varying efficiency and kinetics of the different treatments (Figure 6).

The complete removal of toluene observed within a few weeks suggests that the polluted soil did not contain substantial amounts of toluene, thereby indicating the efficacy of the treatment measures employed (Genovese et al., 2008). The work conducted by Moe et al. (2018) also confirmed the effective removal of toluene in contaminated soil through microbially mediated processes. Furthermore, Wolicka et al. (2009) supported these research findings by showcasing the effective elimination of toluene at high concentrations from petroleum-contaminated soil using the bioremediation potential of aerobic microorganisms.

The estimated ethylbenzene values for week 1 and week 9 were as follows: S1 (60.59% and 87.90), S2 (3.38% and 100%), S3 (41.09% and 100%), and S4 (77.50% and 98.44%) (Table 4). The synergistic treatment of ethylbenzene was observed with the following percentages; S5 (70% and 100%) and S6 (72.56% and 100%) during weeks 1 and 9, respectively (Table 4) (Figure 7).

The data from week 1 to week 9 demonstrate a progressive increase in the percentage of toluene removal from contaminated soil while utilizing different remediation procedures. Both S2 and S5 achieved a complete elimination of ethylbenzene (Wolicka et al., 2009; Soares et al., 2010). Additional results also indicated 100% removal efficiency for S6, as depicted in Figure 6. The results of Aburto-Medina and Ball (2015) support the current investigation, since they recorded the influence of microorganisms on the anaerobic breakdown of ethylbenzene.

