



# **Bioremediation and Phytoremediation of Petroleum Contaminated Soil**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Remediating soil contaminated by petroleum is a significant environmental problem that calls for long-term and practical solutions. In order to solve this problem, this study investigates the potential benefits of combining phytoremediation with bioremediation procedures. Whereas phytoremediation uses specialized plants to collect and detoxify pollutants, bioremediation makes use of microorganisms' inherent ability to degrade materials. Aim of the search is to assess the viability, effectiveness, and ecological consequences of these techniques. Systematic experiments revealed that petroleum pollutants in soil can be efficiently reduced by both phytoremediation and bioremediation. While phytoremediation demonstrates the potential of specific plant species to collect and mitigate contaminants, bioremediation benefits from a broad microbial community that breaks down complex hydrocarbons. A few variables that affect the effectiveness of both strategies are temperature, moisture levels, plant selection, and microbial activity. The use of bioremediation

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and phytoremediation both have benefits and drawbacks, which renders them complimentary methods. Site- specific elements including soil characteristics, pollutant kinds, and weather patterns affect its performance. These techniques are being improved upon by research, which also incorporates cutting-edge technology like genetic engineering and nanoremediation.

**Keywords:** Contaminated soil; petroleum; bacteria; phytoremediation; bioremediation.

## 1. INTRODUCTION

Phytoremediation, a form of bioremediation uses biological agents like bacteria, enzymes, and plants to remove contaminants, is covered in the introduction. The term "remediation" refers to the process of cleaning up or getting rid of contaminants using these biological agents. These living things have the ability to mineralize (completely remove) contaminants or bio transform (change them into less hazardous forms) them [1-5]. This is a procedure that many bacteria and fungus are capable of. Specifically, the utilization of plants to eliminate or repair contaminants is the subject of phytoremediation [6-11]. Certain contaminants can be stored in the shoots or leaves of plants, increasing their bioavailability [12-17]. Pollutants are either discharged into the atmosphere by volatilization or retained in an inactive condition within plant cells [18-22]. Plant portions can then be harvested to take out the contaminants that have been trapped. Microorganisms such as fungi, bacteria, and archaea are important in bioremediation [23-29].

## 2. MATERIALS

**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, which consists primarily of sand, silt, and a lesser quantity of clay, is ideal for planting. Its mineral makeup is composed of roughly 40–20% sand, silt, and clay by weight. It also has the ideal balance, holding moisture while allowing oxygen to reach plant roots and being high in humus (organic matter). Additionally, the soil utilized in this project came from a farm along Umuoma Village, which is located beyond the school's grounds.

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

### 2.1 Setup for Phytoremediation

We bought the okra seeds from the major market in Onitsha (ose). These seeds have previously been sun- dried to promote rapid plant growth. The study's top-loamy soil was taken from the village of Umuoma along the university road at a depth of five centimeters, and the petroleum oil, known as "Bonny light," came from a commercial station located along the university road in Umuoma, Uli Anambra State, Nigeria. In the laboratory, planting containers weighing a consistent 2.5 kg apiece were set up. The groups underwent three replications, with the labels "treatment CS (control), CSB, CSP, and SP (control)". For three consecutive days, 20 milliliters of water were applied to each treatment and the control planting container days at seven o'clock GMT. In both the treatment and control experiments, three okra seeds were then sown in each planting container. At 7:00 GMT, daily watering continued. It was noted and observed when the seedlings in the treatments and the control experiment emerged. The following formula was used to determine the percentage of seedling emergence in each treatment:

$$* 100 * \left( \frac{\text{Total number of seedlings that surfaced}}{\text{Total number of seeds sowed}} \right) = \text{Emergence proportion (E\%)}$$

Furthermore, the following formula was used to find the germination velocity co-efficient (cov) for each treatment:

The formula for the coefficient of velocity (COV) is as follows:  $COV = (A1 + A2 + \dots + A3) / (A1 \cdot T1 + A2 \cdot T2 + \dots + A5 \cdot T5)$ , where A1, A2, ..., and A3 stand for the number of seedlings at various times, and T1, T2, ..., T5 stand for the corresponding time intervals.

