

# Effect of Different Remediation Strategies on the Removal of Polycyclic Aromatic Hydrocarbons from Crude Oil Polluted Soils

Ogu Chinedu<sup>1</sup>, Kariuki David<sup>2</sup>, Wanjohi John<sup>2</sup>, Owhoeke Elechi<sup>3</sup>

<sup>1</sup> Doctoral Program in Chemistry, Faculty of Science, University of Nairobi, Nairobi Kenya, [cogu@students.uonbi.ac.ke](mailto:cogu@students.uonbi.ac.ke)

<sup>2</sup> Department of Chemistry, Faculty of Science, University of Nairobi, P.O. Box 30197 - 00100 Nairobi Kenya, [kkariuki@uonbi.ac.ke](mailto:kkariuki@uonbi.ac.ke)

<sup>3</sup> Department of Industrial and Petrochemical Science, Faculty of Science, University of Port-Harcourt, Rivers State, Nigeria, [elechi.ohwoeke@uniport.edu.ng](mailto:elechi.ohwoeke@uniport.edu.ng)

---

## Abstract

This study investigated the effects of crude oil pollution on the soil in the Niger-Delta region of Nigeria and explored possible remediation methods. The aim of the study was to analyze the physico-chemical parameters of the soil and determine the crude oil contamination on land within the port-harcourt refinery. The study focused on analyzing polycyclic aromatic hydrocarbons with the use of microorganisms. The microorganisms used in this study were *Aspergillus Niger*, *Pseudomonas aeruginosa*, and *Sargassum filipendula*. The synergies of microorganisms and charcoal were also used to check the removal efficacy of polycyclic aromatic hydrocarbon. The total non-carcinogenic polycyclic aromatic hydrocarbons were estimated at  $327.9 \pm 3.46$  mg/kg, and the carcinogenic polycyclic aromatic hydrocarbons was recorded at  $69.07 \pm 4.20$  mg/kg. According to the results, phenanthrene has the highest concentration of non-carcinogenic polycyclic aromatic hydrocarbons at  $175.8 \pm 0.23$  mg/kg, while benzo[a]anthracene has the highest concentration of carcinogenic polycyclic aromatic hydrocarbons at  $20.29 \pm 0.67$  mg/kg. The efficacy of Polycyclic aromatic hydrocarbons removal increased over time, from week 1 to week 9, when employing the six remediation techniques. Anthracene, phenanthrene, dibenz[a,h]anthracene, indeno(1,2,3-cd)pyrene, and benzo[ghi]pyrene were completely removed from polluted soil when treated with individual microorganisms or a synergy of charcoal and microorganisms. Other polycyclic aromatic hydrocarbons discovered in the sampling area have a high percentage of elimination; however, this percentage is not as high as the aforementioned polycyclic aromatic hydrocarbons.

**Keywords:** Charcoal, Pollution, Crude oil, Microorganisms,

---

## 1.0 INTRODUCTION

Oil exploration and exploitation in Nigeria is located in the Niger Delta, and is profitable, as well serve as a source of revenue. However, similar to other industrial operations, it creates environmental hazards and they often take months or years to induce illness and mortality (Amosu & Adeosun, 2021). The sources of oil spillage into the environment are many, encompassing pipeline leakage and rupture, unintentional spills such as tank accidents, discharges from refineries and urban areas, and so on (Bhattacharjee & Dutta, 2022). A wide variety of plant and animal species are able to thrive in the mangrove ecosystem of Niger Delta region. However, the mangrove swamp forests of the Niger Delta are the most common sites of oil spills in Nigeria, and oil spills have had a negative effect on this ecosystem (Ivshina et al., 2015).

An oil spill has a wide range of effects on ecosystem health and biota. Excessive exploration and seismic activities have adverse effects on soil toxicity and crop quality. Environmentalists are expressing a widespread concern about the rapid deterioration of agricultural quality in the Niger Delta. The ongoing exploration for oil without the necessary Environmental Impact Assessment (EIA) is negatively impacting the yield of crops (Akpomuvie, 2011 Khanna et al., 2017). A study by Aghalino and Eyinla (2009) suggested that 96.5% of mangrove seedling and other shoreline vegetation perished within two weeks of being exposed to the oil film. Oil disrupts the functioning of different organ and systems in plants and animals. For example, oil on water surface forms a layer that inhibits oxygen flow, resulting in the suffocating of certain aquatic species. Crude oil contains poisonous components that kill plants and animals and

inflict other sub-lethal effects (Ahmed et al., 2021)

Mangrove forests provide a haven for a wide variety of plant and animal life, including mammals {such as monkeys and antelopes}, crustaceans, fish, reptiles, birds, and mollusks. As a consequence of widespread pollution, groundwater evaluation is moving in the direction of a watershed approach (Ophori, 2005). Due to the contamination of water sources in the Niger Delta, residents have resorted to sinking boreholes to abstract drinking water (Ite et al., 2018). As a result, safeguarding the groundwater supply is crucial to reducing the risks connected with petroleum contamination.

Lowland, woodlands and the estuary zone are also devastated by the Niger Delta oil spill. Both beach-dwelling and benthic creatures are also affected by oil spills. Oil depletes edible crustacean supplies, kills algae, and changes crucial food webs. Coating birds makes them less able to fly or makes their feathers less insulating, making them more vulnerable to cold (Akpan & Ajayi, 2016; Khanna et al., 2017). The oil pollution of coastal fish hatcheries and the subsequent contamination of commercially valued species' meat is a major problem. Hydrocarbon pollutants are worrisome due to their potential to induce cancer, alter genetic structures, and impact embryos and fetuses (Elisha & Felix, 2021). Aside from hydrocarbon-induced chemical pollution, the oil industry's operations are associated with various additional environmental challenges. These include the removal of land for oilfield infrastructure, alterations to water systems caused by the construction of roads and pipelines, and pollution from compounds that are not hydrocarbons (Nababa et al., 2020). Toxicity is generally determined by the kind and type of crude oil, the level of oil contamination, the type of habitat, and particular organisms' selection sensitivity (Akpan & Ajayi, 2016; Nababa et al., 2020).

Crude oil and gas production and transportation are also associated with fire accidents and leakages. In 2004, there was a leak and subsequent fire in the Nigerian Liquefied Natural Gas (NLNG) pipeline that passes through the Okrika and Kala-Akama mangrove wetlands in the Niger Delta (Igbani et al., 2024). The local flora and fauna in the affected area were wiped out as the fire raged unchecked for three days (Zabbey et al., 2004). It is important to remember that situations like these can wipe out whole populations of rare, endangered creatures. Footprints of hippos were found in Bonny during the construction of the NLNG gas project (Zabbey, 2004). Those enormous beasts have gone forever from the Finima region, where the NLNG plant complex currently stands. Whether the vanishing Finima hippos just migrated to a more'safe' and less disturbed area or went extinct altogether is a mystery (Igbani et al., 2024). It is widely acknowledged that anthropogenic disruptions can result in the relocation of species, which can result in ecological disasters Akpan & Ajayi, 2016; Nna et al., 2020).

