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Phytoremediation of crude oil contaminated soil using saw dust and grass amendments

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Abstract
Crude oil contamination has affected Nigeria's arable soil, rendering it desolate. This study investigated the remediation of crude oil-contaminated soil using sawdust and grass to identify effective remediation methods. The contaminated soil sample, containing 820g of soil and crude oil mixture, was amended with sawdust, grass, and their combination. In four weeks, all treated samples showed an increase in pH, except for the control, which experienced a decrease. The growth phase was monitored, and the treatment with sawdust and grass resulted in the highest mean height (18.1) and mean leaf length (7.1). The control had the lowest mean height (2.5) and mean leaf length (1.2). The microorganisms present were identified using standard procedures. The bacterium *Bacillus subtilis* and the fungus *Aspergillus flavus* were identified. The initial hydrocarbon-utilizing count increased from (2.10) to (2.62) for *B. subtilis* and from (1.52) to (2.90) for *A. flavus*. This study suggests that sawdust, grass, and their combination can be effective for remediating crude oil-contaminated soil in consortium with indigenous hydrocarbon-utilizing microorganisms. The findings have implications for developing sustainable remediation strategies for crude oil-contaminated soil, potentially benefiting agricultural productivity and environmental sustainability.

Keywords: Crude oil contamination, soil remediation, sawdust and grass, hydrocarbon-utilizing microorganisms, environmental sustainability

Introduction

Soil is a vital natural resource that plays a critical role in sustaining economic and social development, as well as maintaining ecosystem balance. As the foundation for agriculture, forestry, and other land-based industries, soil is essential for food security, water filtration, and carbon sequestration [1].

Nigeria, like many other countries, relies heavily on crude oil, a naturally occurring liquid fossil fuel, for revenue generation and economic growth [1]. However, soil contamination with oil is a significant environmental concern, as it renders the soil unsuitable for agriculture and has detrimental effects on soil microorganisms, plants, and the surrounding ecosystem [1].

The presence of highly toxic polycyclic aromatic hydrocarbons (PAHs) in the oil poses a substantial risk of surface and groundwater pollution, further exacerbating the environmental impact [2]. Oil pollution in soil has a profound impact on the ecosystem, disrupting the delicate balance between the soil, atmosphere, and living organisms. One of the primary effects of oil pollution is the disruption of the normal oxygen exchange between the soil and atmosphere, which is caused by the hydrophobic properties of oil [3].

Phytoremediation, a rapidly advancing technology, is regarded as one of the best available technologies and is recognized as a highly effective and appealing method for remediating contaminated sites [4]. According to Cluis [4], the advantages of phytoremediation include its cost-effectiveness, aesthetic benefits, and long-term applicability, making it an attractive solution for environmental remediation.

According to Yan *et al.* [5], the term phytoremediation encompasses a range of plant-based technologies that utilize either naturally occurring or genetically engineered plants to address environmental concerns. Phytoremediation has been recognized as a viable solution for mitigating heavy metal pollution in the biosphere, which is often caused by industrial activities and chemical spills. As a green technology, phytoremediation offers a sustainable and environmentally friendly approach to cleaning up contaminated sites [6].

Several studies have investigated the use of phytoremediation in remediating oil-contaminated soil. Yi and Crowley [7] discovered that plants release fatty acids, which act as biosurfactants, substantially increasing the degradation of pyrene and benzo[a]pyrene when applied directly to contaminated soil. According to Greg *et al.* [8], the selection of plant species and soil amendments is crucial for effective phytoremediation of oil-contaminated soil.

The primary research objective, as noted by Greg *et al.* [8], is to identify suitable plant species and management techniques to remediate oil-polluted soil. Uwazie *et al.* [9] investigated the use of phytoremediation in the remediation of soils contaminated with petroleum hydrocarbons and organics.

