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Research Article

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

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Abstract

In this study, we analyzed the combined effect of microorganisms and activated charcoal on the degradation of total petroleum hydrocarbons (TPH) and benzene, toluene, ethylbenzene, and xylene (BTEX) from crude oil contaminated soil along the Port-Harcourt refinery in Rivers State, Nigeria. The microorganisms (bacteria, fungi, algae) only, the activated charcoal, and the combination of both were used on the soil samples for a period of nine weeks. Physicochemical parameters like pH, temperature, organic matter (OM), and total organic carbon (TOC) were also tracked. The findings revealed that pH values were near neutrality, favorable for microbial growth, while the TOC and OM values varied with treatment. The synergetic combination of charcoal and microorganisms gave the maximum TPH removal efficiency (91.45%), outcompeting individual treatments. BTEX removal was also greatly improved, with total degradation of BTEX by week nine. Xylene isomers exhibited almost complete removal with some fluctuations. This research demonstrates the efficiency of coupling microbial consortia and charcoal for bioremediation, providing and inexpensive and green approach to the remediation of crude oil contaminated soils. The findings confirm previous research on microbial hydrocarbon degradation and underscores the efficiency of synergetic approaches for environmental remediation on a large scale in oil contaminated regions like the Niger-Delta.

Keywords: Charcoal, Crude Oil, Microorganisms, Organic Contaminants and Remediation

1. Introduction

Crude oil is a complex mixture of several hydrocarbons, consisting of both low and high molecular weights. This complex mixture consists of fully saturated hydrocarbons, branching hydrocarbons, unsaturated hydrocarbons, cyclic hydrocarbons (both with carbon atoms only and with other atoms), and aromatic compounds [1,2]. Petrol and diesel components such as TPH, are among the most common environmental pollutants, along with benzene, toluene, ethylbenzene, and three isomers of xylene (ortho-, meta-, and para-) (together known as BTEX).

Crude oil and its derivatives are likely to have mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene, and various isomers of xylene that are all known to be 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 [3]. BTEX are volatile aromatic hydrocarbons responsible for health problems such as irritation, headaches, liver and kidney damage, as well as cancer [4].

The Niger Delta region of Nigeria has experienced significant environmental degradation due to oil pollution and gas

flaring, impacting both terrestrial and aquatic ecosystems. The consequences encompass a decrease in the soil's ability to produce, which has ramifications for both living organisms and the economic well-being of individuals in the contaminated region. This, in turn, leads to a high poverty rate and unemployment [5, 6]. Oil spill incidents occurring in other countries, except Nigeria, particularly in more technologically advanced nations, receive prompt and sufficient attention for the implementation of clean-up and remediation measures.

Bioremediation refers to the utilization of biological mechanisms to degrade, decompose, convert, or effectively eliminate pollutants or substances that reduce the quality of soil and water. Bioremediation involves the utilization of microbes or their byproducts (bioaugmentation), nutrients (biostimulation), and plants (phytoremediation) to restore environments contaminated with crude oil [7]. The utilization of organic wastes is replacing the utilization of chemical fertilizer due to its inherent advantages. Although numerous techniques to remediate petroleum contaminated soil have been discussed and advocated, there is a need for a more cost-effective, ecologically friendly technique that uses locally developed hydrocarbon-degrading ingredients. Bioremediation, a form of biological therapy, has been demonstrated to be environmentally friendly and cost- effective, when compared to physical and chemical treatments such as solidification/stabilization, thermal desorption, and burning. The specificity of this study is that it examines the synergetic effect achieved by the combination of microorganisms with activated charcoal for the bioremediation of crude oil pollution in soil. Although previous studies have dealt with the isolated microbial treatments or the adsorptive capability of charcoal, this study combines both strategies uniquely to improve the recovery of TPH and BTEX constituents.

This new approach offers an inexpensive, 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. Material and Method

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 extracting solvent, dichloromethane (DCM) is a product of Merck and 98% purity. It was further distilled to obtain higher purity (99.9%) and also of analytical standard.

2.2. Sampling Site

The site chosen for the experiment was the Port Harcourt refinery depot area in Alesha Eleme, Rivers State. Situated within the Niger Delta region, this area has experienced significant oil- related activities, including extraction, refining, and transportation, leading to frequent occurrences of oil spills. These oil spillages have had a significant impact on Alesha Eleme's environmental landscape. Samples were collected near Port Harcourt Refining Company Limited (PHRC), a subsidiary of the Nigerian National Petroleum Corporation's (NNPC) Port Harcourt (Lat: 4o49'.0012" N and Long: 7o2'0.9996" 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.