The Niger Delta environment is a broad region that encompasses natural systems such as land, rivers and living organisms. The region is distinguished by two key factors: ecological zones and biological diversity. According Okonkwo et al., (2015), the Niger Delta is divided into two natural zones: a tropical rainforest in the north and a coastal area with mangrove vegetation in the south, both of which are connected by numerous rivers, tributaries, and creeks. This region encompasses a land area exceeding 70,000 km<sup>2</sup>, traverses 800 villages engaged in oil production, and experiences the most severe impacts of oil spills and gas flaring (Ateboh & Raimi, 2018). The Niger Delta is known for its significant oil infrastructure, including over 900 oil wells, 100 flow stations and gas plants, more than 1,500 km of trunk lines, and around 45,000 km of oil and gas flow lines. Unfortunately, this region has also gained notoriety for oil pollution, with an average of 221 oil spills every year (Elum et al., 2016). Nigeria annually releases 17.2 billion cubic metres of natural gas into the atmosphere as a byproduct of crude oil extraction in the Niger Delta (GGFR, 2003). The oil leak and gas flaring have a detrimental impact on both the plant and animal components of the delicate environment. Thus, the study focused on the impact of crude oil pollution on Port Harcourt refinery depot area in Alesha Eleme, Rivers in the Niger-Delta Region of Nigeria. The study also provides possible remediating techniques that are cheap, available and eco-friendly.

## **2.0 MATERIAL AND METHOD**

### **2.1. Chemicals and media**

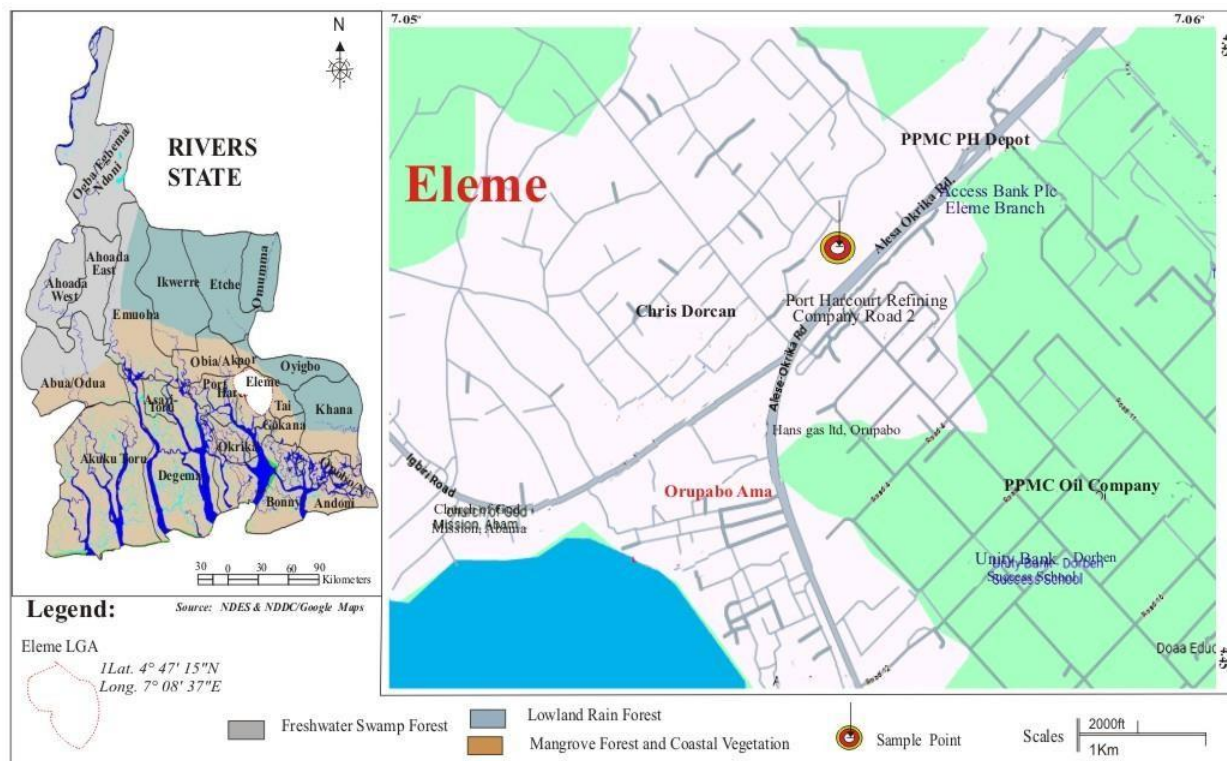
Chemicals: The polycyclic aromatic hydrocarbons used in this work comprised of a mixture of naphthalene,

acenaphthylene, acenaphthene, fluorine, anthracene, phenanthrene, fluoranthene, pyrene 99% purity, JHD, China. The extracting solvent, dichloromethane 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 in Alesha Eleme, Rivers State where crude oil is refined. The Alesha Eleme area in Rivers State, Nigeria, is renowned for being the focal point of the oil industry in Africa. Situated within the Niger Delta region, significant oil-related operations, such as extraction, refining, and transportation occur in this region which has resulted in periodic oil spills. Samples were collected from different location within the refinery (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.

Figure 1: Map of the Sample Site



### 2.3 Collection of Microbes

In the microbiology laboratory at the university, a concentrated suspension of three different microorganisms—fungi (*Aspergillus niger*), bacteria (*Pseudomonas aeruginosa*), and algae (*Sargassum filipendula*)—was 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 centimeters 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.

**Table 1: Experimental sample set**

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)

#### 2.5 Experimental Design

The soil samples, contaminated with pollutants, were treated by combining them with specific biodegrading agents outlined in Table 2.1. 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 Polycyclic aromatic hydrocarbons 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 pollutants 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 Determination of Polycyclic Aromatic Hydrocarbon

The method utilized for this process adhered to the specifications outlined in ASTM D4657. Initially, a contaminated soil sample weighing 10 grams was placed within a Soxhlet extractor along with anhydrous sodium sulfate. The extraction was carried out using 200 milliliters of dichloromethane. Subsequently, the resulting extracted sample, comprising 10 milliliters, was carefully transferred to a specialized column. This column consisted of 20 grams of 1% deactivated silica gel within a slurry of crude oil ether. Atop this column, 5 grams of neutral alumina were added. The

process of fractionating aliphatic and polyaromatic hydrocarbon residues commenced by eluting the aliphatic fraction from the column using 100 milliliters of crude oil ether. Following this, the elution of the aromatic fraction was conducted. This involved using a solution comprised of 10 milliliters of 40% methylene chloride, 60% crude oil ether, and an additional 50 milliliters of methylene chloride. The entire procedure was conducted according to the guidelines and specifications outlined in the ASTM D4657 standard. This meticulous process of extraction and fractionation allowed for the separation and analysis of distinct hydrocarbon fractions, aligning with the standardized methodology for reliable and precise testing.

### 2.7 Gas Chromatography

Polycyclic aromatic hydrocarbons were subjected to gas chromatography analysis. This examination occurred at the Department of Chemistry at the University of Port Harcourt, Nigeria. Throughout this analytical process, the total duration of the analysis spanned between 40 to 50 minutes.