Where T is the number of days it took in order for seeds to grow and A is the number of seeds that germinated; records were kept for four weeks following planting (4WAP) for each treatment. Using the meter rule, the planting height was measured, and the breadth and length of the leaf were also taken, noted, and continuously when a new leaf emerges and reaches a length of 2 cm and a width of 2 cm, it begins to deteriorate and fall off.

## 2.2 Sample Collection

To guarantee that when sampling, aseptic conditions are satisfied as stated by Eziuzor and Okpokwasili (2009), that the composite soil sample was gathered using a sterilized hand trowel and sterile plastic buckets that was been cleaned with a cotton wool that was soaked at 70% ethanol. Following excavation, the soil used was collected from 4 different locations and then sent to Chukwuemeka Odumegwu Ojukwu University's Microbiology Laboratory for a preliminary physicochemical examination and bioremediation investigation. The fuel was purchased from a for-profit gas station located on University Road in Umuoma, Uli, Anambra State, Nigeria.

## 2.3 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 Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Department of Microbiology.

In order to choose bacteria for the creation of a bacterial consortium, the capacity for separated strains to breakdown hydrocarbons was ascertained. The effectiveness of isolated hydrocarbon-degrading strains were screened utilizing hydrocarbons as the only energy source on Bushnell Haas medium with DCPIP indicator. The bacterial strains chosen for the consortium's

preparation were those that could break down petroleum hydrocarbons. Prior to the bioremediation study, physicochemical and microbiological studies were conducted. The Sattar group (2022)

## 2.4 Bacterial Consortium Preparation

We used the process described by Sattar et al. (2022) to form the bacterial consortium.

Initially, we chose new colonies (those that were only 24 hours old) for every bacterial strain that was going to be part of the consortium. Following that, these colonies were placed into flasks filled with nutritional broth that was sterile.

Cotton plugs were used to seal the flasks during incubation to avoid contamination. For seven days, the whole flasks used were all incubated at 150 rpm in a shaking incubator. Every 24 hours, we tracked the bacterial strains' development. Following incubation, a fresh single bacterial colony (chosen from the isolated strains) was placed in the nutrient broth media, and the mixture was centrifuged for 10 minutes at 5000 rpm. After removal of the liquid supernatant, bacterial pellets were still present. A UVVIS spectrophotometer (Spectrum Lab 752N, China) was used to adjust the optical density (O.D.) of each pellet after it had been individually re-suspended in autoclaved distilled water to a value of 1 and Pure cultures of bacterial strains with comparable volumes and an optical density of 1 at 660 nm and 106 cells per milliliter (106 cells/mL) were combined to create the final consortium.

### 2.4.1 Baseline study and soil contamination

The 5 L plastic bucket holding the 2500g of soil was filled with about 250 mL of gasoline. During this stage, samples of the contaminated soil were taken for research purposes. A baseline research looks at the current situation to establish the starting point of a project. It also helps with impact monitoring after development and identifies the expected impact level (Ezekoye et al. 2015).

### 2.4.2 Study design

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

**Table 1. Experimental design of bioremediation**

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

### 2.4.3 Bioremediation experiment

These was carried out ex situ in the Microbiology Laboratory (COOU). Two thousand, five hundred grams (2500 g) of the soil was mixed with 250 mL of petrol and also 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). Every time, a new sterile spatula was used to turn the samples carrying nutrients and control, and 20 milliliters of sterile distilled water was used to wet them 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 throughout the various experimental setups were studied as described below:

#### 2.4.3.1 Physicochemical analysis

##### Physical Analysis:

**pH And Conductivity Determination:** The pH and conductivity were measured using digital millimeter (DSS – 11A, China) by adopting the standard method of AOAC (2012). The study's pH, conductivity, and temperature were measured in the samples that were taken on baseline days 1, 7, 14, 21, and 28. For every position, three values were acquired, and the average of the values was utilized.