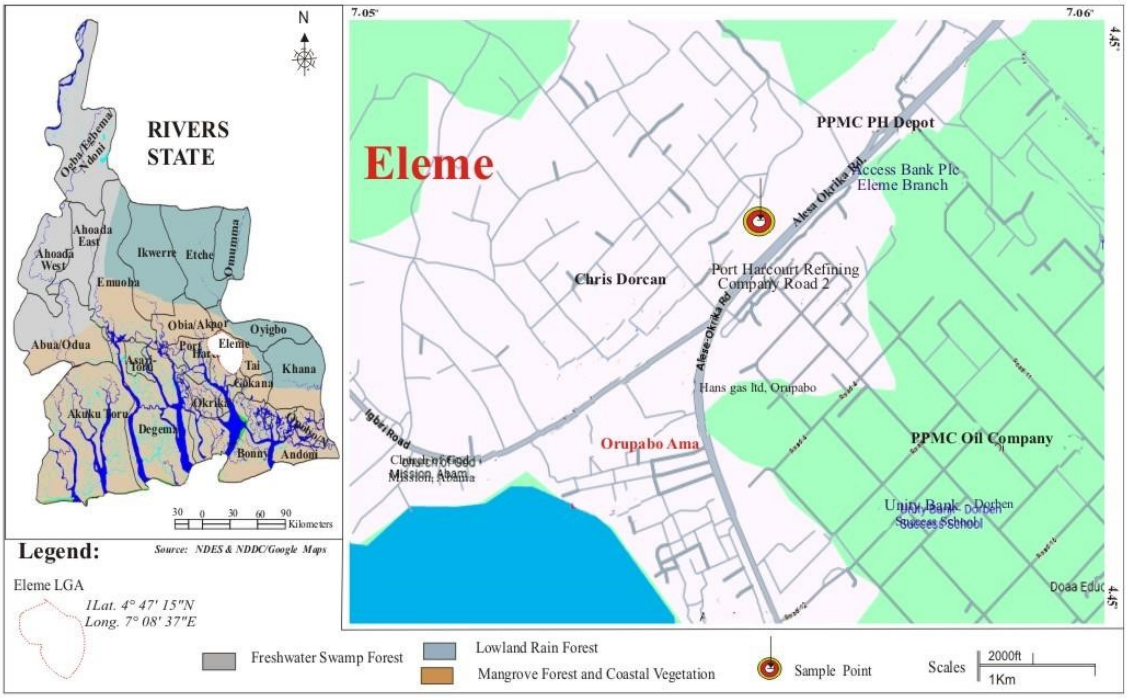

Figure 1: Map of Sampling Site

2.3. Collection of Microbes

In the microbiology laboratory at the university of Port Harcourt, a concentrated suspension of three different microorganisms—fungi (*Aspergillus niger*), bacteria (*Pseudomonas aeruginosa*), and algae (*Sargassum filipendula*)—were prepared. This suspension, containing the specified concentration of each microorganism, was carefully measured and transferred into two separate bottles, each with a capacity of 750 milliliters. To maintain the viability and integrity of these microorganisms for future use, the bottles were promptly refrigerated. 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 environment helped to maintain the characteristics and properties of the microorganisms until they were required for further research or analysis in the laboratory setting.

2.4. Sample Collection and Preparation

The stratified approach was the sampling strategy that was utilized in this particular research. The sampling field was segmented into quadrants, and a total of fifteen (15) samples were taken by the proportionality rule. This means that more samples were gathered from regions that contained a high concentration of contaminants. An application of the composite approach, which involves the blending of sampling units to generate a single sample, was utilized in conjunction with the stratified sampling technique. An oil analysis was performed with a sterile spatula, and the top two centimetres of the sample core were collected and used for the analysis. To prevent any cross-contamination in the samples, the storage was done using PVC bags that were clean and appropriate, which were placed inside tin cans and sampling bottles. The experimental set of samples employed is shown in Table 1.

Experimental Set	Test Experiment
S1	Polluted soil sample (250g) + bacteria culture (0.5ml)
S2	Polluted soil sample (250g) + fungal culture (0.5ml)
S3	Polluted soil sample (250g) + algae culture (0.5ml)
S4	Polluted soil sample (250g) + charcoal (2.5kg)
S5	Polluted soil sample (250g) + synergetic amendment of microbes (0.5ml)
S6	Polluted soil sample (250g) + synergetic amendment of charcoal (2.5g) and microbes (0.5ml)
C1 (Control)	Unpolluted soil sample (250g) + no amendment

Table 1: Experimental Sample Set

2.5. Experimental Design

The soil samples, contaminated with pollutants, were treated by combining them with specific biodegrading agents outlined in Table 1. This amalgamation 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 served to improve oxygen circulation, aiding the activities of microorganisms responsible for breaking down the contaminants in the soil. The experimental setup was then left to settle for intervals of five days. At these designated time points, 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. Notably, the 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 against 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 was used to carefully test how well the biodegradation process worked and how the different treatments affected lowering the levels of contaminants still in the soil. The systematic collection and analysis of samples at regular intervals, in triplicate, ensured a thorough and reliable assessment of the remediation process.