## 3.0 RESULT AND DISCUSSION

### 3.1 Polycyclic Aromatic Hydrocarbons in polluted soil of the sample areas

The Polycyclic aromatic hydrocarbons in the sampled areas are depicted in Table 2

**Table 2: Polycyclic aromatic hydrocarbons in polluted soil in Port Harcourt refinery area in Alesha Eleme.**

Parameters (n=15)	Initial
Naphthalene	25.06±0.27
Acenaphthylene	38.89±0.23
Acenaphthene	20.21±0.52
Fluorene	23.97±0.78
Anthracene	22.78±0.98
Phenanthrene	175.8±0.23
Fluoranthene	7.16±0.22
Pyrene	14.03±0.23
<b>Σ8Polycyclic aromatic hydrocarbons</b>	<b>327.9±3.46</b>
Benzo[a]anthracene	20.29±0.67
Chrysene	8.11±0.75
Benzo[b]fluoranthrene	9.45±0.65
Benzo[k]fluoranthrene	12.06±0.87
Benzo[a]pyrene	4.67±0.21
Dibenz[a,h]anthracene	4.67±0.42
Indeno(1,2,3-cd)pyrene	5.06±0.21
Benzo[ghi] pyrene	4.76±0.42
<b>Σ8HPolycyclic aromatic hydrocarbons</b>	<b>69.07±4.20</b>

According to the results, the total concentration of non-carcinogenic Polycyclic aromatic hydrocarbons in polluted soil of Port Harcourt refinery depot area in Alesha Eleme was estimated at  $327.9 \pm 3.46$  mg/kg. The finding affirmed that phenanthrene has the highest concentration of  $175.8 \pm 0.23$  mg/kg, and others have a concentration that is less than 40mg/kg. This implies that soil of the sampled areas is highly contaminated with phenanthrene. High concentration phenanthrene is toxic to aquatic life, causes irritation of the respiratory system, and inflammation leading to breathing difficulties (Okocha et al., 2023; Ow hoeke et al., 2023). Areguamen et al. (2023) reported 102 mg/kg concentration of 20 polycyclic aromatic hydrocarbons in the sediment of the Ikpoba River, south-south Nigeria. Aralu et al. (2023), in similar research, reported 135.68  $\mu\text{ssg/L}$  concentrations for boreholes located within an unsanitary dumpsite in Nnewi, Anambra State, Nigeria. Parth and Mukherjee (2019) reported an average concentration of 14459  $\mu\text{g/kg}$  for 16 Polycyclic aromatic hydrocarbons [ $\Sigma 16$  Polycyclic aromatic hydrocarbons] in sediment near a municipal solid waste landfill site.

The total concentration of carcinogenic Polycyclic aromatic hydrocarbons in polluted soil of Port Harcourt refinery depot area in Alesha Eleme was estimated at  $69.07 \pm 4.20$  mg/kg. Benzo[a]anthracene has the highest concentration of  $20.29 \pm 0.67$  mg/kg. Benzo[a]anthracene a persistent organic pollutant in the soil, air, and water, and has been linked to skin, bladder, and liver cancers (Nna et al., 2024).

Wang et al. (2012) did a similar study on polycyclic aromatic hydrocarbons at sediment of a river close to a dumpsites and metal scrap sites in Lagos. They found that the total Polycyclic aromatic hydrocarbons concentrations in sediments were between 127 and 10,600 ng/g and 199 to 2420 ng/g, respectively. Wei et al. (2021) reported similar research on Polycyclic aromatic hydrocarbons in waste water disposal sites in Taizhou, Zhejiang Province. The Polycyclic aromatic hydrocarbon concentrations in the water samples were 29.7–2170 ng/g. Ailijiang et al. (2022) in their research on the Polycyclic aromatic hydrocarbons in sediments in urban parks of Northwest China recorded a total Polycyclic aromatic hydrocarbon concentration of 0.033–3.941 mg/kg in Urumqi parks.

Based on the presence and the high concentrations of Polycyclic aromatic hydrocarbons in the Port Harcourt refinery depot area of Niger Delta, there is need for possible remediation techniques, using bioremediation that are available, cheap, and eco-friendly.

3.2 Analysis of Polycyclic aromatic hydrocarbons in crude oil polluted soil treated with different remediating techniques

The percentage removal of Polycyclic aromatic hydrocarbons 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 3.

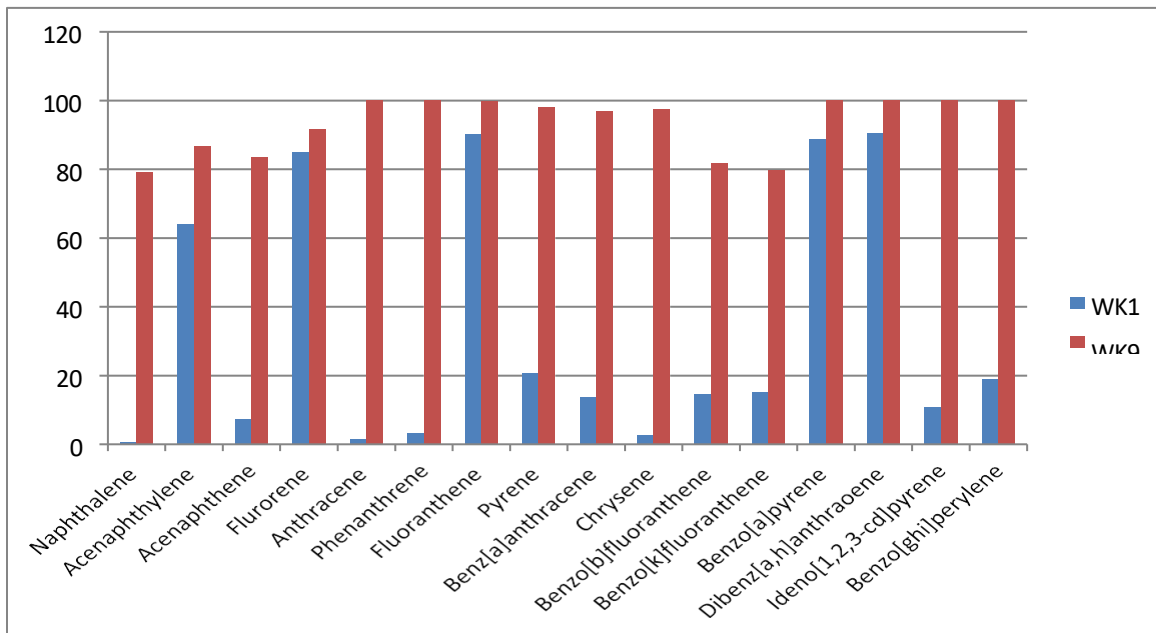
**Table 3: Percentage Remediation of Respective Polycyclic aromatic hydrocarbons from Polluted Soil**

	S1		S2		S3		S4		S5		S6	
	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9	WK1	WK9
<b>Naph</b>	0.67	79.25	1.04	86.39	96.11	80.89	13.13	82.80	41.22	86.41	78.17	82.80
<b>Acen</b>	63.93	86.67	55.45	90.51	78.25	93.44	80.02	71.66	60.65	91.56	30.95	71.66
<b>Ace</b>	7.41	83.62	68.28	85.60	50.21	65.07	29.89	47.65	32.16	93.36	8.85	747.64
<b>Flur</b>	84.96	91.64	68.37	96.41	42.57	92.82	2.96	78.85	71.92	97.37	54.58	78.84
<b>Ant</b>	1.46	100	0.35	100	40.04	100	57.99	100	67.10	100	11.42	100
<b>Phe</b>	3.12	100	87.01	100	68.91	100	87.83	100	81.12	100	92.41	100
<b>Fluorant</b>	90.28	99.80	27.93	97.07	4.05	100	61.45	100	71.64	100	64.66	100
<b>Pyrene</b>	20.81	98.04	50.82	97.93	74.91	98.86	75.05	93.80	14.41	100	23.87	96.18

<b>B[a]A</b>	13.54	97.08	44.01	99.36	69.94	97.49	39.67	95.86	70.37	99.11	11.77	99.52
<b>Chry</b>	2.76	97.54	49.57	97.90	77.44	92.23	75.09	87.18	11.46	97.28	40.93	87.17
<b>B[b]f</b>	14.67	81.87	40.53	83.81	19.26	85.82	57.46	81.27	62.43	64.86	66.45	81.26
<b>B[k]f</b>	15.13	79.79	55.74	93.86	69.17	98.67	58.87	86.48	65.83	80.76	70.39	86.48
<b>B[a]P</b>	88.81	100	33.62	70.24	28.99	69.38	50.11	45.82	54.81	63.38	24.62	45.82
<b>D[a,h]A</b>	90.58	100	57.91	100	62.85	100	11.71	100	78.58	100	28.90	100
<b>I[1,2,3-cd]P</b>	10.67	100	11.55	100	76.68	100	30.04	100	80.43	100	11.18	100
<b>B[ghi]P</b>	19.06	100	78.68	100	66.37	100	88.45	100	82.77	100	12.60	100

