Open Access

Advances in Environmental and Engineering Research

LIDSEN Publishing Inc.

Original Research

From Killer to Solution: Evaluating Bioremediation Strategies on Microbial Diversity in Crude Oil-Contaminated Soil over Three to Six Months in Port Harcourt, Nigeria

Fubara Gift Evans [1], Ukoima Hudson Nkalo [2], Dokuboba Amachree [3], Morufu Olalekan Raimi [4,*]

1. Department of Biology, Faculty of Sciences, Federal University Otuoke, Bayelsa State, Nigeria; E-Mail: fubarage@fuotuoke.edu.ng; ORCID: 0009-0009-4342-9783
2. Department of Forestry and Environment, Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria; E-Mail: rijmanconsult@yahoo.com
3. Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria; E-Mail: dokuboba.amachree@ust.edu.ng; ORCID: 0009-0005-2666-6622
4. Department of Environmental Management and Toxicology, Faculty of Sciences, Federal University Otuoke, Bayelsa State, Nigeria; E-Mail: raimimo@fuotuoke.edu.ng; ORCID: 0000-0001-5042-6729

* **Correspondence:** Morufu Olalekan Raimi; E-Mail: raimimo@fuotuoke.edu.ng; ORCID: 0000-0001-5042-6729

Academic Editor: Manuel Jesús Gazquez

Special Issue: Soil Pollution Assessment and Sustainable Remediation Strategies

Adv Environ Eng Res
2024, volume 5, issue 4
doi:10.21926/aeer.2404023

Received: May 12, 2024
Accepted: October 24, 2024
Published: November 05, 2024

Abstract

The study aimed to evaluate the efficacy of various bioremediation approaches on microbial diversity in crude oil-contaminated soil over three to six months in Port Harcourt, Nigeria. The objective was to assess the impact of different bioremediation strategies on microbial populations, particularly focusing on hydrocarbon-utilizing bacteria and fungi. Microbial populations were quantified using serial dilution and microbial count techniques. The vapor phase transfer mechanism was employed to estimate hydrocarbon-utilizing bacteria and fungi.

Bacterial and fungal colonies were incubated for five days, followed by biochemical tests for isolate identification. Fungal pure cultures were observed under a microscope. The study observed a significant increase in microbial populations in soil free of crude oil pollution when bioremediators such as mushrooms and earthworms were introduced. Mushrooms exhibited a 50% increase in hydrocarbon-utilizing bacteria (HUB), while earthworms showed a 55% increase in HUB over the three to six-month period. The longer lifespan and nutrient absorption capabilities of earthworms facilitated faster growth. Furthermore, significant growth in the microbial population of hydrocarbon-utilizing bacteria and fungi was observed in crude oil-polluted soil after employing bioremediation, with the highest growth observed in soil treated with mushrooms at six months, followed by earthworms at six months. Conversely, the lowest microbial population was recorded in soil polluted with 10% crude oil and remediated with earthworms at three months. The results suggest that mushrooms and earthworms effectively increase microbial populations in crude oil-polluted soil. However, mushrooms demonstrated a higher microbial population increase compared to earthworms, especially in terms of promoting the growth of hydrocarbon-utilizing bacteria (HUB) and hydrocarbon-utilizing fungi (HUF). Based on the findings, it is recommended to prioritize using mushrooms as bioremediation agents in similar environmental restoration efforts due to their superior efficacy in increasing microbial populations, particularly HUB and HUF. This study underscores the potential of mushrooms and earthworms as effective bioremediation agents for restoring microbial diversity in crude oil-contaminated soil, offering insights for sustainable environmental restoration practices in oil-affected regions like Port Harcourt, Nigeria.

Keywords

Crude oil; bioremediation strategies; microbial diversity; contaminated soil; port-Harcourt

1. Introduction

Soil contamination by crude oil represents a significant environmental challenge, with far-reaching consequences for both ecosystem health and human well-being [1-19]. This issue persists in various forms and intensities across the globe, particularly in regions where oil extraction, processing, and transportation activities are prevalent. The persistence of hydrocarbons in the soil leads to disruptions in microbial communities, which are essential for nutrient-cycling processes. These disruptions exacerbate the risk of groundwater contamination, posing a critical threat to both environmental and public health [20-45]. Given the scope and scale of this issue, urgent action is necessary to develop effective remediation strategies, such as bioremediation, which could mitigate these adverse effects [36, 39, 46-53]. The degree of the problem is especially pronounced in regions like Nigeria's Niger Delta, where crude oil spills have extensively polluted the soil, affecting both rural and urban communities. The financial impact of soil contamination is immense, leading to losses in agricultural productivity, increased healthcare costs, and the expense of implementing remediation strategies [6, 13, 14, 18, 22, 54-56]. Crude oil contamination has persisted for decades, exacerbated by inadequate regulatory oversight and frequent pipeline ruptures. Historically, spills have occurred in this region since the onset of oil exploration in the 1950s, and the problem has continued to grow with increasing oil production and transportation activities [12-14, 29, 57-59]. This issue is most prevalent in national and regional contexts, particularly in oil-producing states such as Rivers, Bayelsa, and Delta in Nigeria. Urban areas like Port Harcourt, a hub of oil and gas activity, face acute contamination from spills and industrial emissions, but rural communities are also heavily affected [20]. In rural areas, where farming is a primary livelihood, oil spills devastate soil fertility, leading to food insecurity and loss of income for local populations [26]. The varying degrees of contamination across urban and rural settings illustrate how different groups are impacted differently by crude oil contamination [12, 20, 22-30, 32-35, 37-43, 60-73]. The impacts are also differentiated by socioeconomic status. While wealthier urban populations may access alternative water sources and healthcare, rural and poorer communities are disproportionately affected by contaminated water supplies and the long-term health impacts of exposure to oil pollutants. This disparity highlights the urgent need for tailored interventions that consider the unique vulnerabilities of affected populations [8, 25]. Women and children, in particular, are more vulnerable due to their direct involvement in water collection and subsistence farming, which exposes them to contaminated soil and water [27]. Bioremediation, as a cost-effective and environmentally sustainable approach, offers significant potential for addressing crude oil contamination. This method leverages microbial communities' natural ability to degrade hydrocarbons, restoring soil functionality [13, 14, 74-76]. Bioremediation strategies such as bioaugmentation, biostimulation, and phytoremediation have shown success in various studies, with bioaugmentation introducing hydrocarbon-degrading microorganisms, biostimulation fostering the growth of indigenous microbes through nutrient supplementation, and phytoremediation using plants to absorb and break down hydrocarbons [59]. Research has consistently shown that these bioremediation techniques can enhance microbial diversity in contaminated soils. For instance, studies have demonstrated that crude oil-contaminated soils undergo significant shifts in microbial composition, enriching hydrocarbon-degrading organisms [12-14]. Notably, certain bacteria, such as *Serratia marcescens* and *Raoultella ornithinolytica*, have exhibited robust crude oil degradation capabilities, achieving degradation rates of up to 96.73% in laboratory settings [1, 2, 4, 5, 12-16, 22, 25, 54, 58, 68, 69, 75, 77, 78].

These findings underscore the importance of continued research into bioremediation strategies. However, gaps remain, particularly concerning the long-term effects of bioremediation on soil microbial diversity. Understanding how these strategies impact microbial communities over time is critical for ensuring the sustainability of remediation efforts [29]. Temporal dynamics are pivotal in determining soil resilience and the overall efficacy of remediation interventions,essential for developing more robust soil restoration practices [77, 79]. The city of Port Harcourt exemplifies the urgent need for practical bioremediation. As a central industrial hub in Nigeria’s oil sector, it faces widespread environmental degradation due to frequent oil spills and emissions from refineries and petrochemical plants [60]. The contamination in Port Harcourt is compounded by rapid urbanization and deforestation, further strained the environment [20]. To address these challenges, bioremediation emerges as a critical tool for cleaning up oil spills and restoring the soil's microbial health, ensuring long-term ecosystem sustainability [22]. In this context, the implementation of bioremediation strategies in Port Harcourt has already shown promise. Research conducted in the region highlights that nutrient supplementation in biostimulation efforts encourages the growth of hydrocarbon-degrading bacteria, effectively reducing soil contamination levels [75]. Biostimulation has been identified as the most effective field method, as it enhances the microbial community's ability to break down hydrocarbons [80]. Ultimately, the success of these interventions in Port Harcourt could serve as a model for other oil-contaminated regions in Nigeria and beyond. Researchers can formulate more sustainable and resilient remediation approaches by advancing our understanding of how bioremediation strategies interact with microbial communities in contaminated soils [4, 22]. This research has the potential to contribute significantly to global efforts aimed at mitigating the environmental and public health impacts of crude oil contamination, particularly in vulnerable communities [54].

2. Materials and Methods

2.1 Study Area

The study site, Port Harcourt, Nigeria, is nestled within the southern expanse of the nation, specifically within the sprawling deltaic region known as the Niger Delta. Geographically positioned between Latitude: 4°40' North to 4°57' North and Longitude: 6°53' East to 7°8' East, Port Harcourt graces the banks of the Bonny River, nestled within a coastal lowland punctuated by a labyrinthine network of rivers, creeks, and estuaries. This region experiences the hallmark of tropical monsoon climates, characterized by sweltering temperatures averaging between 25°C and 32°C, accompanied by oppressive humidity levels often surpassing 80%. The climatic rhythm orchestrates a symphony of rainfall, drenching the landscape with 2,400-3,000 mm precipitation during the rainy seasons, starkly juxtaposed against the aridity of the dry seasons spanning from November to February. The climatic tapestry profoundly shapes the local milieu, intricately weaving the fabric of vegetation patterns, agricultural paradigms, and water resource management strategies. March heralds the onset of the rainy season, inundating the region with copious rainfall until October, while the ensuing dry seasons usher in parched landscapes. The environs surrounding Port Harcourt are predominantly cloaked in mangrove forests and swamps, serving as vital ecosystems fostering a rich tapestry of flora and fauna. Regrettably, these ecosystems bear the scars of profound degradation wrought by oil pollution, precipitating a harrowing toll on biodiversity and ecological equilibrium [13, 14, 22]. Culturally and commercially, Port Harcourt is a vibrant nexus of human activity, encompassing an eclectic array of ethnicities such as the Ikwerre, Ogoni, and Ijaw peoples. As Rivers State's capital, Port Harcourt is a linchpin of Nigeria's economic and industrial landscape. Its urban fabric seamlessly interlaces with rural enclaves, teeming markets, and bustling residential and commercial precincts, epitomizing the dynamic synergy of urbanity and rustic charm.