2.6. Soil pH

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

2.7. Determination of Temperature

The 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 measuring the pH using a calibrated pH meter for soil pH analysis. The temperature was determined using liquid in glass thermometer.

2.8. Electrical Conductivity

The electrical conductivity of the soil was measured by assessing 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 the ability of a solution to conduct electricity, was then employed to analyze the electrical conductivity specifically within this filtrate. The reading was recorded.

2.9. Determination of Total Petroleum Hydrocarbon (TPH)

The testing procedure outlined for this assessment adheres to the

guidelines specified by ASTM 166 D3921. Initially, soil samples weighing 10 grammes 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 absence of moisture is crucial for 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 milliliters of methylene chloride was used as the extracting solvent.

This solvent choice is particularly effective in isolating hydrocarbons from soil samples due to its selective properties, allowing for the efficient extraction of the desired compounds. The extraction process involved the solvent passing through the soil samples within the Soxhlet apparatus, facilitating the dissolution and extraction of the total crude oil hydrocarbons present in the soil matrix. This process continued over some time, allowing for thorough extraction and concentration of the target compounds. Following the completion of the extraction process, the resulting solution containing the extracted hydrocarbons was collected and prepared for further analysis, as per the specified ASTM method. The steps in ASTM D3921 provided a consistent and dependable way to get total crude oil hydrocarbons out of soil samples and analyze them. This makes sure that the test results are accurate and can be used again.

2.10. BTEX Degradation Experiment by Microorganisms

For the testing of BTEX, the pH was adjusted to pH 6 and supplemented with 1% v/v BTEX as the sole carbon source. An aliquot (1.0mL) of 96hrs prepared spore suspensions (1.0 x 106 spore/mL) was inoculated into each treatment flask. The control was left uninoculated. Experiments were carried out in triplicates at room temperature on a rotary shaker (Griffin Mechanical Shaker-Gallenkamp, England) (180rpm) for 25 days [8]. An aliquot (5mL) samples were taken aseptically at 5-day intervals. The residual BTEX were extracted using 5mL dichloromethane (DCM) and centrifuged (Griffin-Gallenkamp, England) (5000rpm) for 5minutes. The absorbances were determined at 600nm wavelength using a UV Spectrometer (Bausch and Lumb Inc., A CE 303) and concentrations were determined from a prepared standard curve [9]. Percentage loss of BTEX by the isolates was analyzed using the formula below. The hydrocarbon-utilizing fungi (HUF) were determined by direct counting using Neubauer Hemocytometer [10,11]. The percentage BTEX degradation was calculated as follows;

$$\% \text{ BTEX degradation} = \frac{(\text{TBEX control} - \text{TBEX treatment}) \times 100}{\text{TBTEX control}}$$

Where: TBTEX control represents the total residual BTEX of the control; TBTEX treatment represents the total residual BTEX of each inoculated flask

3. Result and Discussion

3.1. Remediation Techniques and Physical Properties of Contaminated Soil

The physical properties of polluted soil treated or remediated with bacteria (S1), fungi (S2), algae (S3), charcoal (S4), a mixture of microorganisms (fungi, bacteria, and algae) (S5), and a mixture of microorganisms with charcoal (S6) culture are shown in Table 2. The physicochemical properties considered were pH, temperature, organic matters and total organic carbon.

	S1		S2		S3		S4		S5		S6	
	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK
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

Table 2: Effect of Remediation Techniques on Physical Properties of Contaminated Soil

The pH of the contaminated soil after treatment with S1-S6 techniques was estimated at the range of 5.72-8.09, and there were slight reductions in the value of the pH. The pH values of the soil when mixture of microorganisms (Aspergillus niger, Pseudomonas aeruginosa, and Sargassum filipendula), and synergistic microorganisms and charcoal were within the range of 5.76 -5.99, which is slightly acidic when compared with single microorganism used as a remediation technique. Sanchez-Mata et al. (2023) 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 were within the range 29.40-22.05 oC. For each of the techniques used, the temperature at week 1 was higher than that of the week 9. The temperature value range also implied that the microorganisms used as remediation agents can work effectively.

For the S1 and S2 techniques, the organic matter increases as the number of weeks increases. This implies that both the Aspergillus niger and Pseudomonas aeruginosa were capable of decomposing the crude oil molecules and thereby contributed to the increase in organic matter in the treated contaminated soil [12,13].

