Review Article

BIOREMIDATION AND PHYTOREMDIATION OF PETROLEUM CONTAMINATED SOIL

Abstract

The remediation of petroleum-contaminated soil is a critical environmental challenge that requires sustainable and effective solutions. This study explores the combined implementation of bioremediation and phytoremediation techniques as promising approaches for addressing this issue. Bioremediation harnesses the natural degradation capabilities of microorganisms, while phytoremediation employs specialized plants to extract and detoxify contaminants. The purpose of this study is to evaluate the feasibility, efficiency, and ecological implications of these methods, through systematic experimentation, it was observed that both bioremediation and phytoremediation effectively reduce petroleum contaminants in soil. Bioremediation benefits from a diverse microbial community that breaks down complex hydrocarbons, while phytoremediation showcases the potential of certain plant species to extract and mitigate pollutants. The success of both approaches is influenced by factors such as temperature, moisture levels, plant selection, and microbial activity. Both bioremediation and phytoremediation have

advantages and limitations, making them complementary techniques. Their success depends on site-specific factors such as soil properties, contaminant types, and climate conditions. Research continues to refine these methods, combining them with cutting-edge technologies like genetic engineering and nanoremediation.

Keywords

Petroleum, Contaminated soil, Bioremediation, Phytoremediation. Bacteria

1. Introduction

The introduction discusses phytoremediation, a form of bioremediation that utilizes biological entities such as plants, microbes, and enzymes to remove pollutants. When we refer to remediation, we're talking about the use of these biological agents to clean up or eliminate pollutants. These biological entities can break down pollutants through mineralization (complete removal) or transform them into less toxic forms (biotransformation). Many bacteria and fungi are capable of this process.

Phytoremediation specifically focuses on the use of plants to remove or remediate pollutants. Plants can enhance the bioavailability of certain pollutants and store them in their shoots or leaves. Pollutants are either stored in an inactive state within plant cells or released into the atmosphere through volatilization. Later, plant parts can be harvested to remove the trapped pollutants. In bioremediation, microorganisms like bacteria, archaea, and fungi play a key role.

2.0 MATERIALS

4 Plastic Container: The plastic container used weighs 88.7kg it was used to plant the okra and the mixing of the bacteria and the soil together.

Sand (loamy soil): Loamy soil is best for planting which is mostly of sand, silt and a smaller amount of clay. By weight, its mineral composition is about 40-20% concentration of sand –silt-clay, respectively and also it has that perfect balance—it holds moisture but also drains well, allows oxygen to reach plants roots, and is rich in humus (organic matter). And the soil used in this project was gotten from the farm outside the school premises along umuoma village.

Petroleum (1 Liter): The Petroleum product used in this project was gotten a fuel station along the school road umuoma village.

Okra: The okra used in this research work was obtained from market (Onitsha main market (ose).

Bacteria: The bacteria used in this research was obtained in the microbiology department lab, Anambra state university, uli.

Glove: Glove used was to wear in other to collect soil sample for analysis.

Foil: The foil used was for the collection of soil sample.

Meter rule: Mete rule used in this project was used to determine the height of the plant.

3.6 PHYTOREMEDIATION SETUP

The seeds of Okra were purchased from Onitsha main market (ose). These seeds were already sun dried to make the plant grow fast. Top-loamy soil used in the study was collected from the village of umuoma along the university road, within a depth of 5 cm while the crude oil type “Bonny light, the petrol oil was obtained from a commercial petrol station along the university road umuoma, Uli Anambra State, Nigeria... The planting containers with a uniform weight of 2.5 kg each were arranged in the lab. The groups were labeled as treatment CS (control), CSB, CSP and SP (control) and replicated 3 threes times. All the treatments and the control planting container received 20 ml of water for three consecutive days at 7:00 GMT. Following this, three okra seeds were planted in each planting container, both in the treatment and the control experiment. Daily watering continued at 7:00 GMT. The emergence of seedlings in the treatments and the control experiment was observed and recorded.

The seedling emergence percentage in each treatment was calculated using the formula:
Emergency percentage (E %) = (Number of seedlings that emerged / Total number of seeds sown) * 100

Additionally, the co-efficient of velocity (COV) of germination in each treatment was determined using the formula:
Co-efficient of velocity (COV) = (A1 + A2 + … + A3) / (A1*T1 + A2*T2 + … + A5*T5), where A1, A2, … A3 represent the number of seedlings at different times, and T1, T2, … T5 represent the respective time intervals.
Where A is the number of seeds germinating and T is the number of days taken to germinate and records was taken for 4 weeks after planting (4WAP) has been done in each treatment. The seeding height was taken with the use of meter rule and leaf length and width was also taken and recorded and constantly after a new leaf surfaces and reaches the length of 2 cm and width of 2cm it will start to decay and fall off.