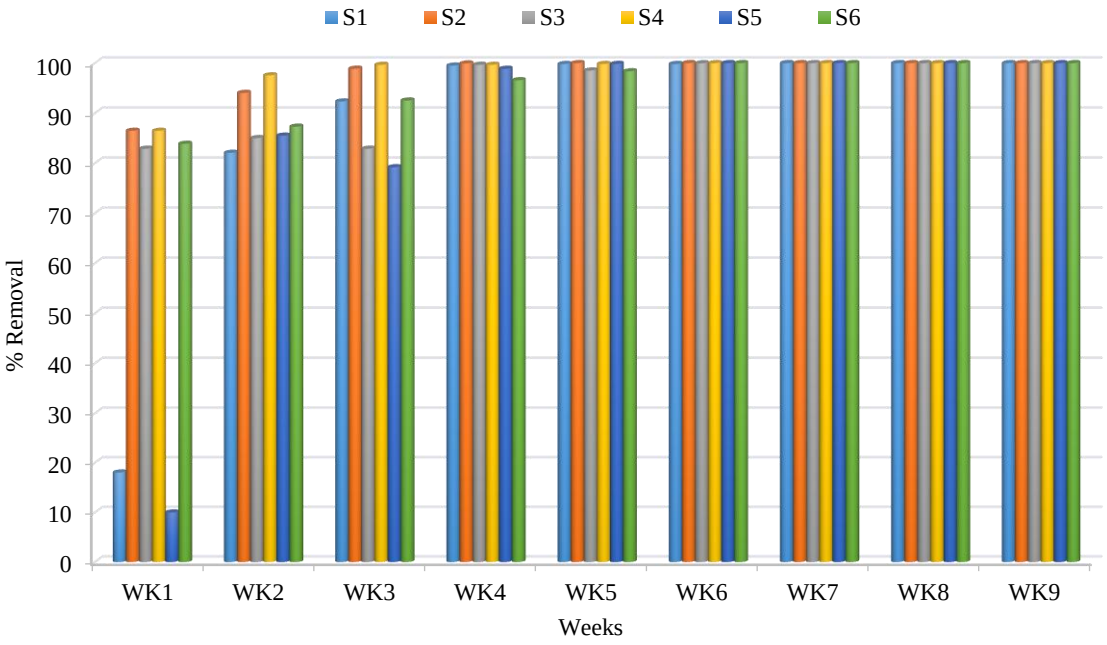

Figure 6. Toluene removal from week 1 to week 9 at various techniques

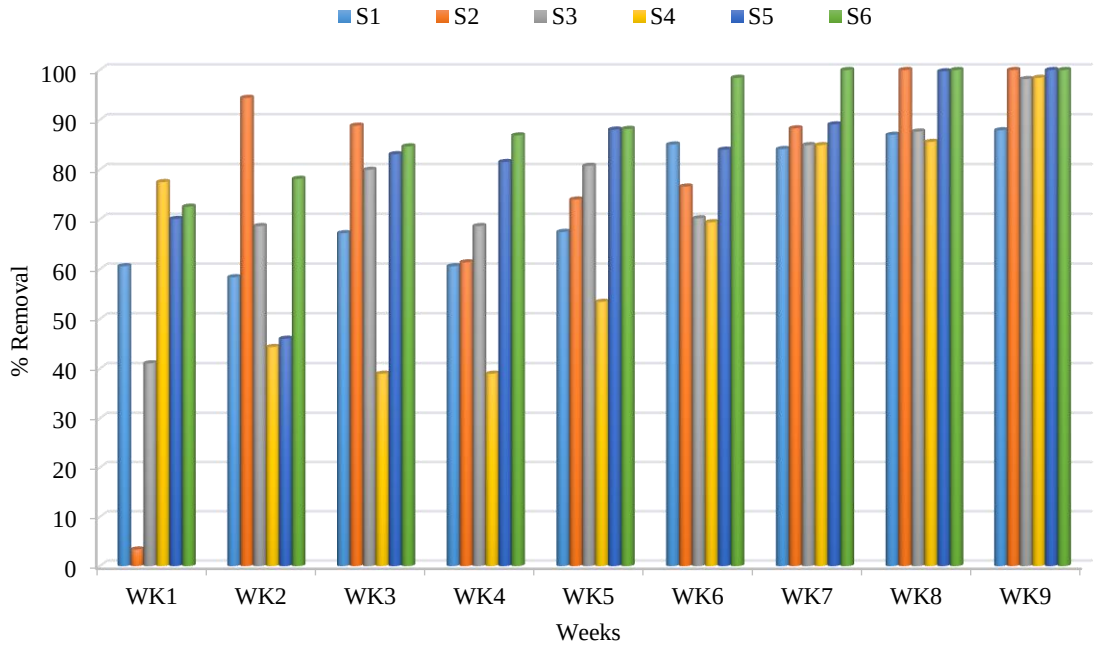

Figure 7. Ethylbenzene removal from week 1 to week 9 at various techniques

The estimated o-xylene values were as follows: S1 (79.05% and 100%), S2 (25.34% and 100%), S3 (1.42% and 100%), and S4 (20.17% and 100%). The synergistic treatment showed o-xylene removal percentages: S5 (72.33% and 99.68%) and S6 (80.61% and 100%) at the weeks 1 and 9, respectively. The data from weeks 1 and 9 show that the use of different remediation methods increased the efficiency of o-xylene removal from contaminated soil. At the seventh week, the rate of o-xylene removal in the S1 treatment was no longer detectable, indicating that the S1 technique achieved complete 100% of o-xylene from polluted soil. By week 9, the S2 and S5 techniques recorded 99.29% and 99.74% of o-xylene, removal respectively. Similarly, at week 9, complete removal of o-xylene was observed for the S3, S4, and S6 techniques (Figure 8). These results highlight the high sensitivity and efficiency of the remediation techniques in removing o-xylene from contaminated soil. The results of this study were in agreement with the findings of Singh and Fulekar's (2009), who reported the successful removal of o-xylene using a

microbial consortium derived from cow dung. Similarly, Wu et al. (2018) investigated o-xylene removal using one- and two-phase partitioning bio-trickling filters, focusing on the steady- and transient-state performance as well as the microbial community dynamics involved in the process. In another study, Taki et al. (2007) observed study where they successfully detected and characterized a significant reduction in o-xylene levels in polluted soil with o-xylene concentrations in contaminated soil through the activity of *Rhodococcus* spp. Additionally, Thakur and Balomajumder (2012) reported the biodegradation of o-xylene by *Azotobacter chroococcum*.

The estimated removal percentages of m-xylene values from crude oil-contaminated soil were as follows: S1 (65.43% and 100%), S2 (70.71% and 100%), S3 (79.44% and 98.42%), and S4 (72.49% and 98.77%) (Table 4). Synergistic treatments m-xylene removal efficiencies of S5 (8.58% and 100%) and S6 (0.59% and 99.99%) during weeks 1 and 9, respectively.

Additionally, the percentage of m-xylene removal increased when microorganisms were applied, and the combined use of charcoal and microorganisms proved effective in treating the crude oil-polluted soil. Complete 100% removal of m-xylene was achieved at week 9 using the S1, S2, and S5 techniques. At week 9, the S3, S4, and S6 techniques recorded 98.42%, 98.77%, and 99.99%, respectively. These results indicate that the microorganism, both independently and in combination with charcoal, are capable of decomposing m-xylene from crude oil-polluted soil.