The organic matter of the contaminated soil after treatment with S3, S4, S5, and S6 techniques contributed to a decrease in organic matter in the crude oil-polluted soil. The algae and charcoal in the S3 and S4 techniques, respectively, decomposed or absorbed the organic matter in the crude oil soil. It also implies that the algae and charcoal in synergic techniques (S5 and S6) could be responsible for the organic matter reduction in crude oil-polluted soil. In similar research, Masciandaro (2013) reported 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 different remediation techniques estimated the total organic carbon (TOC) of crude oil- polluted soil 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%). The S5 synergic approach recorded a TOC removal of 6.86% and 4.06% for wk1 and wk9, respectively, while the S6 technique treated soil with an estimated TOC emoval of 7.76% and 5.11% for wk1 and wk9, respectively.

Total organic carbon (TOC) showed significant increases in S1 and S2 treatments at the end of the experiment. These findings are consistent with previous studies by Tanee and Kinako (2008) and Tanee and Abert (2011), which demonstrated that microbial activity can facilitate the decomposition of hydrocarbons, resulting in an elevation of organic carbon content in the soil [14,15]. This phenomenon can be attributed to the inherently high carbon content present in crude oil, as documented by Speight (2014).

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 in the S3 and S4 techniques, respectively, decreased the TOC in the crude oil soil. 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 [16]. The decrease in the TOC property for the synergy-treated soil was high when compared with the S3 and S4 techniques. This could also be linked to the interactive effect of the remediating agents on the polluted soil [17,18].

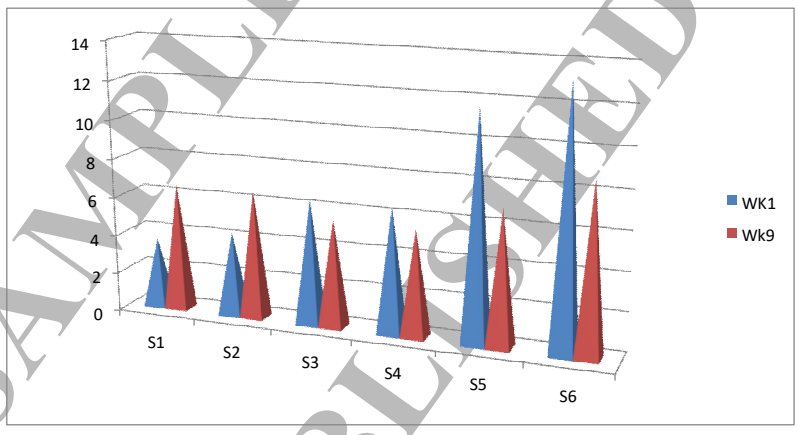

Figure 2: Variation of (a) Organic Matter for wk1 and wk9 at Various Techniques.

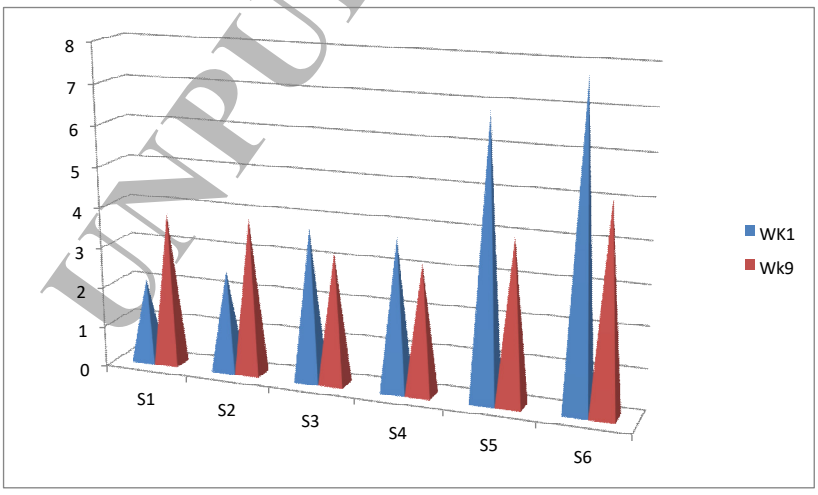

Figure 3: Variation of TOC for wk1 and wk9 at Various Techniques.

3.2. Analysis of TPH in Polluted Soil Treated With Different Remediating Techniques

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

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

Table 3: Percentage Removal of TPH from Contaminated Soil.

The percentage removal of TPH from the contaminated soil from wk.1 to wk. 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% 279 and 91.45%). S4 technique which involves the use of charcoal had the lowest percentage of TPH removed, while S6 technique which involves the use of a synergetic amendment of the microorganisms and charcoal had the highest percentage of TPH removed at week 9 of the experiment.