2.2 Methods

The assessment of microbial populations occurred at two critical time points: three and six months after introducing mushrooms and earthworms individually into native soil. It is important to note that the mushrooms and earthworms were introduced simultaneously to ensure uniform conditions for the bioremediation process. Their populations were monitored throughout the study by regular sampling at predetermined intervals, with adjustments to maintain their health and activity. This included maintaining optimal soil moisture, temperature, and nutrient levels. The objective was to discern and juxtapose the efficacy of each organism in augmenting or diminishing microbial biomass. This endeavor adhered to a meticulously structured methodology delineated below, ensuring methodological rigor and reproducibility. A control condition was established where no mushrooms or earthworms were introduced to ensure that external factors such as temperature, soil moisture, and nutrient availability did not unduly influence microbial populations. The control soil was monitored in parallel, with the same periodic checks to ensure the reliability of the findings.

The outcomes of each bioremediation were meticulously documented to facilitate a rudimentary comparison of occurrence frequencies. Furthermore, the statistical analyses entailed the computation of both mean values and standard errors for each treatment, providing a robust quantitative framework for interpreting the data. The systematic execution of these procedures facilitated the elucidation of temporal dynamics in microbial populations. It engendered a nuanced understanding of the relative effectiveness of earthworms versus mushrooms as bioremediation agents. Such methodological precision is the cornerstone for rigorous scientific inquiry, underpinning the reliability and validity of the ensuing findings.

2.3 Serial Dilution

The procedure commenced with precisely measuring one gram of desiccated soil, meticulously weighed into 9 milliliters of 0.1% Peptone water diluent. This amalgam was subjected to vigorous agitation to ensure thorough homogenization of soil particles within the Peptone water solution. Subsequently, a meticulous tenfold (v/v) serial dilution was executed by transferring one milliliter of the initial solution into successive Peptone water diluents, spanning a dilution range from 10^{-1} to 10^{-7}. This systematic dilution regimen facilitated the creation of a spectrum of diluted samples, enabling the accurate enumeration of microbial populations within the soil matrix.

2.4 Microbial Count

To ascertain the abundance of hydrocarbon-utilizing microbes, we employed the vapor phase transfer mechanism (explained briefly as the process where volatile hydrocarbons transfer from the liquid phase to the gas phase and interact with microorganisms in the growth media), initially developed by Mills *et al.* [81] and subsequently refined by Obire *et al.* [82]. This method enabled the estimation of hydrocarbon-utilizing bacteria and fungi within the medium, utilizing the spread plate technique on a mineral salt medium. The mineral salt medium composition consisted of the following components: KH_2PO_4 (1.0 g/L), Na_2HPO_4 (1.0 g/L), NH_4NO_3 (1.0 g/L), $MgSO_4{\cdot}7H_2O$ (0.2 g/L), and $FeSO_4$ (0.05 g/L), pH adjusted to 7.0. The procedure involved the preparation of sterile filter paper soaked in 2 milliliters of sterile crude oil, which was then aseptically positioned onto the inverted cover of sterile Petri dishes before incubation. Subsequently, soil samples were serially diluted from 10^{-1} to 10^{-7}, enabling the enumeration of hydrocarbon-utilizing bacteria. From the 10^{-2} dilution, a 0.1milliliter aliquot was inoculated onto the media in replicates to determine bacterial counts. Fungal counts were performed using selective media, adding streptomycin and Fungusol miconazole nitrate to inhibit bacterial and fungal growth. Following injection, the cultures were incubated for five days at ambient temperature. This incubation duration allowed for the propagation and manifestation of microbial colonies, facilitating accurate quantification ofhydrocarbon-utilizing bacteria and fungi populations. The number of colonies on each plate was counted and the average count was expressed as colony-forming units per gram (cfu/g) using the formula below [83].

$$\frac{Cf_u}{g} = \frac{N}{D \times V} \quad (1)$$

Where N = number of observed colonies,
D = Dilution factor,
V = volume of sample plate.

2.5 Isolation, Preparation of Pure Culture of Microbial Isolates

Following the initial enumeration, each distinct bacterial colony was meticulously transferred into separate Petri dishes containing nutrient agar, employing the streak-plate method to ensure individual isolation. These Petri dishes were incubated for 24 hours to facilitate bacterial colony growth and proliferation. Fungal colonies were similarly isolated and grown on Sabouroud Dextrose Agar (SDA) media for 72 hours at ambient temperature. This protocol ensured the development of pure cultures, with stock cultures preserved in 10% (v/v) glycerol suspension for long-term storage.

2.6 Identification of Microbial Isolates

Standard microbiological techniques were employed to identify bacterial isolates. Gram staining was conducted to determine the Gram reaction (positive or negative), and a series of biochemical tests were performed to ascertain metabolic capabilities. These tests included the catalase test (for the breakdown of hydrogen peroxide), the oxidase test (to identify cytochrome c oxidase enzyme), and a battery of sugar fermentation tests (using glucose, lactose, and mannitol) to evaluate the bacteria's ability to ferment different sugars. The fungal isolates were identified based on both macroscopic and microscopic features. Macroscopic characteristics included colony color, texture, and growth pattern on Sabouraud Dextrose Agar (SDA). For microscopic analysis, slides were prepared using the lactophenol cotton blue stain, and sporulation patterns, hyphal structures, and conidia were examined under a light microscope. The identification of fungal species was performed with the help of standard identification keys and manuals such as Barnett and Hunter's Illustrated Genera of Imperfect Fungi.

2.7 Enumeration of Microbial Population Growth (Experimental Design)

The microbial population growth was systematically monitored at two critical time points-three and six months post-introduction of mushrooms and earthworms into the soil. The experimental setup was designed to assess the effect of each bioremediator (mushrooms or earthworms) on microbial populations within the soil.

2.8 Experimental Setup

1. **Soil Sampling and Preparation:** Soil samples were collected from the native soil area and were desiccated by air-drying in a clean, sterile environment. The samples were homogenized to ensure uniformity in texture and nutrient composition. From this homogenized batch, experimental treatments were established, each replicated three times:
    - **Treatment 1:** Soil with earthworms only
    - **Treatment 2:** Soil with mushrooms only

  - **Control:** Native soil without any bioremediation addition

  Each treatment was prepared in 2 kg soil batches, with earthworms (10 individuals per batch) and mushrooms (*Pleurotus spp.)* introduced at equal density (5 g/kg of soil). All treatments, including the control, were housed in a controlled laboratory environment where optimal moisture levels (30-40%) and temperature (22-25°C) were maintained.

2. **Sampling at Time Intervals:** Soil samples were collected from each treatment at time zero (before bioremediation introduction) and at subsequent intervals of three months and six months. These intervals were chosen to monitor both short-term and extended changes in microbial populations.
3. **Microbial Enumeration Process:** The experimental process for microbial enumeration involved several key steps, detailed below:
   - **Serial Dilution and Plating:** 1 gram of soil was taken from the treatment batches and suspended in 9 mL of 0.1% Peptone water diluent for each sample to form a homogeneous mixture. Serial dilutions (10^{-1} to 10^{-7}) were performed to obtain a gradient of microbial concentrations.
   - **Hydrocarbon-Utilizing Microbes:** Spread plate techniques were utilized to measure the hydrocarbon-utilizing microbial populations. Diluted samples (from 10^{-2} dilutions) were plated onto mineral salt agar medium enriched with sterile crude oil to facilitate the growth of hydrocarbon-degrading microbes. The plates were incubated at room temperature (22-25°C) for five days, after which visible microbial colonies were counted.
   - **General Microbial Population:** In addition to hydrocarbon-utilizing bacteria, the total microbial population (including bacteria and fungi) was enumerated by plating soil dilutions onto nutrient agar for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. The bacterial plates were incubated at 37°C for 24-48 hours, while fungal plates were incubated at 25°C for 72 hours for colony development.
4. **Experimental Controls:** A control experiment was conducted in parallel to ensure an accurate interpretation of microbial growth. The control soil, with no bioremediators introduced, underwent the same serial dilution and plating procedures to provide baseline data on microbial population changes under natural conditions.

2.9 Statistical Analysis

Statistical analyses were performed using SPSS (version 25.0) and Microsoft Excel. The data were first assessed for normality using the Shapiro-Wilk test. For comparisons between groups (i.e., control, earthworm, and mushroom treatments), analysis of variance (ANOVA) was applied to test for statistically significant differences in microbial counts at different time points (three months and six months). Post-hoc comparisons were conducted using Tukey's HSD test to identify specific group differences. In addition, Pearson correlation analysis was employed to explore potential relationships between microbial biomass and environmental parameters such as temperature, moisture, and nutrient content. A significance level of $P < 0.05$ was adopted for all statistical tests.

3. Results

Table 1 and Figure 1 present the quantification of microbial populations in soil devoid of crude oil contamination (0% crude oil pollution) following the application of two bioremediation, mushrooms and earthworms, over three and six months. The results indicate that mushrooms significantly increased Hydrocarbon-Utilizing Bacteria (HUB) populations by 50% after three months, compared to a 33% increase by earthworms during the same period. However, earthworms demonstrated a greater effect, at the six-month mark, enhancing HUB populations by 55%, while mushrooms contributed a 41% increase. For HydrocarbonUtilizing Fungi (HUF), mushrooms outperformed earthworms at three and six-month intervals, with a notable rise in fungal populations after six months. The results highlight significant differences ($P < 0.05$) in microbial population growth between the bioremediation timeframes. The implications of these findings suggest that mushrooms may provide a rapid, short-term boost in microbial populations, particularly for bacteria, while earthworms offer sustained benefits over longer periods, particularly for HUB growth. This differential temporal efficacy could guide the bioremediation choice based on the treatment duration required. With their continuous nutrient cycling and organic matter digestion, Earthworms show greater potential for long-term soil health improvement, while mushrooms are effective for quickly initiating microbial growth. Both bioremediation play complementary roles in enhancing soil microbial activity, which is critical for the success of bioremediation in environments affected by pollutants.

Table 1 Quantification and Comparison of the Effect of Bioremediators on the Microbial Population at 0% Crude Oil Polluted Soil.