The percentage removal of Polycyclic aromatic hydrocarbons at week 1 and week 9 from soil treated with S1 technique is presented in Table 3 and Figure 2. According to the information in Table 3, there was a general increase in percentage removal of Polycyclic aromatic hydrocarbons with S1 technique from week 1 to week 9. This suggests that the bacteria used in this technique are effective. The percentage removal of Polycyclic aromatic hydrocarbons with S1 technique for wk1 and wk9 was recorded as: naphthalene [0.67% and 79.25%], acenaphthylene [63.93% and 86.67%], acenaphthene [7.41% and 83.62%], fluorene [84.96% and 91.64%], anthracene [1.46% and 100%], phenanthrene [3.12% and 100%], fluoranthene [90.81% and 99.80%], pyrene [20.81% and 98.04%], Benz[a]anthracene [13.54% and 97.08%], chrysene [2.76% and 97.54%], benzo[b]fluoranthene [14.67% and 81.87%], benzo[k]fluoranthene [15.13% and 79.79%], benzo[a]pyrene [88.81% and 100%], dibenz[a,h]anthraene [90.58% and 100%], ideno[1,2,3-cd]pyrene [10.67% and 100%], and benzo[ghi]perylene [19.06% and 100%].

The percentage removal of anthracene, phenanthrene, dibenz[a,h]anthraene, Ideno[1,2,3-cd]pyrene and Benzo[ghi]perylene were more sensitive to bacteria used in the technique. This is on the bases that 100% removal was achieved in week 6. Benzo[a]pyrene was also more sensitive than others Polycyclic aromatic hydrocarbons, on the basis that its 100% removal was achieved in week 7. Polycyclic aromatic hydrocarbons that exhibit complete removal are typically more responsive to the S1 approach and demonstrate a higher rate of polycyclic aromatic hydrocarbon removal. This suggests that the bacteria exhibited a higher level of sensitivity to the aforementioned Polycyclic aromatic hydrocarbons. The results of the study align with the findings of Bisht et al. (2015), who documented the elimination of specific polycyclic aromatic hydrocarbons by the utilization of rhizosphere technology. This study is also consistent with the findings of Ismail et al. (2022), who documented the microbial bioremediation methods for Polycyclic aromatic hydrocarbons.



**Figure 2: Variation of Polycyclic aromatic hydrocarbons for wk1 and wk9 via S1 Techniques**

The percentage removal of Polycyclic aromatic hydrocarbons from crude oil contaminated soil treated for nine weeks with S2 technique is presented in Table 3 and Figure 3. The percentage removal of Polycyclic aromatic hydrocarbons for wk1 and wk9 was recorded as: naphthalene [1.04% and 86.39%], acenaphthylene [55.45% and 90.51%], acenaphthene [68.28% and 85.60%], fluorene [68.28% and 96.41%], anthracene [0.35% and 100%], phenanthrene [87.01% and 100%], fluoranthene [27.93% and 97.07%], pyrene [50.82% and 97.73%], Benz[a]anthracene [44.01% and 93.36%], chrysene [49.57% and 97.90%], benzo[b]fluoranthene [40.53% and 83.81%], benzo[k]fluoranthene [55.74% and 93.86%], benzo[a]pyrene [33.62% and 70.24%], dibenz[a,h]anthraoene [57.91% and 100%], ideno[1,2,3-cd]pyrene [11.55% and 100%], and benzo[ghi]perylene [78.67 and 100%].

The fungi employed in the procedure showed higher sensitivity in removing anthracene, phenanthrene, dibenz[a,h]anthraoene, Ideno[1,2,3-cd]pyrene, and Benzo[ghi]perylene. The achievement of 100% elimination was observed during the fifth week. The significant removal of polycyclic aromatic hydrocarbons is linked to the ability of fungi to degrade these Polycyclic aromatic hydrocarbons in polluted soil (Bisht et al., 2015; Ismail et al., 2022). Fungi have been utilized as a means of bioremediating Polycyclic aromatic hydrocarbons in a previous study (Alao & Adebayo, 2022).

The results of this work are consistent with the findings of Vasudevan et al. (2018), who documented the bioremediation of Dibenz (a, h) Anthracene-A, a pentacyclic polycyclic aromatic hydrocarbon, using bacteria, fungi, and autotrophic eukaryotes. Mao and Guan (2016) additionally corroborated the research by documenting the fungal decomposition of polycyclic aromatic hydrocarbons in polluted soil through the utilization of *Scopulariopsis brevicaulis*. The results align with the study conducted by Agrawal et al. (2018), which confirmed the presence of phenanthrene and pyrene in the ligninolytic fungi *Ganoderma lucidum* obtained from the hardwood stump.

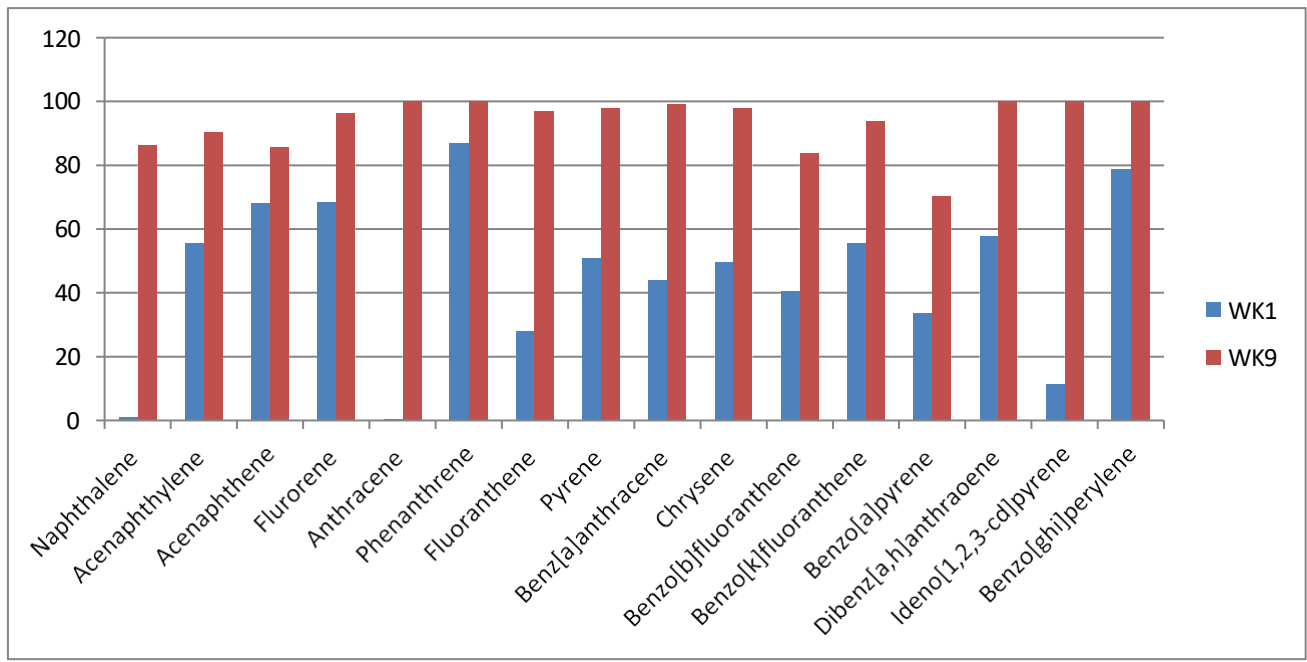
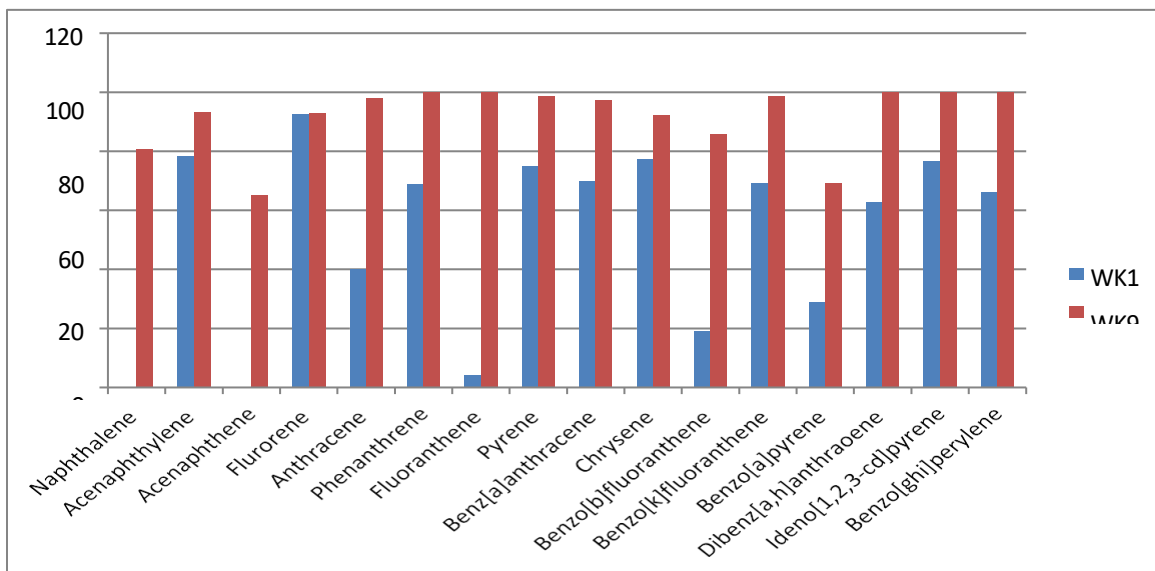