From week 1 to week 9, the percentage of TPH removed increased. This implies the ability of the microorganisms (bacteria, fungi, and algae) to metabolize the hydrocarbons in the polluted soil. Previous studies have demonstrated the potential of charcoal to effectively remove TPH from contaminated soil owing to its adsorptive properties [19,20,21]. Similarly, research involving microorganisms has shown significant TPH removal efficiencies. For instance, Suja et al. (2014) reported a 79% reduction in TPH levels using microorganisms in crude oil contaminated soil [22]. These findings align with the work of, who highlighted the hydrocarbon degrading capabilities of specific bacterial strains [23].

The microorganism mixture and the mixture of charcoal and microorganisms resulted in an increase in the percentage removal of TPH from crude oil-polluted soil. At week 9, the charcoal and microorganism amendment showed a higher percentage of TPH removal than the microorganism mixture. This implies that the microorganisms' synergy with charcoal was more effective than the microorganisms [24,25]. Parhamfar et al. (2020), reported a high percentage removal of TPH using synergy of indigenous isolated bacteria [26]. The result is also consistent with Zuzolo et al. (2021), who reported 89% of TPH removal, using synergy of the plant-bacteria-mycorrhiza [27].

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

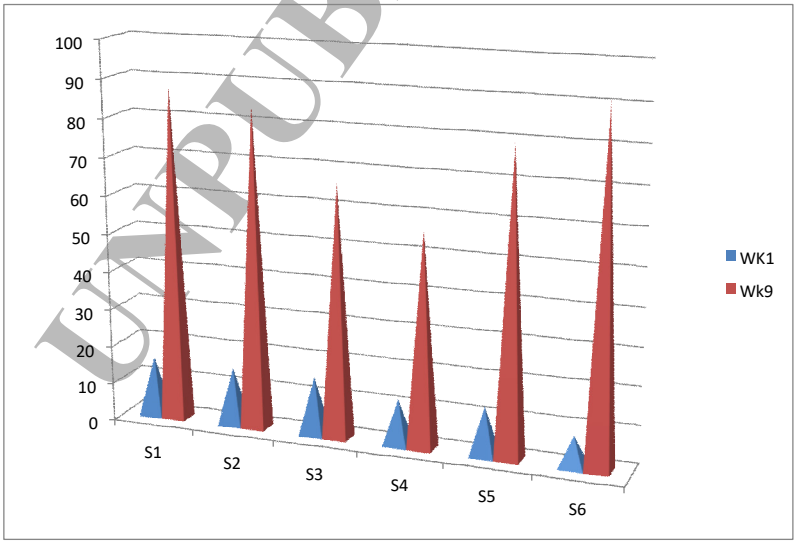

Figure 4: Variation of TPH for wk1 and wk9 at Various Techniques.

3.3. Analysis of BTEX in Crude Oil Polluted Soil Treated With Remediating Techniques

The analysis of BTEX 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.

	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
Ethylbenze ne	60.59	87.90	3.38	100	141.01	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

Table 4: Percentage Removal of BTEX from Contaminated Soil for Week1 and Week 9

The percentage removal of benzene at week 1 and week 9 from soil treated with S1-S6 techniques is presented in Table 4. The estimated benzene values 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 treatment led to a significant removal of benzene, with the following percentages observed; S5 (61.18% and 100%) and S6 (41.15% and 100%) during weeks 1 and 9, respectively.

There were increases in the percentage of benzene removal from different treatment techniques applied on the contaminated soil. The complete benzene removal from the crude oil polluted soil using the established techniques was observed as follows; S1(week 8), S2(week 7), S5(week 8) and S6(week9). For S3 and S4 the percentages of benzene removal were 97.37% and 99.98% at week 9.

The findings of align with the present study, as they documented the impact of microorganisms on the anaerobic degradation of benzene [28]. In a study conducted by Soares et al. (2010), the researchers observed the successful elimination of benzene from polluted soil using the combined application of soil vapor extraction and bioremediation techniques [29]. The research findings were additionally corroborated by Wolicka et al. (2009), who demonstrated the successful removal of benzene at high concentrations from soil contaminated with petroleum products through the utilization of aerobic microorganism's bioremediation [30].