Microbial Pollution (cfu/g)	**Bioremediators**			
	Mushroom 3 Months	**Earthworm 3 Months**	**Mushroom 6 Months**	**Earthworm 6 Months**
HUB	42000 ± 1154^{c}	39000 ± 1154^{c}	63000 ± 1154^{a}	5200 ± 1154^{b}
HUF	5000 ± 115.4^{b}	4300 ± 115.4^{c}	7066 ± 145.2^{a}	6700 ± 115.4^{a}

DMRT shows that the means with various letters differ considerably ($P < 0.05$).

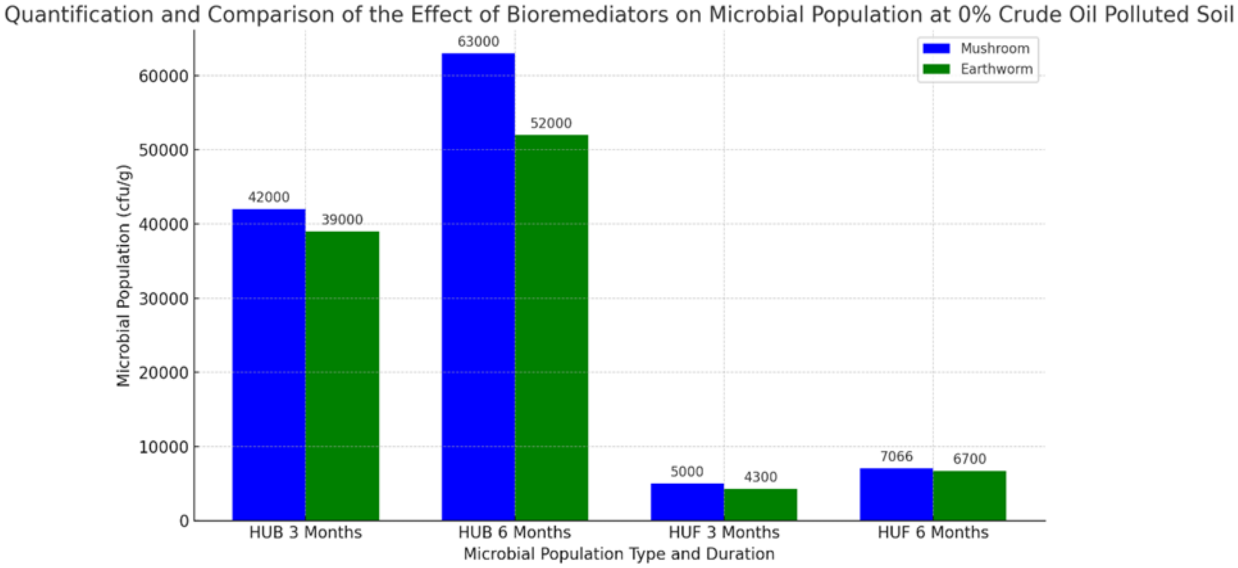

Figure 1 shows the bar chart that compares the microbial population (cfu/g) influenced by mushrooms and earthworms over 3 and 6 months for both Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF) in 0% crude oil polluted soil. The

blue bars represent the results for mushrooms and the green bars represent the results for earthworms. This visual can help you better understand the comparative effects of each bioremediator over time.

Table 2 and Figure 2 presents the impact of bioremediators (mushrooms and earthworms) on the population of Hydrocarbon Utilizing Bacteria (HUB) in soil contaminated with varying concentrations of crude oil (0%, 5%, 7.5%, and 10%) over 3 and 6 months. The data show that at 0% crude oil pollution, the population of HUB increased more significantly in soil treated with mushrooms compared to earthworms, mainly after 6 months, where mushroom treatment led to 63,010 cfu/g compared to 52,007 cfu/g for earthworms. Similar trends were observed across all crude oil concentrations (5%, 7.5%, and 10%), with the population of HUB consistently higher in mushroom-treated soil after both 3 and 6 months. As crude oil concentration increased, HUB populations generally declined in both treatments. However, at each concentration level, mushrooms outperformed earthworms in promoting HUB growth, particularly at 7.5% crude oil pollution after 6 months (mushrooms: 29,003 cfu/g vs. earthworms: 21,007 cfu/g). The results from Table 2 suggest that mushrooms are more effective than earthworms in enhancing the Hydrocarbon Utilizing Bacteria (HUB) population, especially over longer periods (6 months) and at higher concentrations of crude oil pollution. The decrease in HUB populations with increasing crude oil concentration implies that higher pollution levels might limit microbial growth, but the bioremediators still manage to stimulate bacterial proliferation. Mushrooms, in particular, demonstrate a superior ability to support microbial recovery in oil-polluted soils, likely due to their more efficient degradation of hydrocarbons and better support for microbial communities over time. These findings emphasize the importance of selecting the right bioremediator, especially when dealing with varying degrees of soil contamination, and they highlight the potential of mushrooms as a powerful tool in bioremediation efforts.

Table 2 The effects of bioremediators on microbial population of varying concentrations of crude oil polluted soil.

Crude conc. (%)	**Polluted Soil**	**Bioremediators**			
		Mushroom 3 Months	**Earthworm 3 Months**	**Mushroom 6 Months**	**Earthworm 6 Months**
0	35002 ± 1.15^{e}	42006 ± 2.33^{c}	39003 ± 3.17^{d}	63010 ± 6.06^{a}	52007 ± 3.60^{b}
5	20002 ± 1.76^{e}	25003 ± 2.40^{c}	22002 ± 1.76^{d}	30005 ± 2.64^{a}	27004 ± 2.60^{b}
7.5	11002 ± 1.50^{e}	20004 ± 2.40^{c}	17003 ± 1.70^{d}	29003 ± 2.02^{a}	21007 ± 3.71^{b}
10	10003 ± 2.02^{e}	19005 ± 2.70^{c}	13004 ± 2.08^{d}	25003 ± 2.02^{a}	20003 ± 1.76^{b}

DMRT shows that the means with various letters differ considerably ($P < 0.05$).

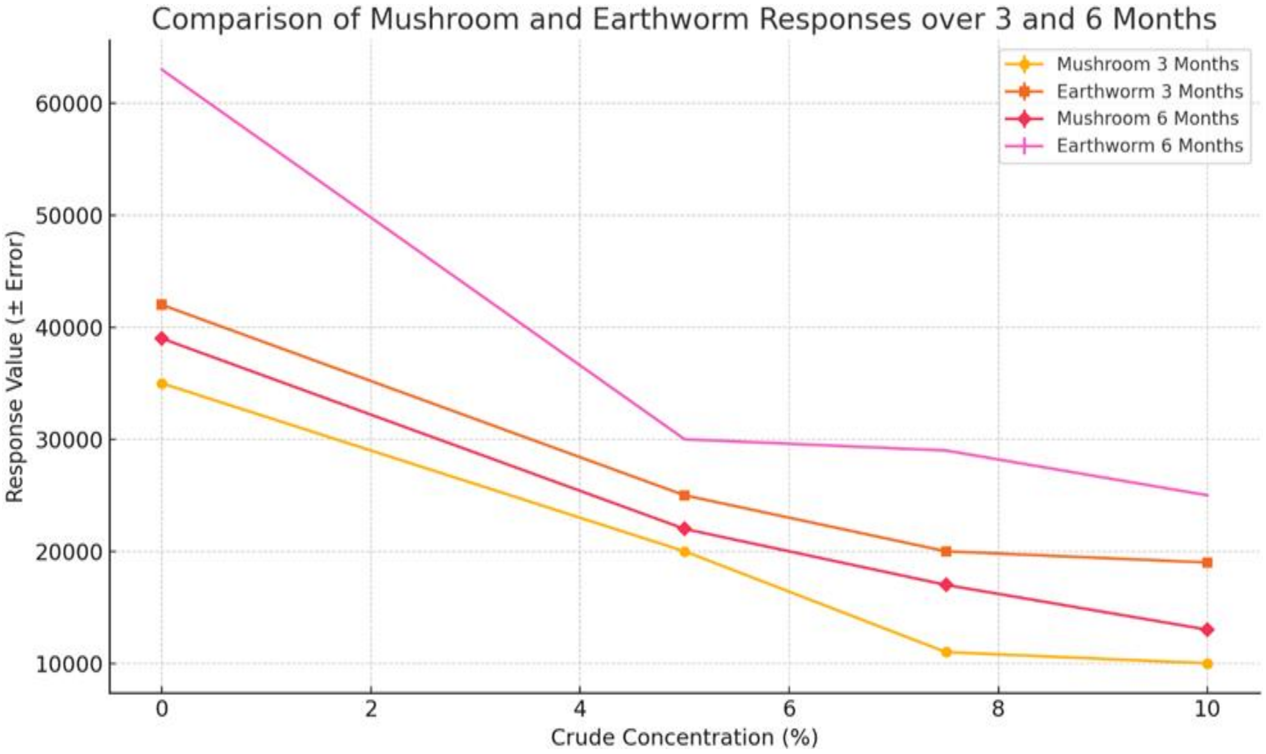

Figure 2 shows the graph that compares the responses of mushrooms and earthworms over 3 and 6 months at different crude concentrations, with error bars included for each dataset.

Table 3 and Figure 3 provide a detailed analysis of the influence of bioremediators-mushrooms and earthworms-on the population of Hydrocarbon-Utilizing Fungi (HUF) across varying crude oil concentrations (0%, 5%, 7.5%, and 10%) over three to six months. The results indicate that at 0% crude oil pollution, mushrooms were more effective in promoting fungal populations, particularly at six months, where a 71.05% increase was observed, compared to a 67.02% increase in earthworm-treated soil. At 5% crude oil concentration, mushroom treatment still resulted in higher HUF populations at three and six months, indicating that mushrooms maintain their effectiveness even in moderately polluted soils. As the crude oil concentration increases to 7.5%, the trend continues with mushrooms, leading to more substantial HUF population increases compared to earthworms. At the six-month mark, the fungi population in mushroom-treated soil rose to 35.05%, compared to 32.04% in earthworm-treated soil. This trend suggests that while both bioremediators are effective, mushrooms outperform earthworms in enhancing HUF populations across different concentrations of crude oil, particularly in highly polluted soils. The more significant increase observed with mushrooms can be attributed to their mycelium network, which can spread more effectively through the soil, breaking down hydrocarbons and promoting fungal growth. These findings have significant implications for bioremediation strategies in oil-contaminated environments. The superior performance of mushrooms, particularly over more extended periods and in more contaminated soils, highlights their potential as a primary agent in fungal bioremediation. However, earthworms also play a crucial role, particularly in environments where organic matter cycling is essential. By fostering fungal growth and breaking down contaminants, both bioremediators contribute to the restoration of soil health. However, mushrooms demonstrate a stronger efficacy in enhancing HUF populations under varying pollution levels. This understanding is critical for optimizing bioremediation approaches and tailoring them to the specific needs of the polluted site.