3.7 SAMPLE COLLECTION

The composite soil sample was collected with a sterile hand trowel into sterile plastic buckets which were cleaned with cotton wool soaked in 70 % ethanol to ensure that aseptic conditions are met during sampling as described by Eziuzor and Okpokwasili (2009). The soil was collected from four sampling points after excavation, which was then transported to Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University for preliminary physicochemical analysis and bioremediation study. The petrol oil was obtained from a commercial petrol station along the university road umuoma, Uli Anambra State, Nigeria

3.8 SOURCE OF BACTERIAL INOCULUM

Two microbes were isolated from oil contaminated soil of mechanic workshops in Uli but applied to petrol contaminated for the degradation experiment. These were *Pseudomonas* sp. 3B and Serratia marcescens 9B were collected from the Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, and Uli Campus.

To select microbes for the preparation of bacterial consortium, the hydrocarbon degrading capacity of isolated strains was determined. The hydrocarbon-degrading efficiency of isolated strains was screened on Bushnell Haas medium with DCPIP indicator by using hydrocarbons as the sole source of energy. The bacterial strains capable of degrading petroleum hydrocarbons were selected for the preparation of the consortium. Physicochemical and microbiological analyses were carried out before bioremediation study Sattar et al. (2022).

3.9 PREPARATION OF THE BACTERIAL CONSORTIUM

To create the bacterial consortium, we followed the procedure outlined by Sattar et al. (2022). First, we selected fresh colonies (24-hour-old colonies) from each bacterial strain intended for the consortium. These colonies were then introduced into flasks containing sterile nutrient broth. The flasks were sealed with cotton plugs to prevent contamination during incubation. All flasks were placed in a shaking incubator and incubated at 150 rpm for a duration of 7 days. We monitored the growth of the bacterial strains every 24 hours.

After incubation, the nutrient broth media containing a fresh single bacterial colony (selected from the isolated strains) were centrifuged at 5000 rpm for 10 minutes. The liquid supernatant was removed, leaving behind bacterial pellets. Each pellet was separately re-suspended in autoclaved distilled water, and the optical density (O.D) was adjusted to 1 at 660 nm using a UV-VIS spectrophotometer (Spectrum Lab 752N, China).

The final consortium was prepared by mixing pure cultures of bacterial strains in equal volumes, each having an O.D of 1 at 660 nm and a bacterial density of 106 cells per milliliter (106 cells/mL).

3.9.1 SOIL CONTAMINATION AND BASELINE STUDY

About 250 mL of petrol was poured into the 5 L plastic bucket containing 2500 g of soil. The polluted soil at this point was sampled for baseline studies. Baseline study is the analysis of current situation to identify the starting point for a project. It is also use to determine the level of impact expected and to enable the monitory of impacts after the development has occurred (Ezekoye *et al*. 2015).

3.9.2 STUDY DESIGN

The study was experimentally designed by adopting the method of Eziuzor and Okpokwasili, (2009) and the details are shown in Table 1 below:

Table 1: Bioremediation design of the study

Experimental setup	Test experiment
Setup 1 (control)	2500 g of soil + 250 mL of petrol + 20 mL of water
Setup 2	2500 g of soil + 250 mL of petrol +30 mL of bacterial consortium + 20 mL of water
Setup 3	2500 g of soil + 250 mL of petrol + three seeds of Okra plant + 20 mL of water
Step 4 (control)	2500 g of soil + two seed of Okra plant + 20ml of water

3.9.3 BIOREMEDIATION EXPERIMENT

These was carried out *ex situ* in the Microbiology Laboratory (COOU). Two thousand, five hundred grams (2500 g) of soil was mixed with 250 mL of petrol and was prepared in 3 setups using 5 L plastic buckets and were left in the laboratory for 6 days. After contamination, 30 mL\ of bacterial consortium and three seeds of Okra plant were added to the petrol polluted soil and the control was not amended either of the additives and it was called zero hour as described by Ezekoye *et al*. (2015). The samples containing nutrients and control were regularly turned using different sterile spatula as well as moistened with 20 mL of sterile distilled water every 2 weeks. Samples were taken for laboratory analysis at 1 week intervals on the 1st, 7th, 14th, 21st and 28th days (Romanus *et al.*, 2015). The bioremediation of petrol oil in the different experimental setups were studied as described below:

3.9.4 Physicochemical analysis

3.9.5 Physical analysis

3.9.6 P^H and Conductivity Determination:

The p^H and conductivity were measured using digital millimeter (DSS – 11A, China) by adopting the standard method of AOAC (2012). The pH, conductivity and temperature of the samples withdrawn at baseline day 1st, 7th, 14th, 21st and 28th day of the study were determined. At each point, three values were obtained and mean of the value were used.