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

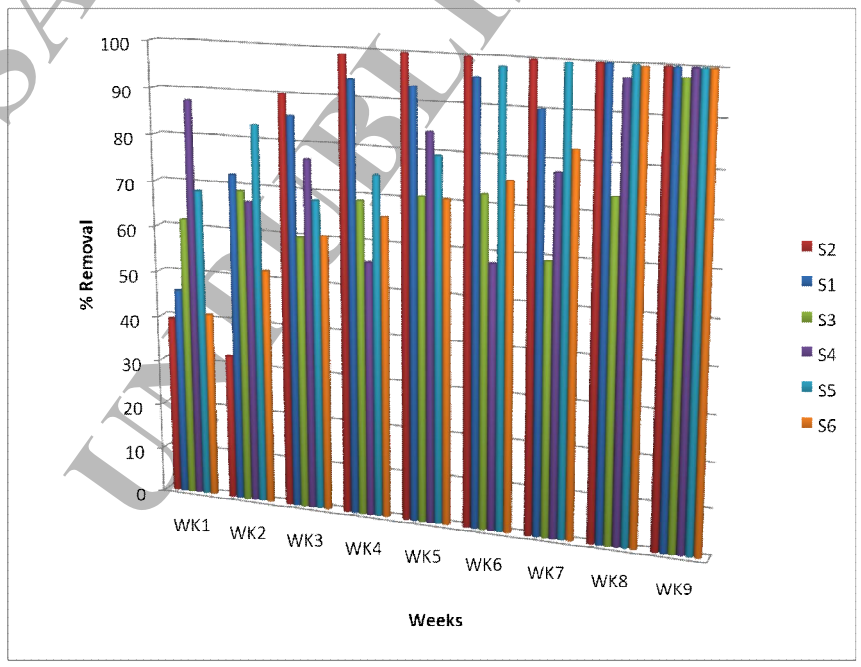

Figure 5: Benzene Removal from Week 1 to Week 9 at Various Techniques.

The estimated toluene values for week 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 treatment of toluene was observed with the following percentages; S5 (10% and 100%) and S6 (83.88% and 100%) during weeks 1 and 9, respectively.

An increase in the percentage of toluene removal from contaminated soil has been found when different remediation methods are used, as evidenced by the data from week 1 to week 9. During week 7, the removal of toluene was achieved by S1, S3, and S6. At the fifth week, the toluene was fully eliminated by S2, and at the sixth week, S4 and S5 were likewise fully removed.

The complete removal of toluene in some weeks suggests that the polluted soil did not contain substantial amounts of toluene, hence indicating the efficacy of the treatment measures employed [31]. The work conducted by Moe et al. (2018) confirmed the effective removal of toluene from contaminated soil through the utilization of microbially- mediated toluene biogenesis [32]. Wolicka et al. (2009) provided further support for the research findings by showcasing the effective elimination of toluene at elevated levels from soil contaminated with petroleum products using the bioremediation capabilities of aerobic microorganisms.

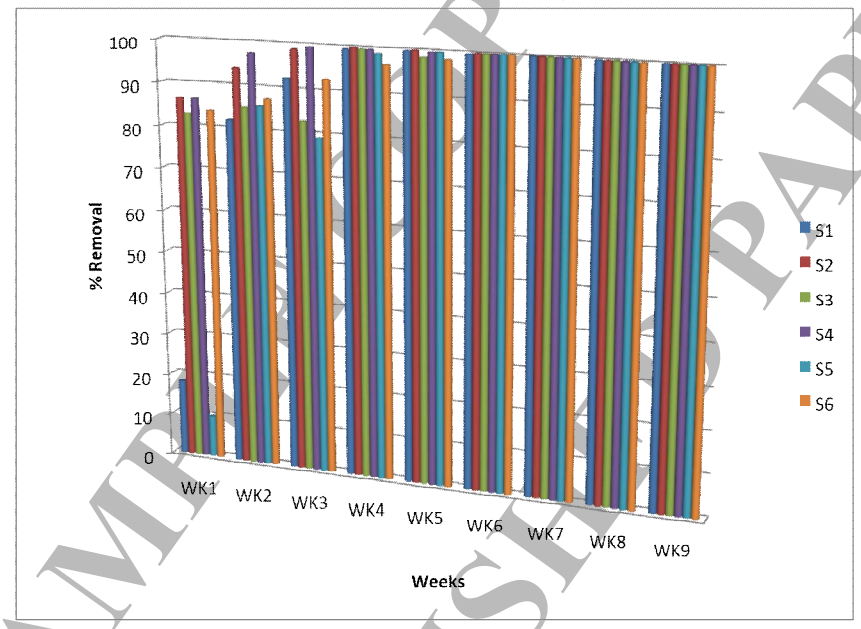

Figure 6: Toluene Removal from Wk1 to Wk9 at Various Techniques.

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%D), and S4 (77.50% and 98.44%) (Table 4). The synergistic treatment of toluene was observed with the following percentages; S5 (70% and 100%) and S6 (72.56% and 100%) during weeks 1 and 9, respectively (Table 4).