Table 3 Evaluation and Comparison of the Influence of Bioremediation on the Population of Hydrocarbon Utilizing Fungi (HUF) in Crude Oil Polluted Soil at a Three to Six Month Interval.

		Bioremediators			
Crude conc. (%)	**Polluted Soil**	**Mushroom 3 Month**	**Earthworm 3 Month**	**Mushroom 6 Month**	**Earthworm 6 Month**
0	4002 ± 1.45^{e}	5003 ± 1.73^{c}	4302 ± 1.52^{d}	7105 ± 2.70^{a}	6702 ± 1.52^{b}
5	2902 ± 1.45^{e}	3304 ± 2.08^{c}	3003 ± 2.30^{d}	3803 ± 1.76^{a}	3404 ± 2.30^{b}
7.5	2302 ± 1.45^{e}	2904 ± 2.08^{c}	2603 ± 2.02^{d}	3505 ± 2.88^{a}	3204 ± 2.40^{b}
10	1503 ± 2.08^{e}	2004 ± 2.08^{c}	1803 ± 2.72^{d}	2904 ± 2.40^{a}	2503 ± 2.08^{b}

DMRT shows that the means of various letters differ considerably (P < 0.05).

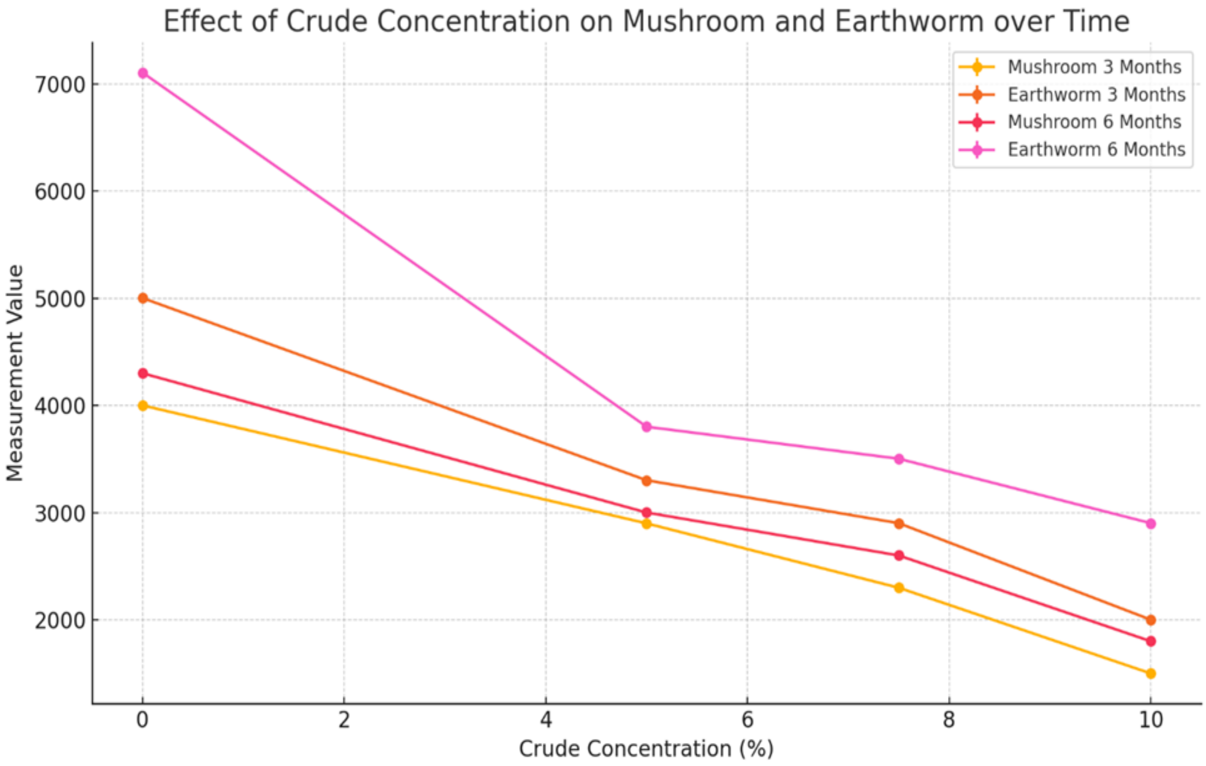

Figure 3 shows the graph that visualizes the effect of crude concentration on mushroom and earthworm measurements over 3 and 6 months. The error bars represent the variability in the measurements, helping to indicate the precision of the values recorded at different crude concentration levels.

4. Discussion

4.1 Comparative Assessment of Mushroom and Earthworm Efficacy in Enhancing Microbial Populations for Bioremediation of Oil-Contaminated Soil over Time

Table 1 provides critical insights into the impact of two bioremediators, mushrooms and earthworms, on microbial populations in soil unaffected by crude oil contamination. Over a period of three and six months, the bioremediators were assessed for their ability to increase Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF) populations. The results suggest that while mushrooms initially promote a rapid rise in HUB populations, earthworms offer a more sustainable increase over time. This dynamic presents intriguing possibilities for environmental remediation, particularly in polluted environments. In comparing these findings with other studies, several parallels and divergences emerge, offering a broader understanding of bioremediation strategies in varying contexts. Several studies, including Obire *et al.* [82], have highlighted the effectiveness of fungi's effectiveness in breaking down crude oil and other pollutants, particularly saprophytic fungi. The present findings reinforce the conclusions of Obire *et al*., demonstrating that mushrooms, a form of fungi, are highly effective at boosting microbial populations, specifically Hydrocarbon Utilizing Fungi (HUF). Mushrooms showed superiority over earthworms in enhancing fungal growth, corroborating the understanding that fungi play a pivotal role in the biodegradation of complex hydrocarbons. This microbial augmentation is vital for ecosystems recovering from oil pollution, where fungi facilitate the breakdown of toxic compounds into less harmful substances. The temporal differences observed in the study, where mushrooms demonstrate rapid efficacy within the first three months, aligns with Muter's [79] observations. Muter identified that bioaugmentation tools like fungi can quickly establish a conducive environment for microbial activity. However, the longevity of this effect may diminish without the introduction of continuous organic material, which could explain why earthworms outperform mushrooms after six months. Earthworms contribute through their ongoing soil processing abilities, continuously providing nutrients that sustain microbial populations over time. This aligns with Muter's hypothesis that bioremediators like earthworms may offer sustained benefits by continuously altering the soil matrix. Also, the long-term efficacy of earthworms is consistent with findings from Praveen and Nagalakshmi [84], who reviewed the role of organisms in bioremediation, emphasizing the importance of nutrient cycling. Earthworms' ability to ingest organic matter and excrete nutrient-rich castings creates a self-sustaining ecosystem that promotes bacterial growth. The study supports this, showing a 55% increase in HUB populations after six months of earthworm treatment.

In contrast, while initially effective, mushrooms may require external nutrient inputs to maintain their bioremediating efficacy over extended periods, suggesting a difference in the mechanismsunderlying their respective success. The findings in Table 1 and Figure 1 show that earthworms significantly enhanced the populations of Hydrocarbon Utilizing Bacteria (HUB) over six months. This is consistent with Omenna *et al.* [85], who emphasized the importance of bacterial populations in breaking down hydrocarbons. Bacteria are crucial for bioremediation because they can degrade various pollutants, including crude oil. By creating favorable soil conditions through their burrowing and organic matter digestion, Earthworms provide an ideal environment for bacteria to thrive. This symbiotic relationship between earthworms and bacteria enhances the overall efficiency of bioremediation. Mushrooms consistently outperformed earthworms in enhancing Hydrocarbon Utilizing Fungi (HUF) populations, a finding that echoes research by Ptaszek *et al.* [86]. Fungi, particularly mushrooms, have specialized enzymes such as lignin peroxidase to break down the complex hydrocarbons in oil-contaminated environments. The ability of mushrooms to enhance HUF populations even in uncontaminated soils suggests they can be leveraged in environments where rapid fungal colonization is needed. This can be especially beneficial in areas where quick fungal activity is required to initiate the breakdown of pollutants. Similarly, the differential effects of mushrooms and earthworms, as shown in the study, suggest that bioremediation strategies should consider the specific goals and timelines of remediation projects. Mushrooms may be more suited for short-term, rapid remediation efforts where immediate microbial activity is necessary to jumpstart the degradation process.

In contrast, with their long-term impact on bacterial populations and soil health, earthworms may be more appropriate for sustained bioremediation projects, particularly in environments requiring ongoing remediation over longer periods. The complementary roles of mushrooms and earthworms in enhancing microbial populations suggest that a combined approach could offer synergistic benefits. This idea is supported by research from Pandolfo *et al.* [58], who found that using multiple bioremediators in tandem can improve outcomes in polluted environments. Earthworms provide long-term benefits through soil structure improvement and continuous nutrient cycling, while mushrooms deliver rapid fungal growth and enzymatic activity. By combining these bioremediators, remediation efforts could achieve faster pollutant breakdown while also ensuring the long-term health and stability of the soil ecosystem. Soil conditions heavily influence the efficacy of both mushrooms and earthworms. In a study by Raimi *et al.* [20], soil health, particularly the availability of organic matter and nutrient levels, was identified as a critical factor affecting bioremediation outcomes. The findings in Table 1 reinforce this, as the bioremediators were applied to soil devoid of crude oil contamination, allowing for more precise observations of their natural effects on microbial populations. In oil oil-contaminated soils, however, the performance of mushrooms and earthworms may differ, with additional challenges posed by toxic hydrocarbons. While the study focuses on soils devoid of contamination, the implications for bioremediation in polluted environments are significant. According to Raimi *et al.* [55], bioremediation, like fungi and earthworms, have shown promise in reducing pollutant levels in contaminated soils. The ability of mushrooms to rapidly enhance fungal populations could be beneficial in environments where quick remediation is needed to mitigate the spread of contaminants. On the other hand, Earthworms could play a key role in the long-term restoration of soil health, ensuring that microbial populations are maintained even after the initial pollutant levels have been reduced. Thus, the study highlights the distinct and complementary roles that mushrooms and earthworms play in enhancing microbial populations in soil. While mushrooms provide rapid, short-term benefits, particularly for fungal populations, earthworms offer long-term improvements in bacterial populations and soil health. These findings align with previous research on bioremediation, supporting the idea that different bioremediation can be tailored to specific remediation timelines and goals. By leveraging the strengths of both mushrooms and earthworms, bioremediation efforts can achieve faster, more sustainable results in restoring polluted environments.