##### Examining Chemicals:

**Nitrate Determination:** Using a spectrophotometer (Astell, UV - Vis Grating, 752 W), the nitrate level was measured at 470 nm using the Brucine method as described by UNEP (2004) and also one milliliter of a clean test tube

was put into soil filtrate, and also another test tube was filled with one milliliter of distilled water as a blank solution. Both test tubes were first filled with a half milliliter of Brucine reagent. Next, two milliliters of concentrated sulfuric acid were added, and the mixture was agitated to ensure homogenization. The resultant solution was measured at 470 nm using a 752 N UV-VIS spectrophotometer after being allowed to cool to ambient temperature until it turned yellow.

### 2.5 Determination of Phosphate

Using a colorimetric approach, the phosphate content was calculated in accordance with UNEP criteria from 2004. At 660 nm, spectrophotometric measurements were made, and the results were compared to water standards that had been generated in the same way. A solution of one-tenth of 2.5% glacial acetic acid was made and used to extract the phosphate. In a 100 mL conical flask, it was combined with 2 grams of the sample and swirled for 10 minutes. A 50 mL sample extract was then obtained and autoclaved for 30 minutes at 121 °C using K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and H<sub>2</sub>SO<sub>4</sub>. Five milliliters of ammonium molybdate were added to the mixture during autoclaving in order to create heteropoly molybdophosphoric acid. At 30 °C, stannous chloride was used to further decrease this in an aqueous sulfuric acid media causing the creation of a molybdenum blue complex at 30 °C, A prepared water standard was used to assess the blue color's intensity using spectrophotometry. This method's detection limits is 0.01mg/L.

### 2.6 Total Organic Carbon Determination

The total organic carbon (TOC) was determined using the colorimetric method developed by Nelson and Sommers in 1975. A neat Pyrex conical flask was filled with one gram of the sample, five milliliters of potassium chromate solution, and seven and a half milliliters of strong sulfuric acid. After that, the mixture was heated

for 15 minutes on an electromagnetic heater to cause reflux. After allowing the sample to reach room temperature, distilled water was used to dilute it to a final volume of 100 mL. Next, using ferroin as an indicator, after then, the sample solution was titrated with 0.2 M ferrous ammonium sulfate in 20 milliliters. Potassium chloride, an oxidant, and sulfuric acid were combined in a blank solution, which was titrated against the sample A value log was kept. The computation adhered to a particular process for determining TOC.

$$\%TOC = \frac{\text{Sample titre} \times 0.003 \times 100}{\text{Blank titre} \times \text{Sample mass}}$$

The formula used to calculate total organic matter (TOM) was  $\% TOM = \% TOC \times 1.724$ .

### 2.6.1 Determination of total petroleum hydrocarbon (Tph) Content:

The total petroleum hydrocarbons (TPH) was measured using the spectrophotometric technique that was created by Akpoveti et al. in 2011. N-Hexane was used as the extractive solvent in this examination, which was carried out at a wavelength of 640 nm. First, a mechanical shaker was used to agitate one gram of the soil sample for ten minutes after it had been dissolved in ten milliliters of hexane. The mixture was then filtered through Whatman's filter paper, and the filtrate was gently diluted by mixing extract a single milliliter from the with fifty milliliters of hexane. Using n-hexane as the blank reference, the absorbance of this diluted solution was measured at 460 nm using a UV-Vis spectrophotometer (model 752N). The total amounts of petroleum hydrocarbons were Throughout a period of 56 days, at intervals of two weeks.

### 2.6.2 Quality control

In order to ensure the accuracy and consistency of the process, we produced procedural blanks and standard solutions the outcomes. During the TPH determination process, we performed replicate studies to get an average result that will be used to evaluate accuracy.

## 3. RESULTS AND DISCUSSION

### 3.1 Result of Phytoremediation

The experiment's findings demonstrate a clear difference between plants grown in soil

contaminated with crude oil and those cultivated in soil that is not. Unlike seedlings planted in untreated soil (control), *Abelmoschus esculentus* treated with oil began to germinate after 6 days.

**Table 2. Seedling emergence (E %) and co-efficient of velocity (Cov) of *Abelmoschus esculentus***

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

Table 2 shows how crude oil affects *Abelmoschus esculentus* seedlings' emergence rate (e %) and the velocity co-efficient (COV). In clean soil, emergence rates of 100% were recorded. At a 5% probability level, notable distinctions were seen between *Abelmoschus esculentus* plants in crude oil-contaminated and uncontaminated soil. The seedling emergence rate (e %) and COV declined as the oil content rose.