Sawdust, a byproduct of wood processing, has been explored as a potential soil amendment for phytoremediation Kayode *et al.* [10]. According to Kayode *et al.* [10], sawdust can be repurposed as a valuable carbon source for wastewater treatment, promoting a cleaner environment and sustainable waste management. This study aims to investigate the remediation of crude oil-contaminated soil using sawdust and grass amendments.

Materials and methods

Sample Collection and Preparation

Soil samples were obtained from the agricultural farm of Enugu State University of Science and Technology, while crude oil was sourced from Eleme Petrochemical Company in Rivers State, Nigeria. Sawdust was collected from the Timber Market in Trans Ekulu, Enugu State, and lemon grass samples were randomly gathered from the agricultural farm of Enugu State University of Science and Technology.

Soil samples were carefully collected at a depth of 0-15 cm using a hand-dug soil auger and transferred into clean, sterile containers. The samples were then transported to the

laboratory in storage containers for analysis. Prior to analysis, large debris and unwanted particles were removed from the collected soil samples. The contaminated sawdust, grass, and soil were removed, as done by Rita *et al.* [1].

Preparation of Crude Oil-Polluted Soil

A modified method based on Stanley *et al.* [11] was employed to create crude oil-polluted soil. Specifically, 120g of crude oil was added to 700g of soil samples, and the contents were thoroughly mixed to obtain a composite mixture. This process was performed in triplicate, including a control sample, to ensure reliable results.

Determination of pH Before and After the Degradation of the Crude Oil Polluted Soil

Oludele *et al.* [12] and Awari *et al.* [13] method was employed for the determination of pH before and after degradation. The pH of the oil-contaminated soil and organic wastes was determined using a pH meter. Ten grams of samples was weighed into a 100ml beaker and 25 ml of distilled water was added. The suspension was shaken with the aid of a mechanical shaker for 25-30 min, then allowed to stand for 50 min and stirred occasionally with a glass rod. The electrode was rinsed with water and dried by dabbing with a piece of tissue. The electrode was inserted into the partly settled suspension to be analyzed and the pH range of the solution was measured. The pH meter was standardized at pH 7.0.

Amendment of Crude Oil-Polluted Soil with Sawdust and Grass

A modified method of Rita *et al.* [1] was applied to amend crude oil-polluted soil. The amendment treatments consisted of four different combinations: 1 kg crude oil-contaminated soil + 120 g sawdust, 1 kg crude oil-contaminated soil + 120 g grass, 1 kg crude oil-contaminated soil + 60 g sawdust + 60 g grass, and 1 kg crude oil-contaminated soil (control). Rice seeds were planted in all the samples to assess the effectiveness of the amendments.

Measurement of Leaf Growth

The length and height of leaves from plants grown in amended soil with sawdust, grass, and a combination of sawdust and grass were monitored and recorded daily over a period of 15 days.

Biochemical Characterization of Bacteria

The biochemical tests were performed according to Nwadioha *et al* [14]

Catalase Test

Emulsify the test organism with 1-2 drops of hydrogen peroxide on a glass slide. Observe for bubbling of air, indicating oxygen gas production and catalase positivity.

Coagulase Test

Make a dense suspension of the organism using 1-2 drops of distilled water on a glass slide. Observe for auto-agglutination. Mix with a loopful of EDTA plasma and observe for clumping or agglutination.

Oxidase Test

Add 2-3 drops of oxidase reagent to a filter paper. Smear a colony of the test organism on it using a piece of glass rod. Observe for a dark purple color within 30 seconds, indicating a positive result.

Indole Test

Inoculate a bijou bottle containing peptone water with the test organism. Incubate at 35-37°C for 24-48 hours. Add 0.5mls of Kovac's reagent. Shake gently and examine for a red color on the surface. A pink to red ring color indicates a positive test, while no color change indicates a negative test.