4.2 The Effects of Bioremediators on Microbial Population of Varying Concentrations of Crude Oil-Contaminated Soils

Bioremediation involves the use of living organisms to detoxify polluted environments. Mushrooms (fungi) and earthworms have been recognized as significant bioremediators, especially in hydrocarbon-contaminated soils. In Table 2 and Figure 2, mushrooms exhibited a higher efficacy in increasing the population of HUB than earthworms. Over 6 months, the mushroom-treated soil had a higher bacterial count (63,010 cfu/g) than earthworms (52,007 cfu/g) in non-polluted soils. The trend persisted as crude oil concentration increased (up to 10%). This aligns with the findings of Obire *et al.* [82], who identified fungi as effective in breaking down complex hydrocarbons, which can explain the superior results obtained from mushroom treatment. Meanwhile, earthworms, which enhance soil aeration, also positively impacted, but their contribution was less significant in hydrocarbon degradation than fungi. As the crude oil concentration increased, HUB populations generally declined across both treatments. At 10% pollution, mushrooms still maintained a higher bacterial population than earthworms, but the overall reduction in microbial activity was noticeable. This phenomenon is consistent with the findings of Muter [79], who observed that high concentrations of hydrocarbons tend to inhibit microbial growth due to the toxic nature of petroleum compounds. However, bioremediation such as mushrooms may reduce toxicity by breaking hydrocarbons into less harmful substances, thus sustaining some microbial growth even in more polluted environments. While mushrooms and earthworms contributed to enhancing HUB populations, mushrooms consistently outperformed earthworms, particularly in soils with higher crude oil contamination (7.5% and 10%). According to Pandolfo *et al.* [58], fungi can degrade hydrocarbons, mainly through enzymatic activity that breaks down the complex organic molecules found in crude oil. This enzymatic process is less pronounced in earthworms, whose contribution to bioremediation is primarily through soil aeration and the enhancement of microbial habitat. As such, mushrooms' greater enzymatic capacity explains their superior results. The impact of bioremediators was observed over two different periods (3 months and 6 months). The microbial population increased significantly over time, particularly in mushroom-treated soils. By 6 months, the HUB population in mushroom-treated soils was considerably higher than in earthworm-treated soils at all contamination levels. This observation can be compared to the findings of Olalekan *et al.* [25], who noted that more extended exposure periods allow bioremediators to fully establish themselves in the soil environment, leading to more effective degradation of hydrocarbons over time.

Several studies support the observed trends in the present analysis. For instance, Omenna *et al.* [85] found that bacterial populations tend to decline with increasing crude oil concentration, but bioremediators can mitigate this decline. They also confirmed that fungi (mushrooms) have a greater capacity for hydrocarbon degradation than other bioremediators, like earthworms. Additionally, Obire *et al.* [82] demonstrated that fungi could adapt to various levels of hydrocarbon pollution, further substantiating the findings that mushrooms are more effective bioremediators in polluted soils. HUB plays a crucial role in breaking down hydrocarbons in contaminated soils. The presence of bioremediators like mushrooms and earthworms enhances their population and activity, which is vital for practical bioremediation. Prescott *et al.* [83] emphasized the symbiotic relationship between fungi and bacteria in bioremediation, where fungi break down hydrocarbons into more minor compounds that bacteria can further degrade. This synergy could explain the higher HUB counts in mushroom-treated soils, as observed in Table 2 and Figure 2. Though effective in improving soil structure and aeration, Earthworms are less suited for hydrocarbon-rich environments. According to Praveen and Nagalakshmi [84], earthworms thrive better in environments with lower toxicity levels, and the nature of the contaminants limits their ability to enhance bioremediation. In the present study, earthworms exhibited lower HUB populations at higher crude oil concentrations, suggesting they may be less effective than fungi in highly contaminated soils. The findings in Table 2 and Figure 2, supported by other studies, highlight the potential for using mushrooms as a primary bioremediator in hydrocarbon-contaminated soils. While earthworms contribute to soil health and can aid bioremediation, their impact is less pronounced in the presence of hydrocarbons. This suggests that bioremediation strategies should focus more on fungal treatments for hydrocarbon degradation, particularly in highly polluted environments. Combining both bioremediators could offer a synergistic effect, where mushrooms degrade hydrocarbons, and earthworms improve soil conditions, fostering microbial growth.

4.3 Comparative Analysis of Bioremediators on Hydrocarbon-Utilizing Fungi (HUF) Populations in Crude Oil-Polluted Soils: Insights from Earthworms and Mushrooms

Bioremediation is critical for addressing soil pollution, particularly in oil-contaminated areas. This section discusses the influence of two bioremediators, mushrooms and earthworms, on the Hydrocarbon-Utilizing Fungi (HUF) population under varying crude oil concentrations. The analysis, based on Table 3 and Figure 3, compares the effectiveness of these two organisms at promoting fungal growth over three and six months. In addition, the findings are compared with related studies to draw broader conclusions about the bioremediation potential of these bioremediators in polluted environments. At 0% crude oil pollution, the study found that mushrooms were more effective in promoting the population of Hydrocarbon Utilizing Fungi (HUF) than earthworms, with a 71.05% increase observed at six months, compared to 67.02% in earthworm-treated soil. The difference is likely due to the ability of mushrooms, particularly their mycelium networks, to better colonize soil and provide more favorable conditions for fungal growth. This trend is consistent with previous research by Obire *et al.* [82], who found that fungi associated with organic materials, such as cow dung and poultry droppings, thrived in environments treated with organic amendments. The ability of mushrooms to break down complex organic matter aligns with their success in promoting HUF populations even in the absence of crude oil pollution. At 5% crude oil concentration, mushrooms outperformed earthworms in enhancing HUF populations. The increase in fungal populations observed in mushroom-treated soil demonstrates that mushrooms retain their efficacy despite low to moderate pollution levels. The results suggest that the mycelium of mushrooms not only helps in soil colonization but also aids in breaking down hydrocarbons, an essential function in bioremediation. Studies like those by Muter [79] highlight the role of bioaugmentation tools in addressing pollution, showing that mushrooms, due to their enzymatic properties, promote microbial activity even in polluted soils. The relatively high fungal population in mushroom-treated soil suggests a better adaptation of fungi to the mildly polluted environment. As the crude oil concentration increased to 7.5%, the effectiveness of mushrooms over earthworms remained apparent. The HUF population increased to 35.05% in mushroom-treated soil, compared to 32.04% in earthworm-treated soil. The ability of mushrooms to enhance fungal growth in more heavily polluted environments can be attributed to their enzymatic pathways that degrade hydrocarbons.

Similar results were observed by Odipe *et al.* [71], who examined the response of microbial communities in polluted water systems. The more significant increase in HUF populations under mushroom treatment highlights their superior potential for bioremediation in heavily polluted soils. At the highest crude oil concentration (10%), mushrooms and earthworms showed reduced efficacy in promoting fungal populations, but mushrooms still had a slight edge over earthworms. The increase in the fungal population in mushroom-treated soil suggests that even under high pollution stress, mushrooms maintain a degree of bioremediation efficiency. This observation aligns with findings by Pandolfo *et al.* [58], who reported that specific microbial communities, including fungi, are more resilient to high hydrocarbon concentrations due to their ability to metabolize complex pollutants. The continued performance of mushrooms at higher pollution levels underscores their robustness as bioremediation.

The findings from Table 3 and Figure 3 align with several studies that have explored the role of bioremediators in soil restoration. For instance, Prescott *et al.* [83] emphasized the critical role of fungi in bioremediation, particularly in environments contaminated with hydrocarbons. The superior performance of mushrooms compared to earthworms is consistent with research highlighting fungal organisms' capacity to thrive in polluted environments due to their unique enzymatic mechanisms. Similarly, studies by Olalekan *et al.* [40] also supported the view that bioremediators such as mushrooms significantly enhance microbial activities, promoting faster degradation of pollutants compared to other organisms. Their biological structure and mechanisms can explain the superiority of mushrooms in enhancing HUF populations. The mycelium network facilitates hydrocarbon degradation by producing ligninolytic enzymes that break down complex organic pollutants. This network allows for greater colonization of polluted soils, promoting microbial activity even in challenging conditions. Research by Praveen and Nagalakshmi [84] supports this explanation, showing that fungal organisms are better suited for bioremediation in hydrocarbon-polluted environments due to their ability to produce specialized enzymes. While earthworms were less effective than mushrooms in promoting HUF populations, they still significantly enhanced soil health and microbial activity. Earthworms contribute to bioremediation by breaking down organic matter and improving soil aeration, which can indirectly support fungal growth. Studies like those by Olalekan *et al.* [25] have shown that earthworms, through their burrowing activities, enhance the degradation of pollutants by improving oxygen flow in the soil, creating a more favorable environment for microbial activity. However, their lower performance compared to mushrooms in this study suggests that their role may be more complementary than primary in promoting HUF populations. The results of this study, as outlined in Table 3 and Figure 3, highlight the effectiveness of mushrooms as bioremediators in crude oil-polluted soils. The superior performance of mushrooms in enhancing HUF populations across varying concentrations of crude oil pollution underscores their potential as a primary bioremediation agent, particularly in heavily polluted environments. While earthworms contribute to soil restoration, their role appears to be more secondary than mushrooms. These findings align with broader bioremediation research, suggesting that a combination of bioremediators, with a focus on fungi like mushrooms, may offer the most effective solution for restoring polluted soils.