3.9.7 Chemical analysis

3.9.8 Nitrate Determination:

The Brucine method stated by UNEP (2004) was employed for the measurement of nitrate content at 470 nm on spectrophotometer (Astell, UV - Vis Grating, 752 W). One millilitre of soil filtrate was measured into a clean test tube and 1 mL of distilled water was measured into another test tube as blank solution. Half millilitre of Brucine reagent was gently introduced into both test tubes after which 2 mL of concentrated sulphuric acid was then added and shaken to homogenize. The resulting solution was allowed to cool to room temperature until the solution turned yellow and was measured at 470nm on 752 N UV – VIS spectrophotometer.

3.9.9 Phosphate Determination

Phosphate content was estimated using a colorimetric method following the guidelines outlined by UNEP in 2004. The measurements were taken spectrophotometrically at 660 nm and compared to identically prepared water standards. To extract the phosphate, a solution of one-tenth of 2.5% glacial acetic acid was prepared and used. It was mixed with 2 grams of the sample in a 100 mL conical flask and stirred for 10 minutes. Subsequently, a 50 mL sample extract was taken and autoclaved with K2S2O8 and H2SO4 for 30 minutes at 121 ºC. After autoclaving, five milliliters of ammonium molybdate was added to the mixture to form a heteropoly molybdophosphoric acid. This was further reduced with stannous chloride in an aqueous sulfuric acid medium at 30 ºC, resulting in the formation of a molybdenum blue complex. The intensity of the resulting blue color was measured spectrophotometrically against a prepared water standard. This method has a detection limit of 0.01 mg/L.

3.9.9.1 TOTAL ORGANIC CARBON DETERMINATION

For the determination of total organic carbon (TOC), we followed the colorimetric method established by Nelson and Sommers in 1975. One gram of the sample was carefully placed in a clean Pyrex conical flask, and 5 mL of potassium chromate solution and 7.5 mL of concentrated sulfuric acid were added. The mixture was then heated on an electrothermal heater for 15 minutes to reflux. Afterward, the sample was allowed to cool to room temperature and then diluted to a final volume of 100 mL using distilled water.

Subsequently, 20 milliliters of the sample solution were titrated with 0.2 M ferrous ammonium sulfate, utilizing Ferroin as an indicator. A blank solution, containing oxidant (potassium chromate) and sulfuric acid, was titrated against the sample, and the titration value was recorded. The calculation followed a specific procedure for TOC determination.

$$\%TOC = \frac{\text{Titre value of blank} - \text{Sample titre} \times 0.003 \times 100}{\text{Sample weight}}$$

The total organic matter (TOM) was determined thus: % TOM = % TOC X 1.724

3.9.9.2 TOTAL PETROLEUM HYDROCARBON (TPH) CONTENT DETERMINATION

The determination of total petroleum hydrocarbons (TPH) was conducted following the spectrophotometric method developed by Akpoveti et al. in 2011. This analysis was performed at a wavelength of 640 nm using N-Hexane as the extractive solvent. Initially, one gram of the soil sample was dissolved in 10 mL of hexane and agitated for ten minutes using a mechanical shaker. Subsequently, the solution was filtered through Whatman’s filter paper, and the filtrate was then diluted by taking 1 mL of the extract and adding it to 50 mL of hexane.

The absorbance of this diluted solution was measured at 460 nm using a UV-Vis spectrophotometer (model 752N), with n-hexane serving as the blank reference. Total petroleum hydrocarbon levels were determined at two-week intervals over a span of 56 days.

3.9.9.3 Quality control: To maintain quality control, we prepared procedural blanks and standard solutions, which were integrated to guarantee the precision and consistency of the results. We conducted replicate analyses during the determination of TPH to obtain an average value that will be used to assess accuracy.

4.0 RESULT AND DISCUSSION

4.1 PHYTOREMEDIATION RESULTS

The results of this experiment show marked different between the plants grown in crude oil polluted soil and non-crude oil-polluted soil. Abelmoschus esculentus planted in soil treated with oil started germinating after 6 days compared to 4 days for plants in untreated soil (control).

Table 2. Result of phytoremediation

Treatment	E %	COV
(Concentration ml)		
1	100	
2	100	
3	66	
4	33	

Table 2 illustrates the impact of crude oil on the emergence rate (e %) and co-efficient of velocity (COV) of Abelmoschus esculentus seedlings. A 100% emergence rate was observed in uncontaminated soil. Significant differences were noted between Abelmoschus esculentus plants in crude oil-polluted and uncontaminated soil at a 5% probability level. As the oil concentration increased, both the seedling emergence rate (e %) and COV decreased. Additionally, it was observed that the leaves of Abelmoschus esculentus turned yellow, some leaves fell, growth was stunted, and some plants in crude oil-treated soil died within four weeks of planting, while others remained alive.

Table 3 Displays the impact of varying levels of crude oil on the growth of Abelmoschus esculentus seedlings. Significant distinctions were observed between plants cultivated in uncontaminated soil and those in soil with similar crude oil concentrations. According to statistical analysis, the plants in uncontaminated soil outperformed those in oil-polluted soil regardless of the oil concentration. However, the plants’ performance in oil-polluted soil depended on the concentration. When compared statistically, plants in uncontaminated soil fared better than those in soil contaminated with 250 ml of crude oil. Consequently, the performance of plants in crude oil-polluted soil is contingent on the concentration.