Figure 3: Variation of Polycyclic aromatic hydrocarbons for wk1 and wk9 via S2 Techniques

The percentage removal of Polycyclic aromatic hydrocarbons from crude oil contaminated soil treated for nine weeks with S3 technique is presented in Table 3 and Figure 4 was recorded as: naphthalene [96.11% and 80.89%], acenaphthylene [78.25% and 92.82%], acenaphthene [50.21% and 65.07%], fluorene [42.57% and 92.821%], anthracene [40.04% and 100%], phenanthrene [68.91% and 100%], fluoranthene [4.05% and 100%], pyrene [74.91% and 98.83%], Benz[a]anthracene [69.94% and 97.49%], chrysene [77.44% and 92.23%], benzo[b]fluoranthene [19.26% and 85.82%], benzo[k]fluoranthene [69.17% and 98.67%], benzo[a]pyrene [28.99% and 69.38%], dibenz[a,h]anthraoene [62.85% and 100%], ideno[1,2,3-cd]pyrene [76.68% and 100%], and benzo[ghi]perylene [66.34 and 100%].

The utilization of algae in the experimental protocol shown enhanced efficacy in the elimination of phenanthrene, Fluoranthene, dibenz[a,h]anthraoene, Ideno[1,2,3-cd]pyrene, and Benzo[ghi]perylene. Complete eradication was achieved by the fifth week. The percentage of anthracene elimination occurs during week 7, indicating its susceptibility to algae. The capacity of algae to breakdown polycyclic aromatic hydrocarbons in contaminated soil has been found to be associated with the notable elimination of these compounds (Bisht et al., 2015; Ismail et al., 2022). In a prior study conducted by Alao and Adebayo (2022), algae were employed as a method for bioremediating polycyclic aromatic hydrocarbons.

The outcomes of this study align with the research conducted by Vasudevan et al. (2018), whereby they observed the potential of bacteria and algae in the bioremediation of Dibenz (a, h) Anthracene- A, a pentacyclic polycyclic aromatic hydrocarbon. According to Mao and Guan (2016), the research findings were further supported by their documentation of the algae breakdown of polycyclic aromatic hydrocarbons in contaminated soil. The observed results are consistent with the findings of Agrawal et al. (2018), who verified the existence of phenanthrene and pyrene in the algae derived from the hardwood stump.

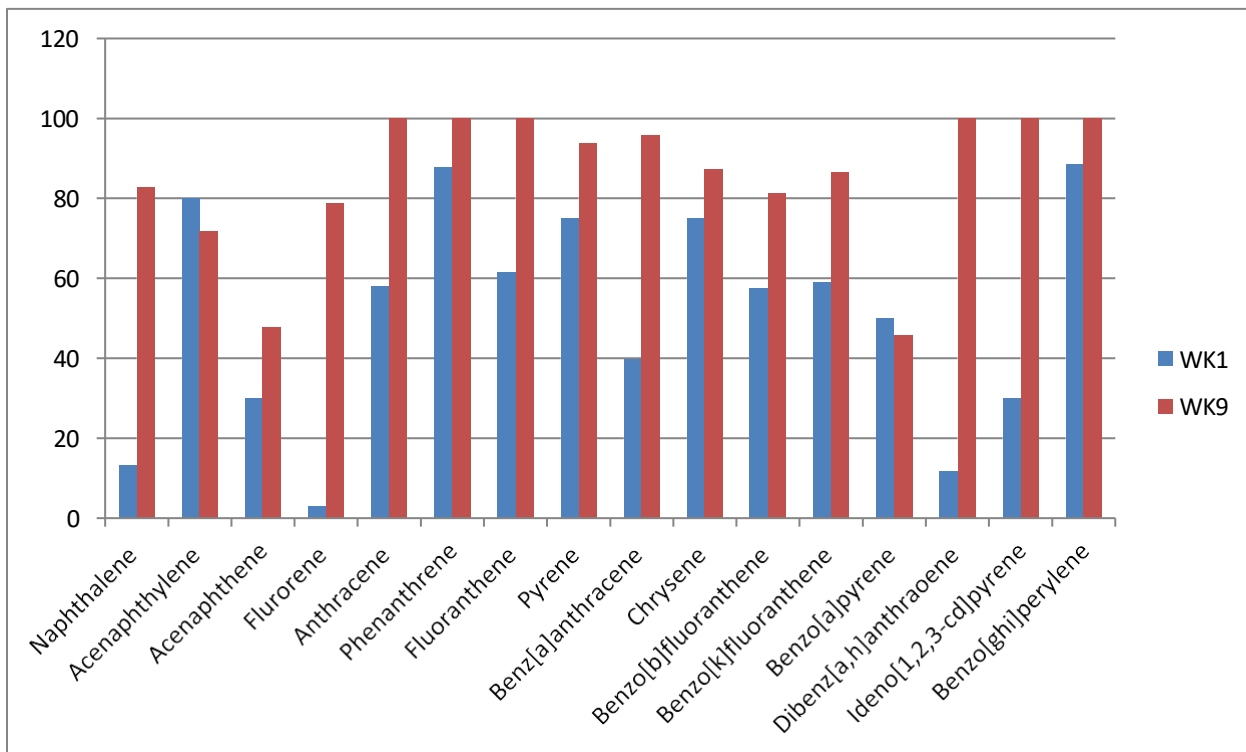


**Figure 4: Variation of Polycyclic aromatic hydrocarbons for wk1 and wk9 via S3 technique**

The percentage removal of Polycyclic aromatic hydrocarbons from crude oil contaminated soil treated for nine weeks with S4 technique is presented in Table 3 and Figure 5 and was recorded as: naphthalene [13.13% and 82.80%], acenaphthylene [80.02% and 71.66%], acenaphthene [29.89% and 47.65%], fluorene [2.96% and 78.85%], anthracene [57.99% and 100%], phenanthrene [87.83% and 100%], fluoranthene [61.45% and 100%], pyrene [75.05% and 93.80%], Benz[a]anthracene [38.67% and 95.85%], chrysene [75.09% and 87.18%], benzo[b]fluoranthene [57.46% and 81.27%], benzo[k]fluoranthene [58.87% and 86.48%], benzo[a]pyrene [50.11% and 45.82%], dibenz[a,h]anthraoene [11.71% and 100%], ideno[1,2,3-cd]pyrene [30.04% and 100%], and benzo[ghi]perylene [88.45 and 100%].