5. Conclusion

The findings underscore the pivotal role of bioremediation agents, particularly mushrooms and earthworms, in enhancing microbial populations within crude oil-polluted soils, offering significant implications for environmental restoration. This study demonstrates that both agents contribute to stimulating colony-forming units (CFUs) of bacteria and fungi, essential for breaking down hydrocarbons. Notably, mushrooms exhibited superior efficacy in promoting HydrocarbonUtilizing Bacteria (HUB) and HydrocarbonUtilizing Fungi (HUF) over six-month intervals, outperforming earthworms in enhancing microbial activity. These results suggest the potential for mushrooms to be prioritized in environmental restoration efforts due to their ability to accelerate bioremediation processes. The symbiotic interaction between these bioremediators and hydrocarbon-degrading microorganisms further highlights their effectiveness. Earthworms aid soil aeration and nutrient cycling, while mushrooms introduce enzymes that facilitate hydrocarbon breakdown. These biological agents foster a robust and efficient degradation process that sustainably restores polluted soils. This dual approach demonstrates nature's inherent ability to reclaim contaminated environments, reinforcing the value of biological agents in mitigating environmental damage.

As the demand for sustainable solutions to pollution management grows, this research aligns with global efforts to reduce the environmental impact of industrial activities, such as oil exploration. Applying earthworms and mushrooms presents an eco-friendly and cost-effective method of rehabilitating oil-contaminated soils. This low-energy intervention supports soil recovery and long-term ecosystem resilience in areas prone to frequent spills. In conclusion, the study highlights the effectiveness of mushrooms and earthworms as powerful bioremediation agents capable of restoring oil-polluted environments through natural processes. Their ability to stimulate microbial populations and enhance hydrocarbon degradation emphasizes the potential of biological interventions in addressing soil contamination. These findings pave the way for further research into optimizing and scaling these bioremediation techniques for broader environmental restoration projects.

6. Recommendation

In light of the findings from this study, it is recommended that earthworms and mushrooms be integrated into bioremediation practices for crude oil-polluted soils, especially in ecologically sensitive areas. Governmental agencies, environmental bodies, and industries should collaborate to develop frameworks and protocols that facilitate the large-scale use of these bioremediators in contamination hotspots. In particular, regulatory policies should encourage industries to adopt eco-friendly soil restoration technologies, providing incentives for employing bioremediation strategies that leverage natural organisms. There is also a need for further exploration of different species of earthworms and mushrooms to determine which are most effective under varying environmental conditions. This includes conducting long-term field studies to assess the durability and adaptability of these organisms in diverse climates and soil types. Such studies could help fine-tune the species selection and their applications in large-scale remediation projects.

Additionally, stakeholders should invest in research and development to optimize the synergistic effects of these bioremediators with hydrocarbon-degrading fungi. Public-private partnerships could focus on improving soil conditions and environmental health by applying earthworms and mushrooms to post-spill recovery efforts. Research should also investigate potential risks associated with their introduction into non-native ecosystems to avoid unintended consequences. Lastly, educational initiatives should promote awareness about the environmental and economic benefits of bioremediation using natural organisms. Integrating these techniques into environmental management curricula at universities and offering workshops for industry professionals could accelerate the adoption of these sustainable practices.

7. Significance Statement

This study is significant in advancing sustainable and eco-friendly solutions for addressing crude oil contamination in soils, offering crucial insights into the potential of natural organisms as bioremediation agents. The comparative analysis of earthworms and mushrooms highlights their distinct roles in accelerating hydrocarbon breakdown and fostering soil recovery, contributing to global efforts in environmental restoration. Given the ongoing threat of oil spills, the findings provide timely and relevant strategies for mitigating soil contamination and restoring ecological balance in polluted regions. By emphasizing the synergistic interactions between bioremediators and hydrocarbon-degrading fungi, the research reveals the resilience of natural ecosystems in self-repairing from industrial damage. The ability of earthworms to enhance soil aeration and nutrient cycling, combined with mushrooms' enzymatic contributions to hydrocarbon breakdown, underscores the collaborative efficiency of biological agents. These insights pave the way for integrating bioremediation into broader environmental management practices, particularly in regions dependent on petroleum extraction. The practical implications of this study extend to offering a cost-effective, scalable, and environmentally sound alternative to traditional chemical or mechanical remediation methods. Using earthworms and mushrooms in bioremediation provides a low-energy, eco-friendly solution that reduces the environmental footprint of restoration activities. This approach supports the recovery of contaminated ecosystems and aligns with global trends toward green technology in pollution management. Ultimately, the significance of this research lies in its potential to inform future policies and practices that prioritize long-term ecosystem health over short-term industrial gains. By promoting the use of biological interventions, the study challenges conventional remediation methods, advocating for more sustainable, context-specific approaches. It contributes to the growing body of knowledge aimed at safeguarding terrestrial ecosystems, highlighting the need for collective action in addressing the global challenge of soil contamination. Thus, graphically it is represented (Figure 4 below) as

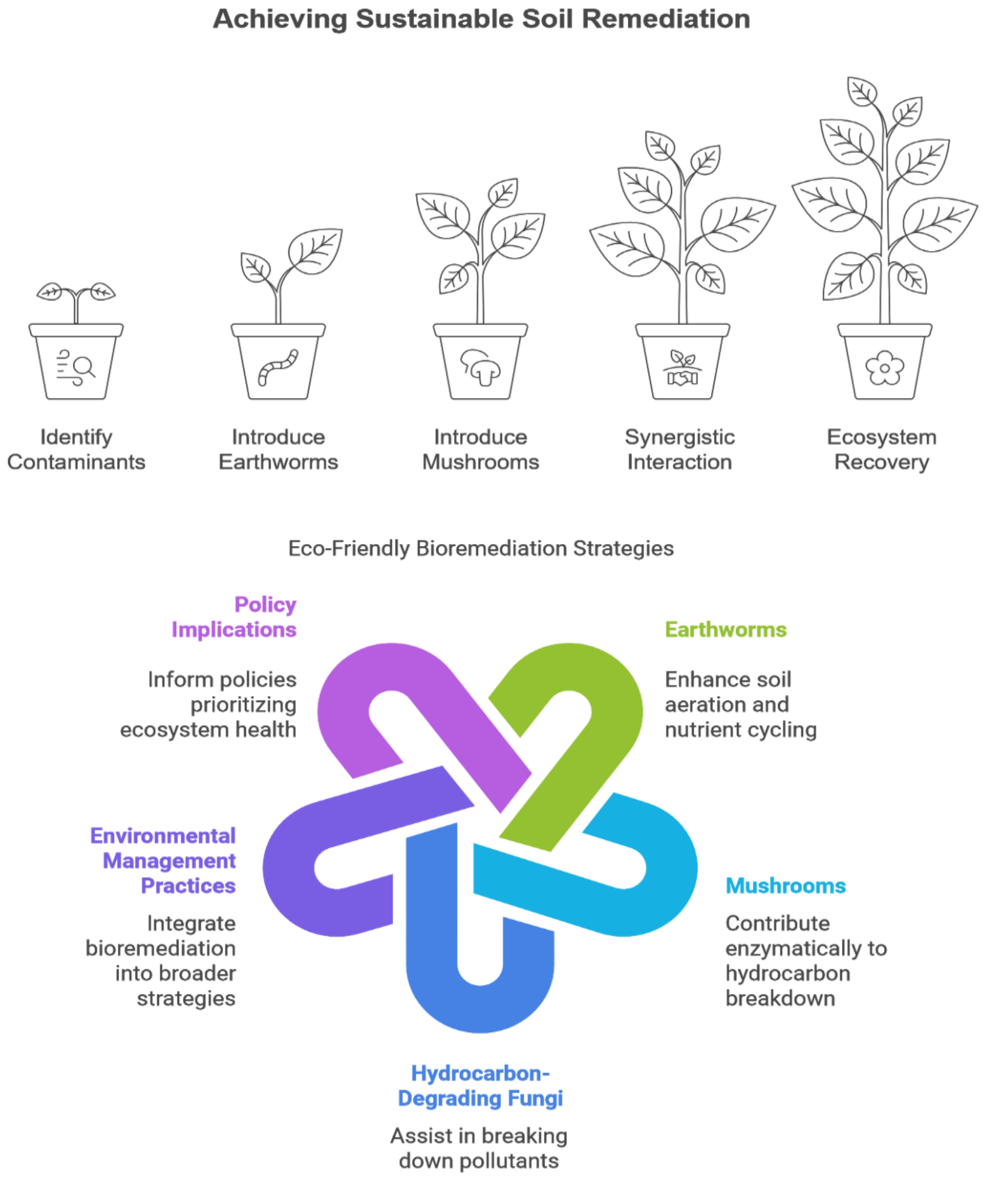

Figure 4 Graphical overview of sustainable and eco-friendly soil bioremediation strategies.

List of Abbreviation

HUB	Hydrocarbon-utilizing bacteria
HUF	Hydrocarbon-utilizing fungi
CFUs	Colonies forming units
SDA	Sabouroud Dextrose Agar

Acknowledgments

The authors would like to express their appreciation to all anonymous reviewers, for feedback and discussions that helped to substantially improve this manuscript.

Author Contributions

All authors contributed equally to conceptualization, validation, writing review and editing.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Competing Interests

The authors declare no conflict of interest.

References

1. Ayibatonyo MN, Ilemi JS, Igoniama EG, Akayinaboderi AE, Morufu OR. Fecundity estimation of Atlantic mudskipper periophthalmus barbarus in Ogbo-okolo mangrove forest of Santa Barbara River, Bayelsa State Niger Delta, Nigeria. bioRxiv. 2024. doi: 10.1101/2024.02.01.578404.
2. Ayibatonyo MN, Bob-Manuel Faye-Ofori G, Morufu OR. Food and feeding of Atlantic mudskipper periophthalmus barbarus in Ogbo-Okolo mangrove forest of Santa Barbara River, Bayelsa State Niger Delta, Nigeria. Qeios. 2024. doi: 10.32388/QNW7VZ.
3. Omoyajowo KO, Raimi MO, Omoyajowo KA, Makengo MB, Adegboyo S, Innocent DC, et al. Towards a reduced pollution society: Systematic review on the role of storytelling, social media, humor and celebrities' influence for research communication. J Appl Sci Environ Manage. 2024; 28: 603-623.
4. Saliu AO, Komolafe OO, Bamidele CO, Raimi MO. The value of biodiversity to sustainable development in Africa. In: Sustainable utilization and conservation of Africa's biological resources and environment. Singapore: Springer; 2023. pp. 269-294.
5. Raheem WB, Fadina OO, Idowu OO, Raimi MO, Austin-Asomeji I. The application of biomaterials in ecological remediation of land pollution: Bioremediation of heavy metals in cement contaminated soil using white-rot fungus pleurotus sajor-caju. 2023. doi: 10.21203/rs.3.rs-2459820/v1.
6. Kader S, Raimi MO, Spalevic V, Iyingiala AA, Bukola RW, Jaufer L, et al. A concise study on essential parameters for the sustainability of Lagoon waters in terms of scientific literature. Turk J Agric For. 2023; 47: 3. doi: 10.55730/1300-011X.3087.
7. Kader S, Raimi MO, Spalevic V, Iyingiala AA, Bukola RW, Jaufer L, et al. A concise study on essential parameters for the sustainability of Lagoon waters in terms of scientific literature. Turk J Agric For. 2023. doi: 10.20944/preprints202303.0099.v1.
8. Raimi MO, Abiola OS, Atoyebi B, Okon GO, Popoola AT, Amuda KA, et al. The challenges and conservation strategies of biodiversity: The role of government and non-governmental organization for action and results on the ground. In: Biodiversity in Africa: Potentials, threats and conservation. Singapore: Springer; 2022. pp. 473-504.
9. Raimi MO, Austin AI, Olawale HS, Abiola OS, Abinotami WE, Ruth EE, et al. Leaving no one behind: Impact of soil pollution on biodiversity in the global south: A global call for action. In: Biodiversity in Africa: Potentials, threats and conservation. Singapore: Springer; 2022. pp. 205-237.
10. Ifeanyichukwu CE, Christian LO, Morufu OR, Iyingiala AA. Hydrocarbon-based contaminants in drinking water sources and shellfish in the Soku oil and gas fields of South-South Nigeria. Open J Yangtze Oil Gas. 2022; 7: 213-230.