Table 3. Impact of varying levels of crude oil on the growth of Abelmoschus esculentus seedlings

Treatment	Seedling heights	Seedling heights
Weeks	Contaminated soil + okra	Not-contaminated soil
0	0	0
1	2 cm	4.73cm
2	2.5cm	8.54
3	3.5cm	16.5
4	4.5 cm	32.3

4.2 BASE LINE FEATURES OF DIESEL IMPACTED SOIL

The baseline physicochemical and microbiological properties of the diesel-impacted soil are summarized in Table 4. According to the results, the following parameters were observed: pH 7.11, conductivity 0.57 mS/cm, temperature 16.50 °C, nitrate 12.09 µg/kg, phosphate 3.90 mg/kg, total organic carbon 6.72%, total organic matter 740.44 mg/kg, and total residual oil content 740.44 mg/kg.

Table 4: Base line physicochemical properties of petrol impacted soil

Parameter	Value
Ph	7.11
Conductivity (mS/ cm)	0.57
Nitrate (NO_3) (µg/ kg)	16.50
Phosphate (PO_4) (mg/ kg)	12.09
Total organic carbon (% TOC)	3.90
Total organic matter (% TOM)	6.72

Total residual oil content (mg/ kg)	740.44

4.3 BIOREMEDIATION PROFILE

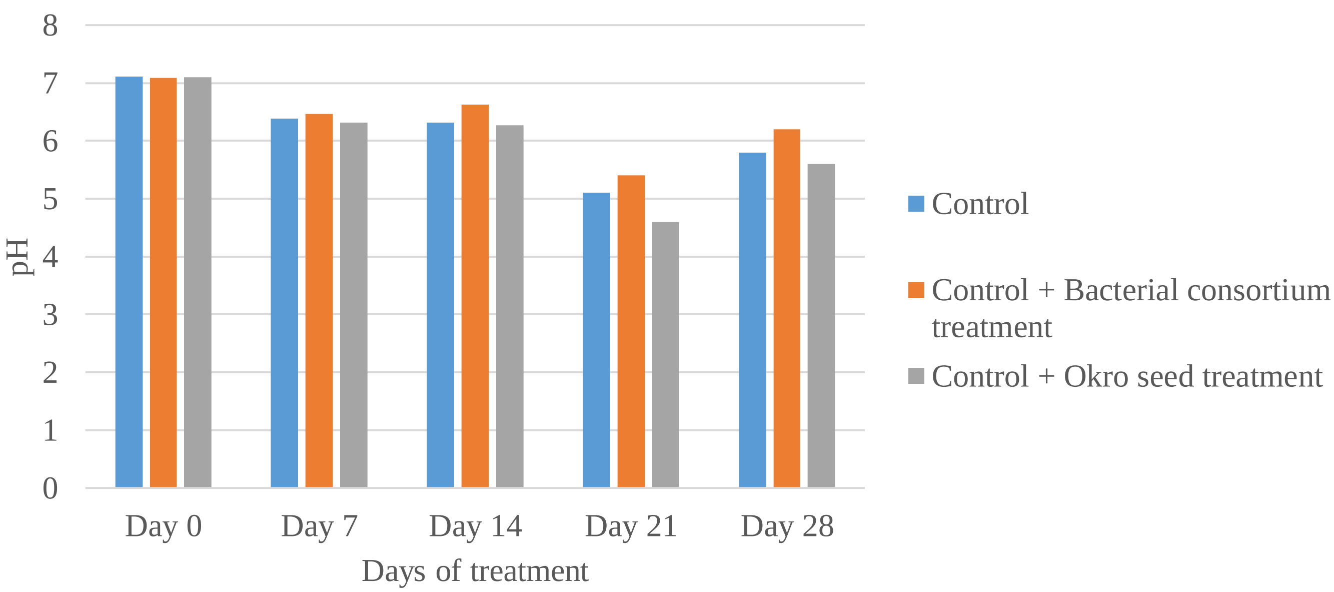

Figure 1: pH variations during bioremediation treatment of petrol contaminated soil