The experimental technique demonstrated improved effectiveness in the removal of phenanthrene, Fluoranthene, dibenz[a,h]anthraoene, Ideno[1,2,3-cd]pyrene, and Benzo[ghi]perylene through the use of charcoal. By the fifth week, complete eradication was likewise accomplished. The ability of charcoal to adsorb polycyclic aromatic hydrocarbons in soil that has been contaminated has been observed to be linked to its significant porosity and adsorptive characteristics (Bisht et al., 2015; Ismail et al., 2022).

The findings of this study were consistent with the research carried out by Vasudevan et al. (2018), in which they found the capacity of bacteria and algae to bioremediate Dibenz (a, h) Anthracene- A, a pentacyclic polycyclic aromatic hydrocarbon. In a study conducted by Banerjee et al. (2024), the researchers examined the role of microorganisms and microbial methods in the remediation of carcinogenic polycyclic aromatic hydrocarbons. Asemoloye et al. (2019) documented the cooperative interactions between plants and bacteria in the rhizosphere for the purpose of removing Polycyclic aromatic hydrocarbons.

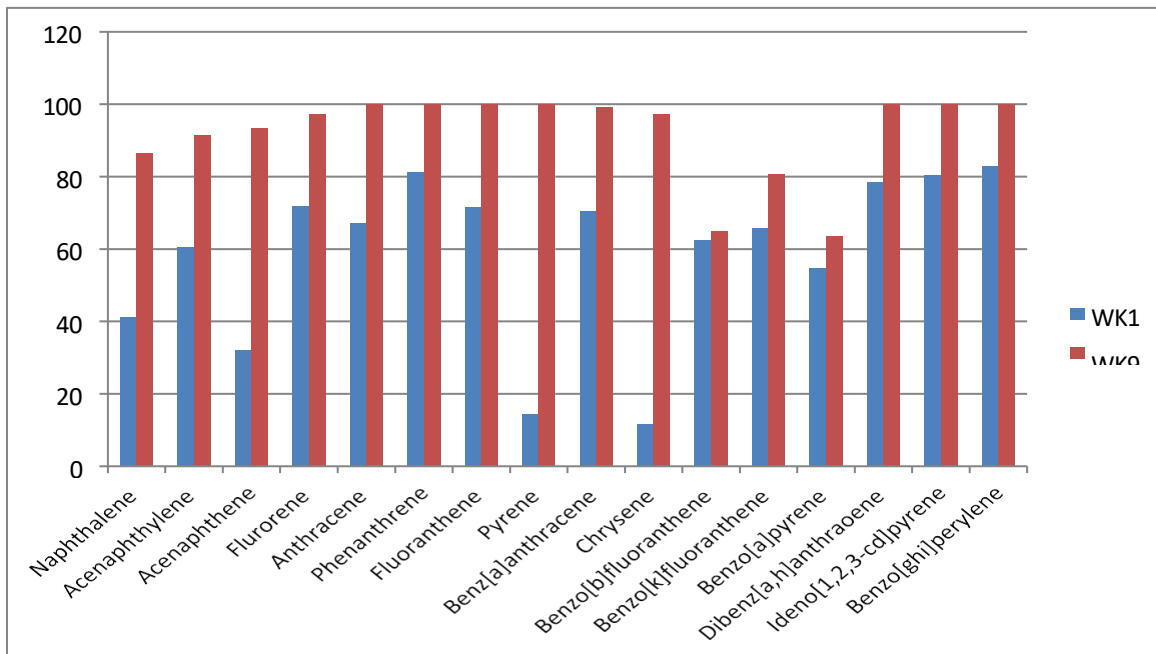


**Figure 5: Variation of Polycyclic aromatic hydrocarbons for wk1 and wk9 via S4 technique**

Table 3 and Figure 6 display the percentage of polycyclic aromatic hydrocarbons that have been eliminated from soil polluted with crude oil after a nine-week treatment using the S5 approach, and was recorded as: naphthalene [41.22% and 86.41%], acenaphthylene [60.65% and 91.56%], acenaphthene [32.16% and 93.36%], fluorene [71.92% and 97.37%], anthracene [67.10% and 100%], phenanthrene [81.12% and 100%], fluoranthene [71.64% and 100%], pyrene [14.14% and 100%], Benz[a]anthracene [70.37% and 99.11%], chrysene [11.46% and 97.28%], benzo[b]fluoranthene [62.43% and 64.86%], benzo[k]fluoranthene [65.83% and 80.76%], benzo[a]pyrene [54.81% and 63.38%], dibenz[a,h]anthraoene [78.58% and 100%], ideno[1,2,3-cd]pyrene [80.43% and 100%], and benzo[ghi]perylene [82.77 and 100%].

The experimental technique implemented the application of microbial synergy to showcase the effectiveness of phenanthrene, fluoranthene, dibenz[a,h]anthraoene, Ideno[1,2,3-cd]pyrene, and Benzo[ghi]perylene in the total removal of hydrocarbon-contaminated environments. The aforementioned elements were entirely eliminated by the fifth week. The association between the capacity of synergistic microorganisms on polycyclic aromatic hydrocarbons (Polycyclic aromatic hydrocarbons) in contaminated soil and the degradation of crude oil hydrocarbons has been observed in previous studies (Eskandary et al., 2017; Asemoloye et al., 2019).

In their study, Gupta et al. (2016) documented a significant level of biodegradation of polycyclic aromatic hydrocarbons (Polycyclic aromatic hydrocarbons) derived from soil and sediment contaminants using a microbial consortium. The results of this study support the findings of Zhou et al. (2023), who conducted research on the removal of polycyclic aromatic hydrocarbons (Polycyclic aromatic hydrocarbons) from soil utilizing immobilized microbial consortia in conjunction with enhanced remediation techniques. In a study conducted by Banerjee et al. (2024), the authors examined the role of microorganisms and microbial methods in the remediation of carcinogenic polycyclic aromatic hydrocarbons.



**Figure 6: Variation of Polycyclic aromatic hydrocarbons for wk1 and wk9 via S5 technique**

The percentage removal of Polycyclic aromatic hydrocarbons from crude oil contaminated soil treated for nine weeks with S6 technique is presented in Table 2, and its values were recorded as: naphthalene (78.17% and 82.80%), acenaphthylene (30.95% and 71.66%), acenaphthene (8.85% and 747.64%), fluorene (54.58% and 78.84%), anthracene (11.42% and 100%), phenanthrene (92.41% and 100%), fluoranthene (64.66% and 100%), pyrene (23.87% and 96.18%), Benz[a]anthracene (11.77% and 99.52%), chrysene (40.93% and 87.17%), benzo[b]fluoranthene (66.45% and 81.26%), benzo[k]fluoranthene (70.39% and 86.48%), benzo[a]pyrene (24.62% and 45.82%), dibenz[a,h]anthraoene (28.90% and 100%), ideno[1,2,3-cd]pyrene (11.18% and 100%), and benzo[ghi]perylene (12.60% and 100%) (see Figure 6).