11. Raimi MO, Odubo TR. Dutch diseases and resources curse: Key regulatory challenges and opportunities associated with extractive industries in Nigeria. Proceedings of the 8th National Conference on Political Stability, Security and Economic Development, Abuja; 2022 July 21-22; Abuja. Nigeria: University of Abuja.
12. Olalekan MR, Albert O, Iyingiala AA, Sanchez DN, Telu M. An environmental/scientific report into the crude oil spillage incidence in Tein community, Biseni, Bayelsa state Nigeria. J Environ Chem Toxicol. 2022; 6: 01-06.
13. Deinkuro NS, Charles WK, Raimi MO, Nimlang HN. Oil spills in the Niger Delta Region, Nigeria: Environmental fate of toxic volatile organics. 2021. doi: 10.21203/rs.3.rs-654453/v1.
14. Deinkuro NS, Charles WK, Raimi MO, Nimlang HN. Environmental fate of toxic volatile organics from oil spills in the Niger Delta Region, Nigeria. Int J Environ Eng Educ. 2021; 3: 89-101.
15. Okoyen E, Raimi MO, Omidiji AO, Ebuete AW. Governing the environmental impact of dredging: Consequences for marine biodiversity in the niger delta region of Nigeria. Insights Min Sci Technol. 2020; 2: 555586.
16. Olalekan RM, Omidiji AO, Williams EA, Christianah MB, Modupe O. The roles of all tiers of government and development partners in environmental conservation of natural resource: A case study in Nigeria. MOJ Ecol Environ Sci. 2019; 4: 114-121.
17. Ateboh PE, Olalekan RM. Corporate civil liability and compensation regime for environmental pollution in the Niger Delta. Int J Recent Adv Multi Res. 2018; 5: 3870-3893.
18. Raimi MO, Sabinus CE. Influence of organic amendment on microbial activities and growth of pepper cultured on crude oil contaminated Niger delta soil. Int J Econ Energy Environ. 2017; 2: 56-76.
19. Raimi MO, Abiola I, Alima O, Omini DE. Exploring how human activities disturb the balance of biogeochemical cycles: Evidence from the carbon, nitrogen and hydrologic cycles. Res World Agric Econ. 2021; 2: 23-44.
20. Raimi MO, Oyeyemi AS, Mcfubara KG, Richard GT, Austin-Asomeji I, Omidiji AO. Geochemical background and correlation study of ground water quality in ebocha-obrikom of rivers state, Nigeria. Trends Appl Sci Res. 2023; 18: 149-168.
21. Olalekan AS, Adewoye SO, Henry SO, Olaniyi OA, Raimi MO. Comprehensive understanding of hydrogeochemical evaluation of seasonal variability in groundwater quality dynamics in the gold mining areas of Osun state, Nigeria. Int J Hydro. 2023; 7: 206-220.
22. Glory R, Sylvester CI, Morufu OR, Austin AI. Public and environmental health implications of artisanal petroleum refining and risk reduction strategies in the Niger Delta region of Nigeria. J Biol Res Biotechnol. 2023; 20: 1685-1698. doi: 10.4314/br.v20i3.6.
23. Kader S, Jaufer L, Bashir O, Olalekan Raimi M. A comparative study on the stormwater retention of organic waste substrates biochar, sawdust, and wood bark recovered from psidium guajava L. Species. Agric For. 2023; 69: 105-112.
24. Stephen OA, Olayinka AS, Olawale HS, Olalekan RM. Comparative assessment of seasonal variations in the quality of surface water and its associated health hazards in gold mining areas of Osun State, south-west Nigeria. Adv Environ Eng Res. 2023; 4: 011.
25. Olalekan AS, Adewoye SO, Henry SO, Olaniyi OA, Raimi MO. Comprehensive understanding of hydrogeochemical evaluation of seasonal variability in groundwater quality dynamics in the gold mining areas of Osun state, Nigeria. medRxiv. 2022. doi: 10.1101/2022.11.06.22282015.

26. Raimi M, Sawyerr H. Preliminary study of groundwater quality using hierarchical classification approaches for contaminated sites in indigenous communities associated with crude oil exploration facilities in rivers state, Nigeria. Open J Yangtze Oil Gas. 2022; 7: 124-148.
27. Raimi MO, Sawyerr OH, Ezekwe IC, Gabriel S. Toxicants in water: Hydrochemical appraisal of toxic metals concentration and seasonal variation in drinking water quality in oil and gas field area of rivers state, Nigeria. London, UK: IntechOpen; 2022.
28. Raimi O, Ezekwe C, Bowale A, Samson T. Hydrogeochemical and multivariate statistical techniques to trace the sources of ground water contaminants and affecting factors of groundwater pollution in an oil and gas producing wetland in rivers state, Nigeria. Open J Yangtze Gas Oil. 2022; 7: 166-202.
29. Raimi MO, Sawyerr OH, Ezekwe CI, Salako G. Many oil wells, one evil: Comprehensive assessment of toxic metals concentration, seasonal variation and human health risk in drinking water quality in areas surrounding crude oil exploration facilities in rivers state, Nigeria. Int J Hydro. 2022; 6: 23-42.
30. Afolabi AS, Raimi MO. When water turns deadly: Investigating source identification and quality of drinking water in Piwoyi community of fed-eral capital territory, Abuja Nigeria. Online J Chem. 2021; 1: 38-58.
31. Morufu OR, Henry OS, Clinton IE, Gabriel S. Many oil wells, one evil: Potentially toxic metals concentration, seasonal variation and human health risk assessment in drinking water quality in Ebocha-Obrikom Oil and Gas Area of Rivers State, Nigeria. medRxiv. 2021. doi: 10.1101/2021.11.06.21266005.
32. Afolabi AS, Morufu OR. Investigating source identification and quality of drinking water in Piwoyi community of federal capital territory, Abuja Nigeria. Durham: Research Square LLC; 2021.
33. Raimi MO, Clinton IE, Olawale HS. Problematic groundwater contaminants: Impact of surface and ground water quality on the environment in Ebocha-Obrikom oil and gas producing area of Rivers State, Nigeria. Proceedings of the 2nd International E-Conference on Geological and Environmental Sustainability; 2021 July 29-30; London, UK.
34. Raimi OM, Samson TK, Sunday AB, Olalekan AZ, Emmanuel OO, Jide OT. Air of uncertainty from pollution profiteers: Status of ambient air quality of sawmill industry in Ilorin Metropolis, Kwara State, Nigeria. Res J Ecol Environ Sci. 2021; 1: 17-38.
35. Raimi MO, Sawyerr HO, Ezekwe CI, Opasola AO. Quality water not everywhere: Exploratory analysis of water quality across ebocha-obrikom oil and gas flaring area in the core niger delta region of Nigeria. 2021. doi: 10.21203/rs.3.rs-953146/v1.
36. Adedoyin OO, Olalekan RM, Olawale SH, Emmanue OO. A review of environmental, social and health impact assessment (Eshia) practice in Nigeria: A panacea for sustainable development and decision making. MOJ Public Health. 2020; 9: 81-87.
37. Raimi MO, Omidiji AO, Adio ZO. Health impact assessment: A tool to advance the knowledge of policy makers understand sustainable development goals. Proceedings of the Association for Environmental Impact Assessment of Nigeria (AEIAN) on Impact Assessment: A Tool for Achieving the Sustainable Development Goals; 2019 November 7-8; Port Harcourt, Nigeria. Port Harcourt: University of Port Harcourt.
38. Raimi MO, Omidiji AO, Adeolu TA, Odipe OE, Babatunde A. An analysis of bayelsa state water challenges on the rise and its possible solutions. Acta Sci Agric. 2019; 3: 110-125.