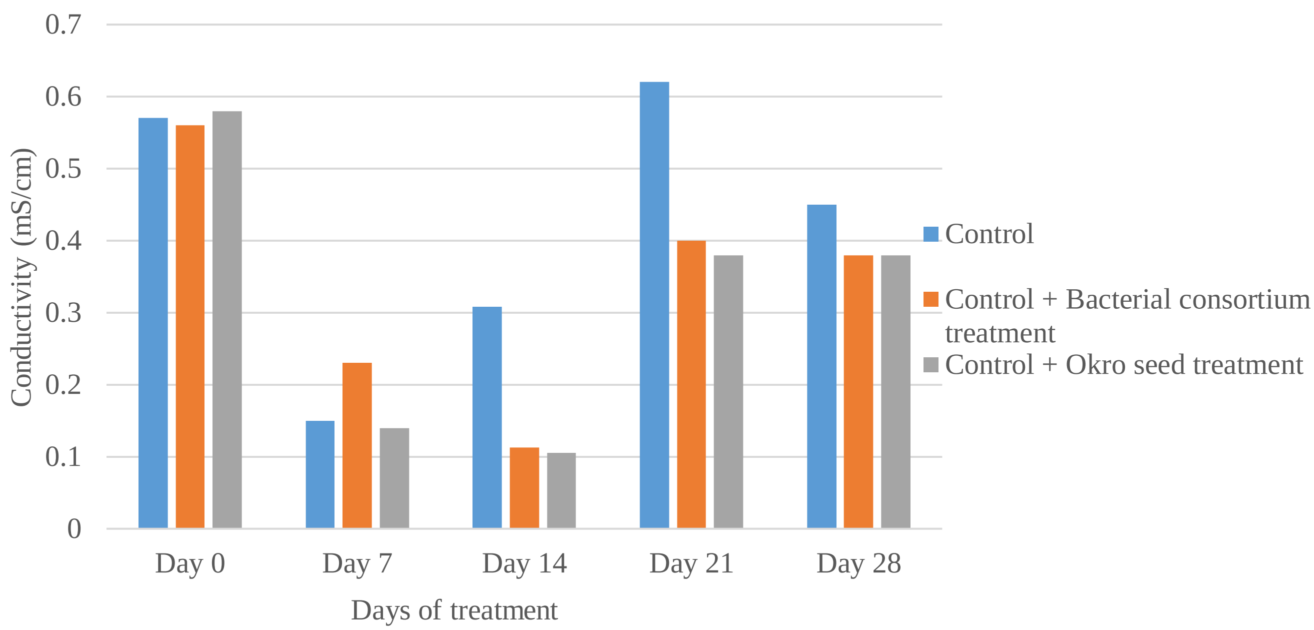

Figure 2: Conductivity variations during bioremediation treatment of petrol contaminated soil

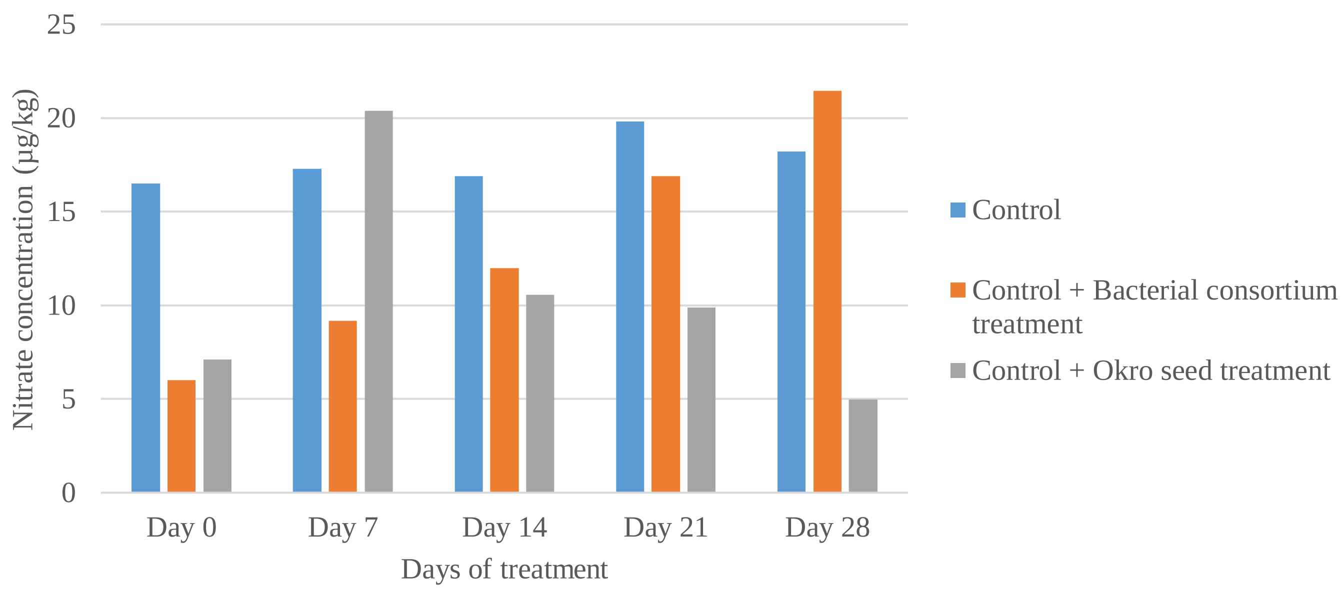

Figure 3: Nitrate variations during bioremediation treatment of petrol contaminated soil