Gram Staining Technique

The Gram staining procedure involves preparing a thin smear of the bacterial sample on a slide, allowing it to air dry completely, and then heat-fixing the smear by passing the slide 3-4 times through the flame of a Bunsen burner. The smear is then covered with crystal violet primary stain for 45-60 seconds, rinsed with water, covered with Lugol's iodine mordant for 45-60 seconds, rinsed again, decolorized with acetone for 1-3 seconds, rinsed, counterstained with safranin for 1-2 minutes, rinsed, and finally air dried before being examined under a microscope using a 40x objective lens [14].

Fungal Identification and Characterization

The sub-cultured fungi were identified based on their cultural and morphological characteristics, including mycelia, spore type, and fruiting bodies. A lactophenol cotton blue wet mount was prepared and examined under a compound microscope at 40x magnification. The observed structures were compared to a fungal atlas for identification. Additionally, Candida albicans was specifically identified using the germ tube test [15, 16].

Table 1: Composition Ratio of Crude Oil Spill Mixture

Sample	Crude Oil Ratio	Soil Ratio	Soil Type
A	120g	700g	Loamy
B	120g	700g	Loamy
C	120g	700g	Loamy
D	120g Control	700g Control	Loamy

Table 2: Amendment Combinations and Ratios for Crude Oil – Contaminated Soil

Sample	Crude Oil Contaminated Soil	Amendments
A	820g Crude Oil and Soil	120g Sawdust
B	820g Crude Oil and Soil	120g Grass
C	820g Crude Oil and Soil	60g Sawdust, 60 Grass
D	820g Crude Oil and Soil	NIL

Table 3: pH Values of Crude Oil Contaminated Soil Amended with Different MaterialsBefore and After Four Weeks

Week	Treatment	Initial PH	Final PH
1	A (Sawdust)	6.2	6.9
2	B (Grass)	6.3	6.7
3	C (Sawdust and Grass)	6.1	7.2
4	D (Control)	6.5	6.4

Table 4: Measurements of Growth Phase of Amended Soil and Control in Four Weeks

Week	Treatment	Mean Height (cm)	Mean Leaf Length (cm)
1	A (Sawdust)	15.2	6.2
2	B (Grass)	12.5	5.5
3	C (Sawdust and Grass)	18.1	7.1
4	D (Control)	2.5	1.2

Table 5: Cultural and Biochemical Identification of Bacteria from Sawdust and Grass Treatment

Growth Appearance	Gram Reaction	Indole	Catalase	Citrate	Wagulate	Oxidase	Suspecter Ornaisation
Milkished, Roughed and Opaque	+	+	—	—	—	+	*Bacilcus subtilis*

Key: +Positive - Negative

Table 6: Cultural and Microscopic Identification of Fungi from Sawdust and Grass Treatment

Cultural Characteristics	Microscopy	Suspected Organism
Consist of Dense Felt Yellow Conidiophores	Conidiophores are Hygiene and Warse Vescile Ore Glucose	*Asperigilus flavus*

Table 7: Enumeration of Hydrocarbon Degrading Bacterial and Fungal counts in Amended Soil with Sawdust and Grass after Four Weeks

Hydrocarbon Utilizing Bacterium Count (CFU)	Hydrocarbon Utilizing Fungal Count (CFU)
Initial Count 2.10 Final Count 2.62	Initial Count 1.52 Final Count 2.90

Discussion

Table 1 showed the composition ratio of 120g of crude oil and 700g of soil, indicating a loamy soil composition, which is consistent with the findings of Rita *et al.* (2023), who investigated the effects of 1kg of crude oil-contaminated soil amended with varying weights of cow dung, sawdust, and poultry manure.

Table 2 presents the various amended combinations, revealing that samples A to D comprised 820g of crude oil-contaminated soil, with differing proportions of sawdust and grass amendments, including 120g of sawdust-grass, 60g of sawdust, and 60g of grass, while the control sample remained unamended. These findings are consistent with the research of Rita *et al.* [1] and Obemeata *et al.* [17], who similarly investigated the effects of various amendments on engine oil contaminated soil.