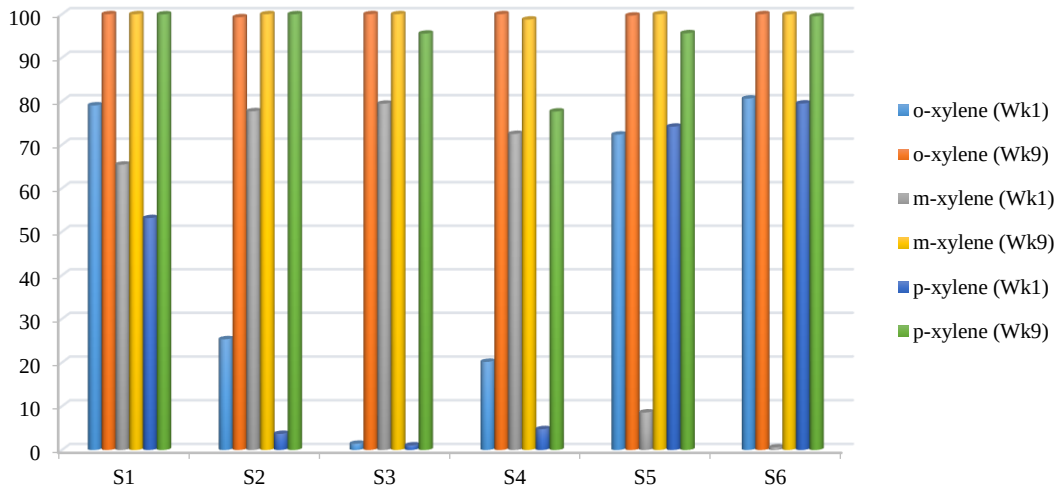

Figure 8. Xylene for week 1 and week 9 at various techniques

The findings of the present study are consistent with those conducted by Hess et al. (1997), which identified a significant concentration of m-xylene in a laboratory aquifer column contaminated with diesel fuel. Their research further demonstrated a marked reduction in m-xylene levels through the activity of degrading bacteria. Similarly, Yao et al. (2022) reported an efficient biodegradation of m-xylene using microorganisms. The results of this study also align with the findings of Ortega-González et al. (2013), who observed that a bacteria group from the rhizosphere soil of *Cyperus* sp. was capable of degrading up to 88% of m-xylene isomers.

The estimated percentages of p-xylene removed from crude oil-contaminated soil were as follows: S1 (53.16% and 99.96%), S2 (30.68% and 99.99%), S3 (1.04% and 95.54%), and S4 (4.76% and 77.62%) (Table 4). The synergistic treatments for p-xylene were recorded as S5 (74.16% and 95.63%) and S6 (83.88% and 100%) during weeks 1 and 9, respectively (Figure 8).

The use of microorganisms enhanced the removal efficiency of p-xylene. Additionally, a combining charcoal with microorganisms proved effective in treating crude oil contaminated soil. At week 9, the S1 and S2 treatments achieved 99.96% removal, while the S3, S4, S5, and S6 reached 95.54%, 77.62%, 95.63%, and 99.52%, respectively. This suggests that the microorganism, either individually or in combination with charcoal, can effectively degrade p-xylene in contaminated soil with crude oil (Yao et al., 2022). The results of this study align with the findings of Sui et al. (2005), who documented a significant reduction in p-xylene through microbial remediation. Similarly, Jeong et al. (2006) demonstrated the use of *Pseudomonas* sp. for p-xylene

removal. These results are also consistent with Prenafeta-Boldú et al. (2012), who achieved substantial removal of both ethylbenzene and p-xylene. Interactions between fungi and bacteria occur during the process of biodegrading TEX hydrocarbons. Miri et al. (2022) further investigated p-xylene biodegradation using three psychrophilic *Pseudomonas* strains, emphasizing gene expression analysis.

4. CONCLUSION

This study demonstrated that the combined application of microbial consortia and activated charcoal significantly enhanced the removal of total petroleum hydrocarbons (TPH) from crude oil-contaminated soil in the Niger Delta. Treatments involving both charcoal and microorganisms (S6) showed the highest TPH reduction and improved restoration of soil physicochemical properties, including pH, total organic carbon, and moisture content.

While preliminary observations suggested notable reductions in BTEX compounds, these results were derived from spectrophotometric measurements at 600 nm, a method not suitable for accurate quantification of volatile mono-aromatic hydrocarbons. Therefore, all BTEX-related findings should be interpreted as indicative of microbial activity rather than validated chemical degradation. Future studies will employ gas chromatography (GC-FID or GC-MS) to confirm BTEX removal and elucidate compound-specific degradation pathways.

Overall, the synergistic use of charcoal and microbial agents presents a promising strategy for soil remediation, particularly in resource-constrained settings. However, further validation using robust analytical techniques and expanded statistical analysis is essential to confirm the efficacy and reproducibility of this approach.

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AUTHOR CONTRIBUTIONS

Ogu Chinedu: Writing-original draft, investigation, formal analyses, data curation. David Kariuki: Writing-review and editing, supervision, methodology, validation. John Wanjohi: Writing-review and editing, supervision. Elechi Owhoeke: Validation, conceptualization.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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