The experimental technique demonstrated the successful removal of fluoranthene, dibenz[a,h]anthraoene, Ideno[1,2,3-cd]pyrene, and Benzo[ghi]perylene from a hydrocarbon-contaminated environment by the synergistic combination of charcoal and microbes. This complete removal was accomplished by the fifth month of the experiment. The total removal of anthracene was achieved by the sixth month of the trial. Consequently, the combination of charcoal and microbes was found to be highly responsive to the aforementioned Polycyclic aromatic hydrocarbons in the soil contaminated with crude oil. The breakdown and adsorption of crude oil hydrocarbons can be attributed to the synergistic combination of charcoal and microbes, as demonstrated by Subashchandrabose et al. (2019) and Alao & Adebayo (2022).

Eskandary et al. (2017) reported a significant reduction in Polycyclic aromatic hydrocarbons from polluted soil with the combined application of plant and isolated microorganisms. The results are consistent with the study conducted by Ali et al. (2022), which reported the bioremediation of Polycyclic aromatic hydrocarbons by the utilization of a combination of clay and microorganisms. The finding supports the findings of Asemoloye et al. (2019), who reported the collaborative interactions between plants and microorganisms in the rhizosphere for the purpose of removing Polycyclic aromatic hydrocarbons.

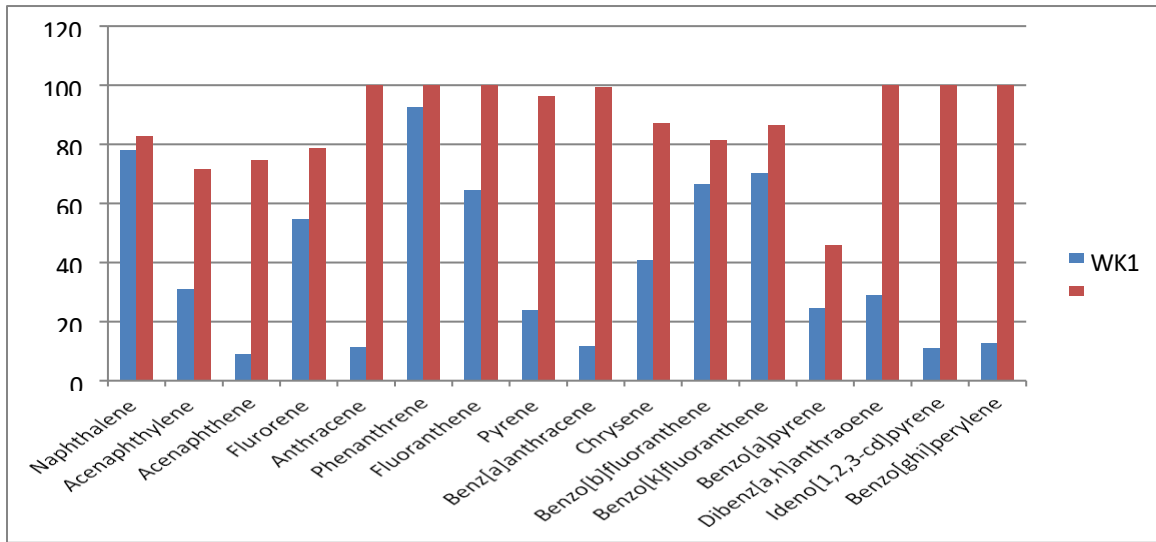


Figure 7: Variation of Polycyclic aromatic hydrocarbons for wk1 and wk9 via S6 technique

#### 4.0 CONCLUSIONS

The use of microorganisms in the remediation of crude oil contaminated land is a widespread approach. This study focuses on the removal of Polycyclic Aromatic hydrocarbon from crude oil contaminated site and assessing the remediation efficacy of microbes and a synergetic approach of a combination of microorganism and charcoal to enhance the polycyclic aromatic hydrocarbon removal. The results show a progressive increase in Polycyclic aromatic hydrocarbon removal efficiency from week one to week nine, with a complete removal of anthracene, phenanthrene, dibenz[a,h]anthracene, indeno(1,2,3-cd)pyrene and benzo[ghi]pyrene. Other polycyclic aromatic hydrocarbons were not totally removed but were reduced to non-toxic levels.

The study therefore demonstrates the effectiveness of using charcoal and microbial synergy for the removal of polycyclic aromatic hydrocarbons in soils and also suggests a promising eco-friendly solution for restoring crude oil contaminated soils in the Niger delta region.

#### Acknowledgements

We thank the staff of the university of Port-Harcourt laboratory, where the samples were analyzed for the facilities and support.

#### Authors 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 Owwoeke:** validation, conceptualization.

#### Declaration of Competing Interest.

The authors have no conflict of interest to declare regarding the publication of this paper.

#### REFERENCES

1. Aghalino, S. O., & Eyinla, B. (2009). Oil exploitation and marine pollution: Evidence from the Niger Delta, Nigeria. *Journal of human ecology*, 28(3), 177-182.
2. Agrawal, N., Verma, P., & Shahi, S. K. (2018). Degradation of polycyclic aromatic hydrocarbons (phenanthrene and pyrene) by the ligninolytic fungi *Ganoderma lucidum* isolated from the hardwood stump. *Bioresources and Bioprocessing*, 5(1), 1-9.
3. Ahmed, I., Ali, A., Sarma, J., Gogoi, R., Hussain, I., Nath, S., & Firdousie, N. (2021). Impact of Oil Spill on Aquatic Environment. *Food and Scientific Reports*, 2, 37-39.
4. Ailijiang, N., Zhong, N., Zhou, X., Mamat, A., Chang, J., Cao, S., ... & Li, N. (2022). Levels, sources, and risk assessment of Polycyclic aromatic hydrocarbons residues in soil and plants in urban parks of Northwest China. *Scientific reports*, 12(1), 21448.