Table 3 presents the pH values of the samples before and after the experiment. Initially, all samples exhibited slightly acid pH levels. The lowest initial pH values were recorded for the samples treated with sawdust and grass (6.1), sawdust alone (6.2), grass alone (6.3), and the control sample (6.5). Following the experiment, the final pH values of the samples ranged from neutral to slightly alkaline, with the highest pH observed for the sawdust and grass treatment (7.2) and the lowest pH for the control sample (6.4). These findings are consistent with previous studies by Onuorah *et al.* [18] and Rita *et al.* [1], which reported changes in pH levels upon addition of microorganisms and amendments to crude oil-contaminated soil and media. Similarly, Obemeata *et al.* [17] observed changes in pH levels, with mean pH ranges varying in pre-exposed soil, two weeks after pollution, and four weeks after remediation.

Table 4 indicates that the treatment combining sawdust and grass resulted in the highest mean height (18.1) and mean leaf length (7.1), while the control sample had the lowest measurements (2.5 and 1.2, respectively). The superior performance of the sawdust-grass treatment may be attributed to the synergistic effects of these materials in biodegrading and bioaccumulating total petroleum hydrocarbons and polyaromatic hydrocarbons. This study's findings align with previous research by Uwazie [9], who used lemon grass to remediate crude oil-contaminated soil. Additionally, the study by Oshiotse *et al.* [19] on the potential uptake of heavy metals by Pteridium aquilinum plants is consistent with the present study's results. Furthermore, the research by Greg [8] on plant species growing on petroleum-contaminated sites also supports the current study's findings.

Another study by Nnamdi and Suripino [20] demonstrated the effectiveness of composting crude oil-contaminated soil using sawdust as a bulking agent, achieving 93% degradation of total petroleum hydrocarbons after twenty two weeks. A study by Kayode *et al.* [21] found that sawdust-based adsorbents can effectively remove various pollutants, including endocrine-disrupting chemicals, pesticides, dyes, and heavy metals, with varying adsorption capacities. The study explored the adsorption mechanisms, strategies for improvement, and potential engineering applications of these sawdust-based adsorbents.

Tables 5 and 6 identified the bacterial and fungal species present in the amended soil with sawdust and grass, which exhibited the highest growth phase. Bacillus subtilis, a Gram-positive bacterium, was identified through cultural and biochemical tests. Aspergillus flavus, a fungus, was identified through cultural and microscopic examination. The presence of these microorganisms, in combination with sawdust and grass, facilitated rapid growth improvement. In contrast, the unamended control (D) showed decreased growth, likely due to the presence of hydrocarbon-utilizing bacteria and fungi, which can break down hydrocarbons with or without external amendments, albeit with lower growth yields. These findings are consistent with Aneke [22], who reported on the proliferation rate of Aspergillus flavus in soil, and Rita *et al.* [1], who identified Bacillus subtilis and Aspergillus flavus in spent engine oil-contaminated soil.

Table 7 presents the initial and final counts of hydrocarbon-utilizing bacteria and fungi after four weeks of amendment with sawdust and grass. The initial count of Bacillus subtilis was 2.10, increasing to 2.60, while the initial count of hydrocarbon-utilizing fungi was 1.52, rising to 2.90. These findings are consistent with Rita *et al.* [1], who reported increases in the initial and final counts of Bacillus subtilis and Aspergillus flavus using sawdust as an amendment. Additionally, Obemeata *et al.* [17] observed a similar trend, with bacterial counts increasing from 1.4 × 10^3 to 21.07 × 10^3 at two weeks after pollution, and further increasing to 1.67 × 10^3 to 80.13 × 10^3 at four weeks after remediation, following biostimulation with sawdust and cow blood on spent engine oil-polluted soil.

Conclusion

This study showed the effectiveness of using sawdust and grass to remediate crude oil-polluted loamy soil. The results showed that amendments with these materials led to increased plant growth, with the combination of sawdust

and grass yielding the highest growth rate. This suggests that sawdust and grass can play a crucial role in cleaning up petroleum hydrocarbon contamination soil.

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