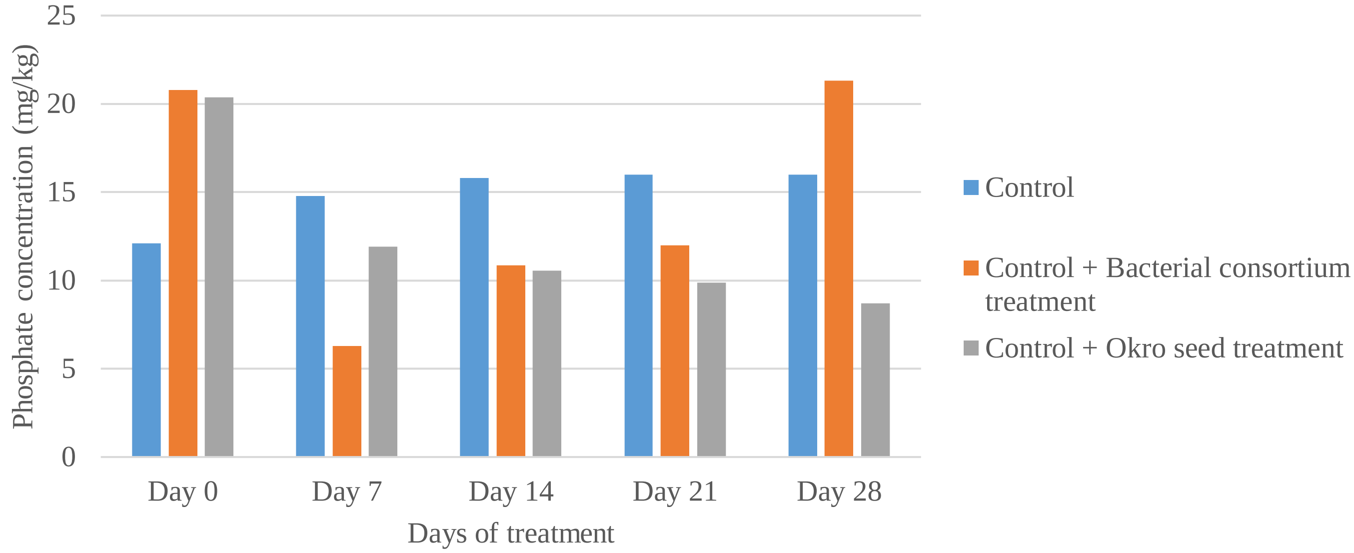

Figure 4: Phosphate variations during bioremediation treatment of petrol contaminated soil

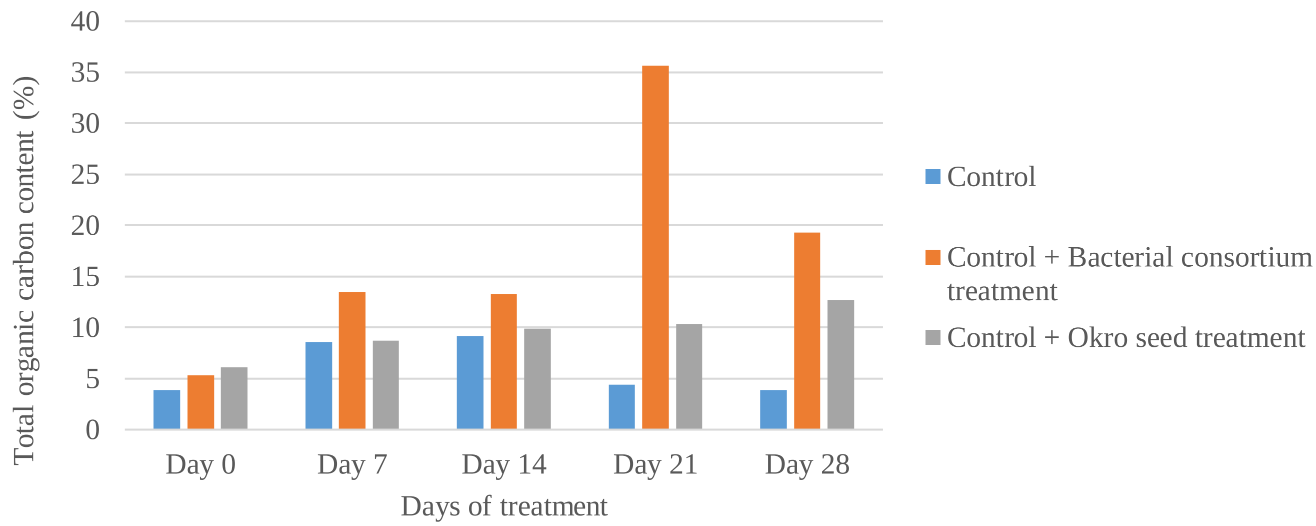

Figure 5: Total organic carbon variations during bioremediation treatment of petrol contaminated soil