5. Akpan, D., & Ajayi, O. (2016). Adverse effect of water contamination or pollution to human health and safety in the Nigeria delta–Nigeria: an environmental case study. *Journal of Environment and Earth Science*, 6(10), 2224-3216.
6. Alao, M. B., & Adebayo, E. A. (2022). Fungi as veritable tool in bioremediation of polycyclic aromatic hydrocarbons-polluted wastewater. *Journal of Basic Microbiology*, 62(3-4), 223-244
7. Ali, M., Song, X., Ding, D., Wang, Q., Zhang, Z., & Tang, Z. (2022). Bioremediation of Polycyclic aromatic hydrocarbons and heavy metals co-contaminated soils: challenges and enhancement strategies. *Environmental Pollution*, 295, 118686.
8. Akpomuvie, O. B., & Orhioghene, B. (2011). Tragedy of commons: Analysis of oil spillage, gas flaring and sustainable development of the Niger Delta of Nigeria. *Journal of Sustainable Development*, 4(2), 200-210.
9. Amosu, C. O., & Adeosun, T. A. (2021). Consequence of oil and Waste Spills on the Environment of Ogoniland, Rivers State, Nigeria. *Indian Journal of Management and Language (IJML)*, 1(2), 15-28.
10. Aralu, C. C., Okoye, P. A. C., Abugu, H. O., Eboagu, N. C., & Eze, V. C. (2023). Characterization, sources, and risk assessment of Polycyclic aromatic hydrocarbons in borehole water from the vicinity of an unlined dumpsite in Awka, Nigeria. *Scientific Reports*, 13(1), 9688.
11. Asemoloye, M. D., Jonathan, S. G., & Ahmad, R. (2019). Synergistic plant-microbes interactions in the rhizosphere: a potential headway for the remediation of hydrocarbon polluted soils. *International journal of phytoremediation*, 21(2), 71-83.
12. Bhattacharjee, S., & Dutta, T. (2022). An overview of oil pollution and oil-spilling incidents. *Advances in Oil-Water Separation*, 3-15.
13. Bisht, S., Pandey, P., Bhargava, B., Sharma, S., Kumar, V., & Sharma, K. D. (2015). Bioremediation of polyaromatic hydrocarbons (Polycyclic aromatic hydrocarbons) using rhizosphere technology. *Brazilian Journal of Microbiology*, 46, 7-21.
14. Edna Ateboh, P., & Raimi, M. O. (2018). Corporate civil liability and compensation regime for environmental pollution in the Niger Delta. *International Journal of Recent Advances in Multidisciplinary Research*, 5(06), 3870-3893.
15. Elisha, O. D., & Felix, M. J. (2021). Destruction of coastal ecosystems and the vicious cycle of poverty in Niger Delta Region. *J. Glob. Agric. Ecol*, 11(2), 7-24.
16. Elum, Z. A., Mopipi, K., & Henri-Ukoha, A. (2016). Oil exploitation and its socioeconomic effects on the Niger Delta region of Nigeria. *Environmental Science and Pollution Research*, 23, 12880-12889.
17. Eskandary, S., Tahmourespour, A., Hoodaji, M., & Abdollahi, A. (2017). The synergistic use of plant and isolated bacteria to clean up polycyclic aromatic hydrocarbons from contaminated soil. *Journal of Environmental Health Science and Engineering*, 15, 1-8.
18. Gupta, G., Kumar, V., & Pal, A. K. (2016). Biodegradation of polycyclic aromatic hydrocarbons by microbial consortium: a distinctive approach for decontamination of soil. *Soil and Sediment Contamination: An International Journal*, 25(6), 597-623.
19. Igbani, F., Tatah, G. W., & Odekina, M. U. (2024). A Review on the Effects of Crude Oil Spill on Aquatic Life (Fish) in The Niger Delta, Niger
20. Ite, A. E., Harry, T. A., Obadimu, C. O., Asuaiko, E. R., & Inim, I. J. (2018). Petroleum hydrocarbons contamination of surface water and groundwater in the Niger Delta region of Nigeria. *Journal of Environment Pollution and Human Health*, 6(2), 51-61.
21. Ismail, N. A., Kasmuri, N., & Hamzah, N. (2022). Microbial bioremediation techniques for polycyclic aromatic hydrocarbon (Polycyclic aromatic hydrocarbons)—a review. *Water, Air, & Soil Pollution*, 233(4), 124.
22. Ivshina, I. B., Kuyukina, M. S., Krivoruchko, A. V., Elkin, A. A., Makarov, S. O., Cunningham,
23. C. J., ... & Philp, J. C. (2015). Oil spill problems and sustainable response strategies through new technologies. *Environmental Science: Processes & Impacts*, 17(7), 1201-1219.
24. Khanna, L. S., Ramnath, K., Monica, J., Muthu, D., & Venkatasubramanian, C. (2017). Comprehensive study on the detrimental effects of fossil fuel exploration and pipe laying in deltaic region. In *IOP Conference Series: Earth and Environmental Science* (Vol. 80, No. 1, p. 012052). IOP Publishing.
25. Mao, J., & Guan, W. (2016). Fungal degradation of polycyclic aromatic hydrocarbons (Polycyclic aromatic hydrocarbons) by *Scopulariopsis brevicaulis* and its application in bioremediation of polycyclic aromatic hydrocarbon-contaminated soil. *Acta Agriculturae Scandinavica, Section B–Soil & Plant Science*, 66(5), 399-405.
26. Nababa, I. I., Symeonakis, E., Koukoulas, S., Higginbottom, T. P., Cavan, G., & Marsden, S. (2020). Land cover dynamics and mangrove degradation in the Niger Delta region. *Remote Sensing*, 12(21), 3619.
27. Nna, P. J., Legborsi, J. and Orié, K. J. (2020). Comparative Study on the Phytoconstituents and Antimicrobial Analysis of *Jatropha curcas* Leaf and Stem Bark. *Direct Res. J. Chem. Mater. Sci.* Vol. 7(3), Pp. 37-43.
28. Nna, P. J., Orié, K. J., & Kalu, N. A. S. (2024). Source Apportionment and Health Risk of Some Organic Contaminants in Water and Suspended Particulate Matter from Imo River, Nigeria. *Journal of Applied Sciences and Environmental Management*, 28(2), 291-303.
29. Okocha, B. I., Orié, K. J., Duru, R. U., & Ngochindo, R. L. (2023). Analysis of the active metabolites of ethanol and ethyl acetate extract of *Justicia carnea*. *African Journal of Biomedical Research*, 26(1), 109-117.
30. Okonkwo, C. N. P., Kumar, L., & Taylor, S. (2015). The Niger Delta wetland ecosystem: What threatens it and why should we protect it?. *African Journal of Environmental Science and Technology*, 9(5), 451-463.
31. Ophori, D. U. (2005). A preliminary analysis of regional groundwater movement in the Niger Delta, Nigeria. *Journal of Environmental Systems*, 32(2).
32. Owhoeko, E., Ali, A., Nnaemeka, O. J., Orié, K. J., Ehiwario, J. N., & Rashid, A. (2023). Index model equation analysis: A case study of the risk and source of inorganic contaminants in roadside uncontaminated soil of the Egi oil producing area, Niger Delta.

*International Journal of Sediment Research*, 38(6), 891-900.

33. Parth, V., & Mukherjee, S. (2019). Polycyclic aromatic hydrocarbons species in soil and its probabilistic cancer risk to residents near municipal solid waste landfill site. *International Journal of Human Capital in Urban Management*, 4(4).
34. Subashchandrabose, S. R., Venkateswarlu, K., Venkidusamy, K., Palanisami, T., Naidu, R., & Megharaj, M. (2019). Bioremediation of soil long-term contaminated with Polycyclic aromatic hydrocarbons by algal-bacterial synergy of *Chlorella* sp. MM3 and *Rhodococcus wratislaviensis* strain 9 in slurry phase. *Science of the total environment*, 659, 724-731.
35. Vasudevan, V., Gayathri, K. V., & Krishnan, M. E. G. (2018). Bioremediation of a pentacyclic polycyclic aromatic hydrocarbon, Dibenz (a, h) Anthracene-A long road to trip with bacteria, fungi, autotrophic eukaryotes and surprises. *Chemosphere*, 202, 387-399.
36. Wei, B., Liu, C., Bao, J., Wang, Y., Hu, J., Qi, M., ... & Wei, Y. (2021). Uptake and distributions of polycyclic aromatic hydrocarbons in cultivated plants around an E-waste disposal site in Southern China. *Environmental Science and Pollution Research*, 28, 2696- 2706.
37. Zabbey, N., Sam, K., & Onyebuchi, A. T. (2017). Remediation of contaminated lands in the Niger Delta, Nigeria: Prospects and challenges. *Science of the Total Environment*, 586, 952- 965.
38. Zhou, H., Gao, X., Wang, S., Zhang, Y., Coulon, F., & Cai, C. (2023). Enhanced bioremediation of aged polycyclic aromatic hydrocarbons in soil using immobilized microbial consortia combined with strengthening remediation strategies. *International Journal of Environmental Research and Public Health*, 20(3), 1766