39. Raimi MO. 21st Century Emerging Issues in Pollution Control. Proceedings of the 6th Global Summit and Expo on Pollution Control; 2019 May 6-7; Amsterdam, Netherlands.
40. Raimi MO, Adeolu AT, Enabulele CE, Awogbami SO. Assessment of air quality indices and its health impacts in ilorin metropolis, Kwara State, Nigeria. Sci Park J Sci Res Impact. 2018; 4: 060-074.
41. Morufu R, Clinton E. Assessment of trace elements in surface and ground water quality. Mauritius: LAP Lambert Academic Publishing; 2017.
42. Raimi MO, Pigha TK, Ochayi EO. Water-related problems and health conditions in the oil producing communities in central senatorial district of Bayelsa state. Imperial J Interdiscip Res. 2017; 3: 780-809.
43. Raimi MO, Sabinus CE. An assessment of trace elements in surface and ground water quality in the ebocha-obrikom oil and gas producing area of Rivers State, Nigeria. Int J Sci Eng Res. 2017; 8: 583-621.
44. Raimi MO, Ayibatonbira AA, Anu B, Odipe OE, Deinkuro NS. 'Digging deeper' evidence on water crisis and its solution in Nigeria for Bayelsa State: A study of current scenario. Int J Hydro. 2019; 3: 244-257.
45. Raimi OM, Ezekwe CI, Bowale A. Statistical and multivariate techniques to trace the sources of ground water contaminants and affecting factors of groundwater pollution in an oil and gas producing wetland in rivers state, Nigeria. medRxiv. 2021. doi: 10.1101/2021.12.26.21268415.
46. Alaidaroos BA. Advancing eco-sustainable bioremediation for hydrocarbon contaminants: Challenges and solutions. Processes. 2023; 11: 3036.
47. Morufu OR, Ebikapaye O, Tuebi M, Aziba-anyam GR, Adedoyin OO, Aishat FA, et al. Do weak institutions prolong crises?[# endsars] in the light of the challenges and opportunities beyond covid-19 pandemic and the next normal in Nigeria. Commun Soc Media. 2021; 4. doi: 10.22158/csm.v4n2p1.
48. Raimi MO, Ihuoma BA, Esther OU, Abdulraheem AF, Opufou T, Deinkuro NS, et al. Health impact assessment: Expanding public policy tools for promoting sustainable development goals (SDGs) in Nigeria. EC Emerg Med Crit Care. 2020; 95-107. Available from: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3686075.
49. Ajayi FA, Raimi MO, Steve-Awogbami OC, Adeniji AO, Adebayo PA. Policy responses to addressing the issues of environmental health impacts of charcoal factory in Nigeria: Necessity today; essentiality tomorrow. Commun Soc Media. 2020; 3. doi: 10.22158/csm.v3n3p1.
50. Olalekan RM, Oluwatoyin OA, Olawale SH, Emmanuel OO, Olalekan AZ. A critical review of health impact assessment: Towards strengthening the knowledge of decision makers understand sustainable development goals in the Twenty-First Century: Necessity today; essentiality tomorrow. Res Adv Environ Sci. 2020; 1: 72-84.
51. Raimi MO, Oluwatoyin OA, Olalekan A. Health impact assessment: A tool to advance the knowledge of policy makers understand sustainable development goals: A review. ES J Public Health. 2020; 1: 1002.
52. Suleiman RM, Raimi MO, Sawyerr HO. A deep dive into the review of national environmental standards and regulations enforcement agency (NESREA) act. Int Res J Appl Sci. 2019; 1: 108-125.
53. Omidiji AO, Raimi MO. Practitioners perspective of environmental, social and health impact assessment (ESHIA) practice in Nigeria: A vital instrument for sustainable development. Paper

Presented at the Association for Environmental Impact Assessment of Nigeria (AEIAN) On Impact Assessment: A Tool for Achieving the Sustainable Development Goals in Nigeria, 7th and 8th November, 2019 In University of Port Harcourt [Internet]. Abuja, Nigeria: Association for Environmental Impact Assessment of Nigeria; 2019. Available from: https://aeian.org/wp-content/uploads/2019/08/EIA-Presentations-Portharcourt.pdf.

54. Sylvester CI, Odangowei IO, Matthew CO, Saoban SS, Zaharadeen MY, Muhammad A, et al. Historical perspectives and overview of the value of herbal medicine. In: Herbal medicine phytochemistry: Applications and trends. Cham: Springer; 2023. pp. 3-35.
55. Raimi OM, Sawyerr OH, Ezekwe CI, Olaniyi OA. Quality water, not everywhere: Assessing the hydrogeochemistry of water quality across ebocha-obrikom oil and gas flaring area in the core Niger Delta Region of Nigeria. Pollution. 2022; 8: 751-778.
56. Yuniati MD. Bioremediation of petroleum-contaminated soil: A review. IOP Conf Ser Earth Environ Sci. 2018; 118: 012063.
57. Bidja Abena MT, Chen G, Chen Z, Zheng X, Li S, Li T, et al. Microbial diversity changes and enrichment of potential petroleum hydrocarbon degraders in crude oil-, diesel-, and gasoline-contaminated soil. 3 Biotech. 2020; 10: 42.
58. Pandolfo E, Barra Caracciolo A, Rolando L. Recent advances in bacterial degradation of hydrocarbons. Water. 2023; 15: 375.
59. Mekonnen BA, Aragaw TA, Genet MB. Bioremediation of petroleum hydrocarbon contaminated soil: A review on principles, degradation mechanisms, and advancements. Front Environ Sci. 2024; 12: 1354422.
60. Keme-Iderikumo K, Augustus EA, Raimi MO. Making the invisible visible: The effects of gas flaring on artisanal fisheries in the down-stream area of Taylor Creek, Bayelsa State, Nigeria. Qeios. 2024. doi: 10.32388/UIM59Z.
61. Raufu YO, Olayinka AS, Raimi MO, Olawale SH, Olabisi OL. Assessment of occupational risks of waste scavenging in Ilorin metropolis. AfricArXiv. 2023. doi: 10.21428/3b2160cd.ffbb315b.
62. Rauf YO, Raimi MO. Wastes, wastes, everywhere not a place to breathe: Redressing and undressing Ilorin and Yenagoa City. AfricArXiv. 2023. doi: 10.21428/3b2160cd.52bfd7dd.
63. Clinton-Ezekwe I, Osu I, Ezekwe I, Raimi M. Slow death from pollution: Potential health hazards from air quality in the mgbede oil fields of south-south Nigeria. Open Access J Sci. 2022; 5: 61-69.
64. Raimi MO, Samson TK, Ajayi BS, Adio Z, Emmanuel OO, Ogunleye TJ. Air of uncertainty from pollution profiteers: Status of ambient air quality of sawmill industry in Ilorin Metropolis, Kwara State, Nigeria. 2021. doi: 10.21203/rs.3.rs-270757/v1.
65. Raimi MO, Vivien OT, Oluwatoyin OA. Creating the healthiest nation: Climate change and environmental health impacts in Nigeria: A narrative review. Sustain Environ. 2021; 6: 61-122. doi: 10.22158/se.v6n1p61.
66. Raimi MO, Adio Z, Emmanuel OO, Samson TK, Ajayi BS, Ogunleye TJ. Impact of sawmill industry on ambient air quality: A case study of Ilorin Metropolis, Kwara State, Nigeria. Energy Earth Sci. 2020; 3. doi: 10.22158/ees.v3n1p1.
67. Raimi MO, Odipe OE, Anu B, Omini DE, Akpojubaro EH, Owobi OE. Leaving no one behind? Drinking water challenge on the rise in Niger Delta region of Nigeria: A Review. Merit Res J Environ Sci Toxicol. 2020; 6: 031-049.

68. Raimi MO, Bilewu OO, Adio Z, Halimat A. Women contributions to sustainable environments in Nigeria. J Sci Res Allied Sci. 2019; 5: 35-51.
69. Raimi MO, Suleiman RM, Odipe OE, Tolulope SJ, Modupe O, Olalekan AS, et al. Women role in environmental conservation and development in Nigeria. Ecol Conserv Sci. 2019; 1: 555558.
70. Williams EA, Raimi MO, Yarwamara EI, Modupe O. Renewable energy sources for the present and future: An alternative power supply for Nigeria. Energy Earth Sci. 2019; 2. doi: 10.22158/ees.v2n2p18.
71. Odipe OE, Raimi MO, Suleiman F. Assessment of heavy metals in effluent water discharges from textile industry and river water at close proximity: A comparison of two textile industries from Funtua and Zaria, North Western Nigeria. Madridge J Agric Environ Sci. 2018; 1: 1-6.
72. Raimi MO, Nimisngha D, Odipe OE, Olalekan AS. Health risk assessment on heavy metals ingestion through groundwater drinking pathway for residents in an oil and gas producing area of Rivers State, Nigeria. Open J Yangtze Oil Gas. 2018; 3: 191-206.
73. Yusuf OR, Opasola OA, Adewoye SO, Raimi OM and Balogun EM. Assessment of occupational risks of wastes scavenging in Ilorin metropolis. J Agric Earth Environ Sci. 2023; 2: 01-08.
74. Cai P, Ning Z, Liu Y, He Z, Shi J, Niu M. Diagnosing bioremediation of crude oil-contaminated soil and related geochemical processes at the field scale through microbial community and functional genes. Ann Microbiol. 2020; 70: 36.
75. Udume OA, Abu GO, Stanley HO, Vincent-Akpu IF, Momoh Y, Eze MO. Biostimulation of petroleum-contaminated soil using organic and inorganic amendments. Plants. 2022; 12: 431.
76. Tan HW, Pang YL, Lim S, Chong WC. A state-of-the-art of phytoremediation approach for sustainable management of heavy metals recovery. Environ Technol Innov. 2023; 30: 103043.
77. Liu S, Sun R, Cai M, Kong Y, Gao Y, Zhang T, et al. Petroleum spill bioremediation by an indigenous constructed bacterial consortium in marine environments. Ecotoxicol Environ Saf. 2022; 241: 113769.
78. Adeniran MA, Oladunjoye MA, Doro KO. Soil and groundwater contamination by crude oil spillage: A review and implications for remediation projects in Nigeria. Front Environ Sci. 2023; 11: 1137496.
79. Muter O. Current trends in bioaugmentation tools for bioremediation: A critical review of advances and knowledge gaps. Microorganisms. 2023; 11: 710.
80. Cai Z, Li M, Zhu Z, Wang X, Huang Y, Li T, et al. Biological degradation of plastics and microplastics: A recent perspective on associated mechanisms and influencing factors. Microorganisms. 2023; 11: 1661.
81. Mills A, Breuil C, Colwell R. Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Can J Microbiol. 1978; 24: 552-557.
82. Obire O, Anyanwu E, Okigbo R. Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents. J Agric Technol. 2008; 4: 81-89.
83. Prescott LM, Harley JP, Klein DA. Microbiology. 6th ed. New York, NY: McGraw-Hill; 2005.
84. Praveen R, Nagalakshmi R. Review on bioremediation and phytoremediation techniques of heavy metals in contaminated soil from dump site. Mater Today Proc. 2022; 68: 1562-1567.
85. Omenna EC, Omage K, Ezaka E, Azeke MA. Tolerance, taxonomic and phylogenetic studies of some bacterial isolates involved in bioremediation of crude oil polluted soil in the southern region of Nigeria. Heliyon. 2023; 9: e15639.

86. Ptaszek N, Pacwa-Płociniczak M, Noszczyńska M, Płociniczak T. Comparative study on multiway enhanced bio-and phytoremediation of aged petroleum-contaminated soil. Agronomy. 2020; 10: 947.