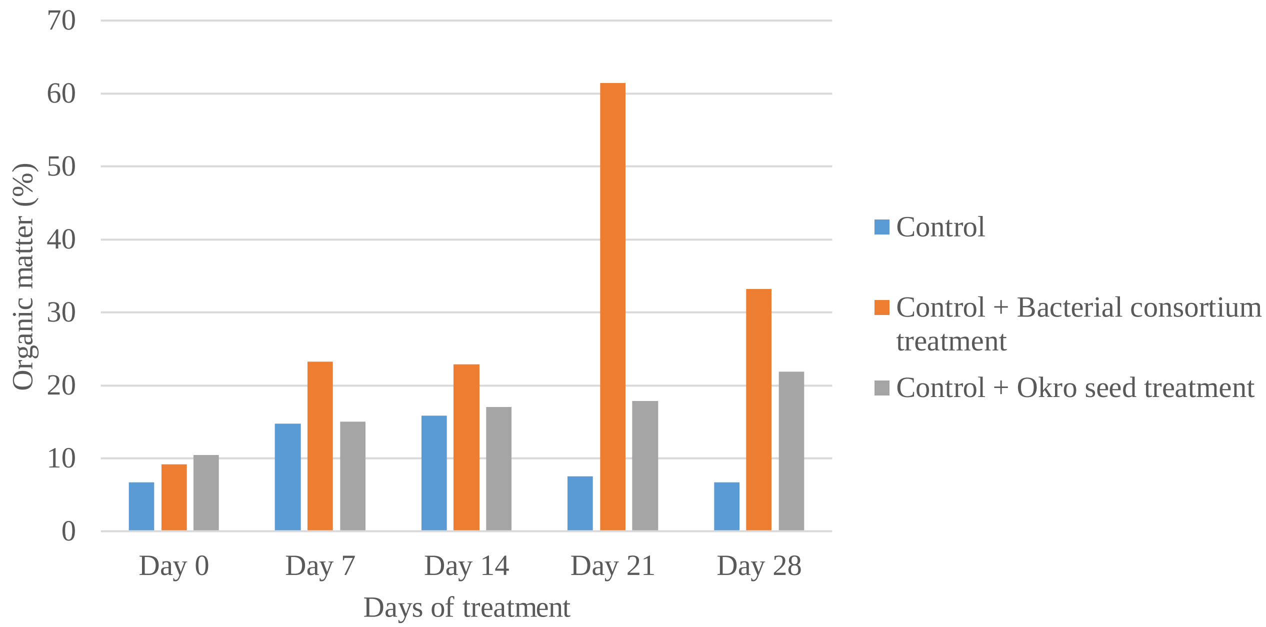

Figure 6: Organic matter variations during bioremediation treatment of petrol contaminated soil

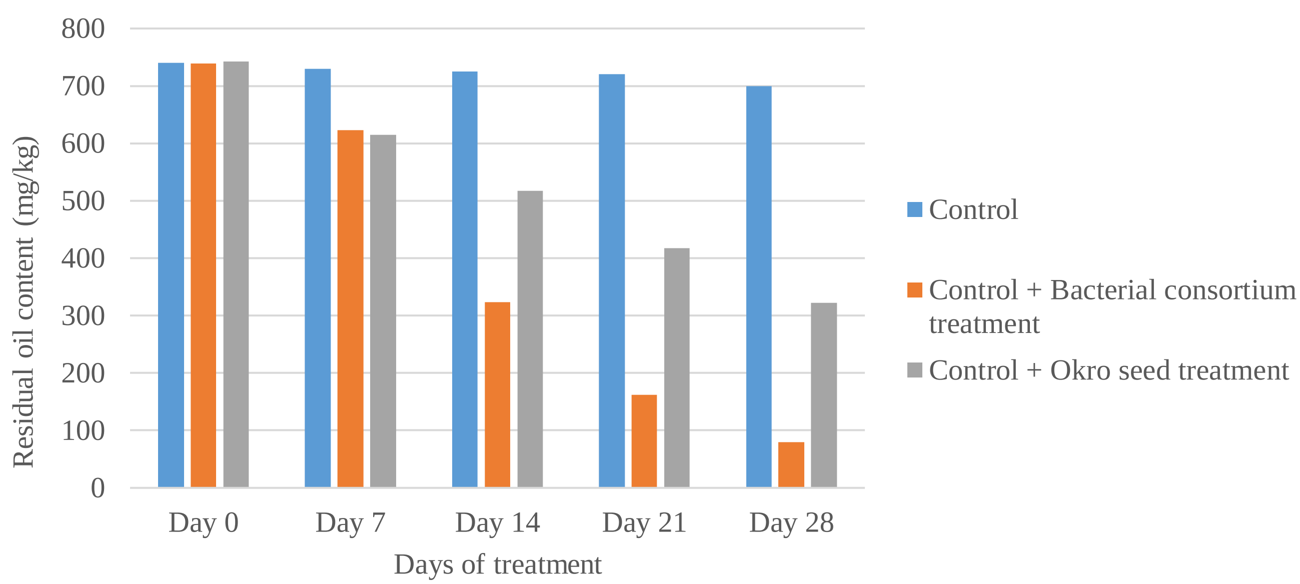

Figure 7: Residual oil content variations during bioremediation treatment of petrol contaminated soil