The data from week 1 to week 9 demonstrates an 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. Additional results also indicated 100% estimation for S6, as depicted in Figure 6. The results of Aburto-Medina and Ball (2015) are consistent with the current investigation, since they recorded the influence of microorganisms on the anaerobic breakdown of ethylbenzene.

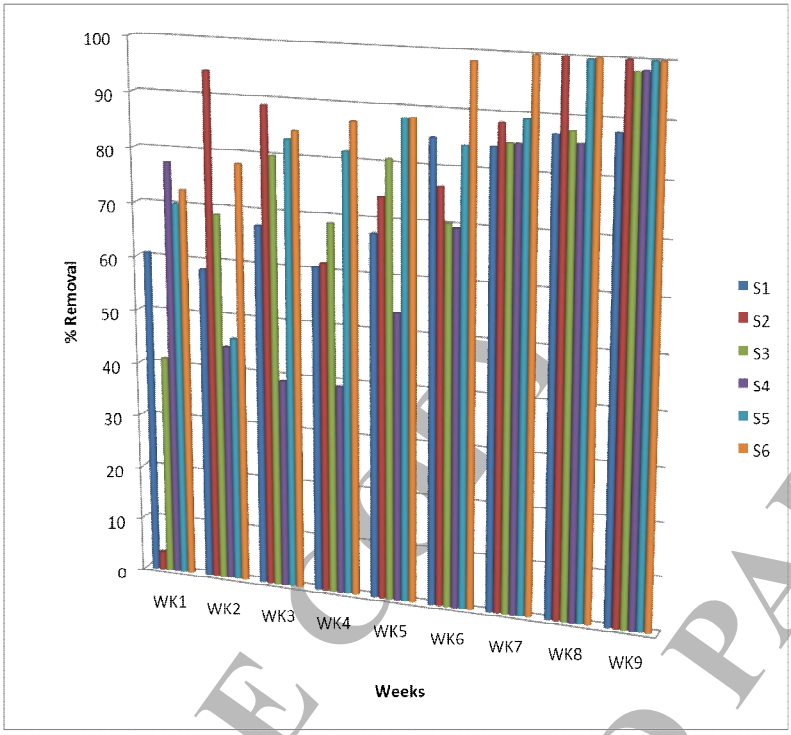

Figure 7: Ethylbenzene Removal from Wk1 to Wk9 at Various Techniques.

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 of o-xylene was observed with the following percentages: S5 (72.33% and 99.68%) and S6 (80.61% and 100%) during weeks 1 and 9, respectively. The data from weeks 1 and 9 show that using different remediation methods increases the percentage of o-xylene removal from contaminated soil. At the seventh week, the rate of o-xylene removal was not discernible for S1. This implies that the S1 technique removes 100% of o-xylene from polluted soil. At week 9, the S2 and S5 techniques removed 99.29% and 99.74% of o-xylene, respectively. At week 9, the percentage removal of o-xylene was 100% for S3, S4, and S6 (Figure 7). This affirmation reveals the sensitivity of the remediation techniques on o-xylene.

The results of this study were consistent with Singh and Fulekar's (2009) findings, which reported the removal of o-xylene by utilizing a microbial consortium derived from cow dung [33]. Wu et al. (2018) conducted a study where they investigated the removal of o-xylene using one- and two-phase partitioning bio-trickling filters [34]. The study focused on evaluating the steady- or transient-state performance and microbial community associated with this process. Taki et al. (2007), conducted a study where they successfully detected and characterized a significant reduction in o-xylene levels in soil polluted with o-xylene [35]. They achieved this by utilizing Rhodococcus spp. Additionally, Thakur and Balomajumder (2012) reported the biodegradation of o-xylene by Azotobacter chroococcum [36].

The estimated m-xylene values removed 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). We observed the synergistic treatment of m-xylene with the following percentages: S5 (8.58% and 100%) and S6 (0.59% and 99.99%) during weeks 1 and 9, respectively.

Additionally, the percentage of m-xylene removed using microorganisms increased, and the combined use of charcoal and microorganisms effectively treated the crude oil-polluted soil. There was 100% removal of m-xylene at week 9 for S1, S2, and S5 techniques. At week 9, the S3, S4, and S6 techniques recorded 98.42%, 98.77%, and 99.99%, respectively. This implies that the microorganism, both as a single organism and in synergies with charcoal, can decompose m-xylene from crude oil-polluted soil.

The findings align with the study conducted by, which documented a significant presence of m-xylene in a laboratory aquifer column contaminated with diesel fuel [37]. Additionally, the researchers observed a substantial reduction in m-xylene levels through the utilization of degrading bacteria. In a comparable study, Yao et al. (2022) documented a highly effective breakdown of m-xylene through the use of microorganisms [38]. The results of this study agree with those of Ortega-González et al. (2013), who found that a group of bacteria from the rhizosphere soil of Cyperus sp. was able to get rid of 88% of m-xylene isomers [39].

The estimated p-xylene values 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 7). The synergistic treatment of p-xylene with remediation techniques of this study was estimated as follows: S5 (74.16% and 95.63%) and S6 (83.88% and 100%) during weeks 1 and 9, respectively (Figure 7).

Utilizing microorganisms also enhanced the percentage removal of p-xylene. Additionally, a combination of charcoal and microorganisms was employed to treat the soil contaminated with crude oil. At week 9, the S1 and S2 approaches had success rates of 99.96%, while the S3, S4, S5, and S6 approaches had success rates of 95.54%, 77.62%, 95.63%, and 99.52%, respectively. This suggests that the microorganism, either on its own or in combination with charcoal, has the ability to break down p-xylene from soil contaminated with crude oil. 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 [40]. Jeong et al. (2006) established the use of Pseudomonas sp. for p-xylene removal in their research [41]. The research is consistent with the findings of Prenafeta-Boldú et al. (2012), who effectively removed a significant proportion of ethylbenzene and p-xylene by their methods. Interactions between fungi and bacteria occur during the process of biodegrading TEX hydrocarbons [42]. Miri et al. (2022) conducted a study in which they investigated the biodegradation of p-xylene using three psychrophilic Pseudomonas bacteria, focusing on the analysis of gene expression [43].

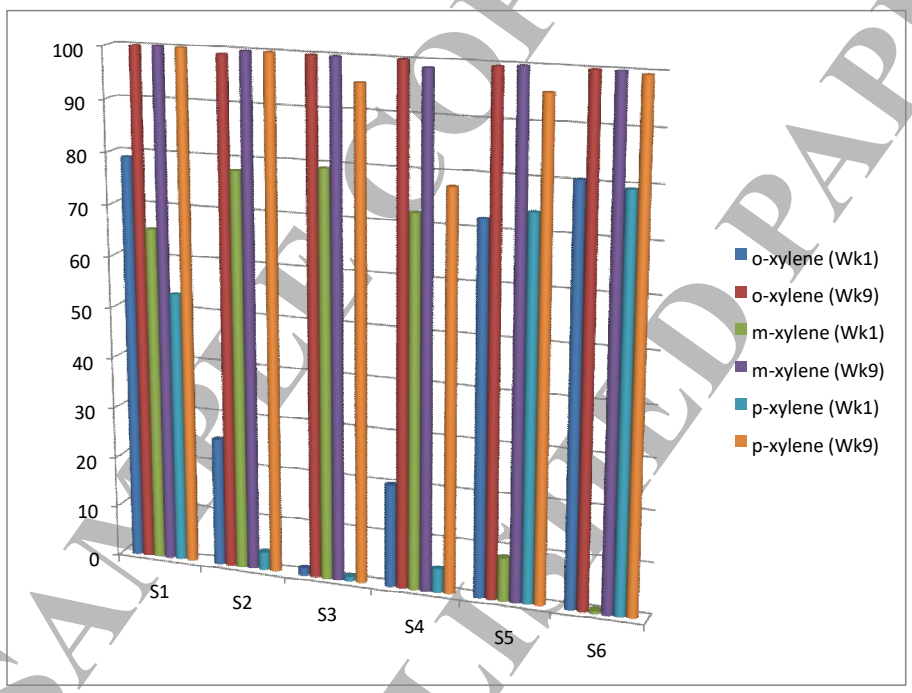

Figure 8: Xylene for wk1 and wk9 at Various Techniques.

4. Conclusions

This study established the synergistic action of combining microorganisms (fungi, bacteria, algae) and charcoal for crude oil-polluted soil remediation, i.e., TPH and BTEX removal. The near-neutral pH values of treated soil, a result of the physicochemical characteristics, promoted microbial growth and enhanced remediation efficiency. Organic matter (OM) and total organic carbon (TOC) levels were enhanced with time, with synergistic treatments (S5 and S6) performing better than individual microbial treatments. The combined treatment (S6) resulted in the highest TPH removal (91.45%) and almost complete degradation of BTEX compounds, with the exception of certain xylene isomers. Although single-microorganism treatments were effective, the synergistic application of microorganisms and charcoal was found to be superior, providing an inexpensive and environmentally friendly solution for the remediation of oil-contaminated soil. This research underscores the promise of integrated bioremediation approaches for large-scale environmental remediation.

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