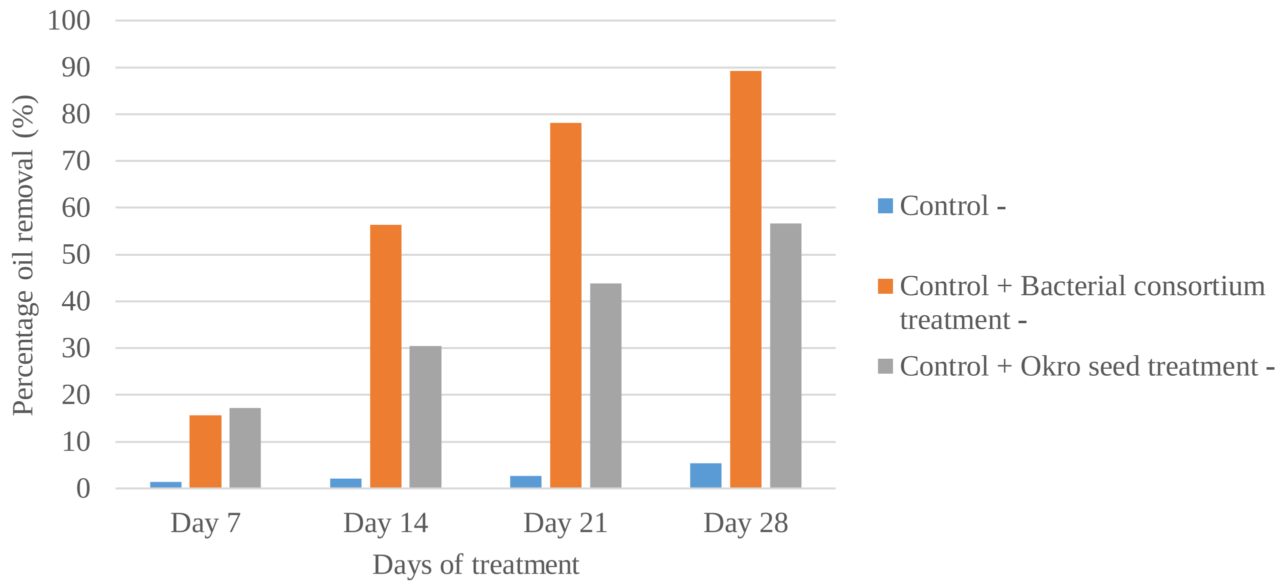

Figure 8: Percentage oil removal during bioremediation treatment of petrol contaminated soil

V CONCLUSIONS

In conclusion, the study experiment on bioremediation and phytoremediation of petroleum contaminated soil has shed light on the effectiveness and potential of these environmentally friendly remediation methods. Both bioremediation and phytoremediation have demonstrated their capabilities in reducing petroleum contaminants in soil, contributing to the restoration of ecological balance. The results from this study highlighted the importance of considering various factors, such as soil conditions, plant selection, and microbial activity, when implementing these methods. Bioremediation, which harnesses the natural degradation capabilities of microorganisms, offers a promising approach for breaking down complex hydrocarbons into less harmful compounds. Phytoremediation, utilizing specialized plants to extract and detoxify contaminants, showcases its ability to mitigate petroleum contamination while promoting plant growth and ecosystem recovery.

Through systematic experimentation, it has become evident that the success of both methods is influenced by factors like temperature, moisture levels, and initial contamination levels. The optimization of these conditions plays a pivotal role in achieving efficient and sustainable remediation outcomes. This study not only adds to the body of knowledge surrounding bioremediation and phytoremediation but also provides valuable insights for environmental practitioners and policymakers. The practical recommendations generated from this research can guide the selection and application of remediation strategies in real-world scenarios, ensuring effective pollution management while minimizing negative impacts on soil health and the ecosystem.

VII RECOMMENDATION

Recommendations derived from this study emphasize the importance of site-specific assessments, optimization of environmental conditions, plant-microbe interactions, and long term monitoring. Integrating microbial inoculation into the rhizosphere of plants enhances the synergistic effects of bioremediation and phytoremediation. Native plant species, diverse planting strategies, and a balanced nutrient management plan contribute to the overall success of the combined methods. Public awareness and community engagement are crucial aspects of promoting sustainable petroleum-contaminated soil remediation. The findings from this study provide valuable insights for environmental practitioners, policymakers, and stakeholders involved in pollution management. By adopting a holistic approach that combines bioremediation and phytoremediation, contaminated sites can be restored while minimizing ecological impact, contributing to a cleaner and healthier environment.

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