*Abelmoschus esculentus* was also found to have yellowing leaves, falling leaves, stunted growth, with some plants dying four weeks after planting in a soil which was treated with a crude oil while others survived [30-36].

The consequences of varying quantities of crude oil on the growth of *Abelmoschus esculentus* seedlings are shown in Table 3. Plants which grew in uncontaminated soil showed notable differences from those which was grown in a soil with comparable levels of crude oil [37-41]. Regardless of the oil concentration, statistical analysis showed that the plants in uncontaminated soil fared and was doing well more than those once in oil- polluted soil. But the effectiveness of the plants in oil-polluted.

### 3.2 Base Line Features of Diesel Impacted Soil

The baseline physicochemical and microbiological properties of the diesel-impacted soil was summarized in Table 1. 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.

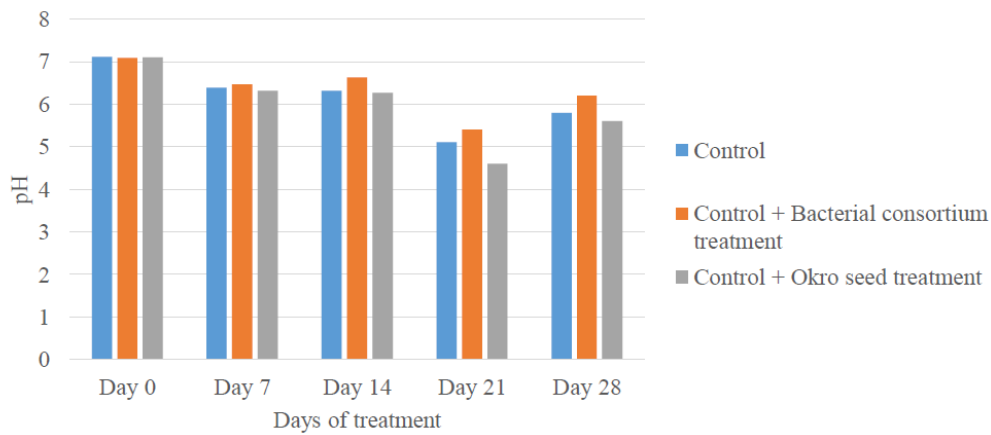
**Table 3. Result of seedlings heights on contaminated and non-contaminated soil**

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

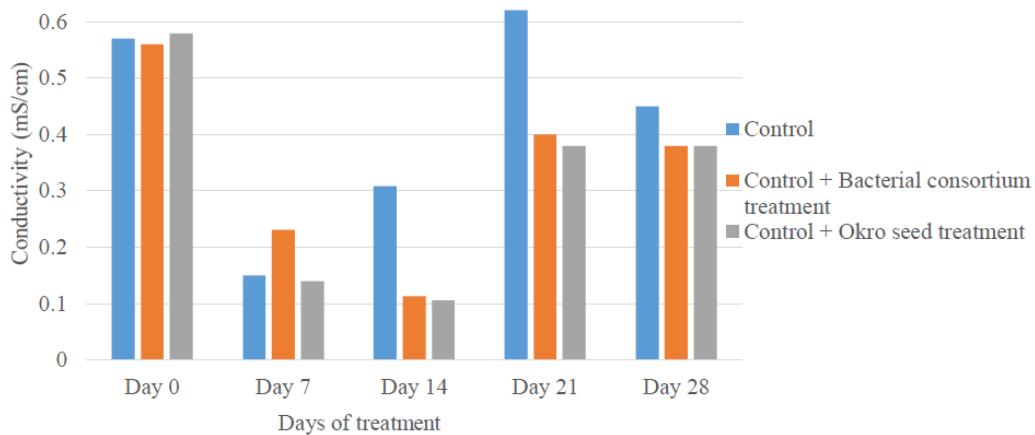
**Table 4. Base line physicochemical properties of petrol impacted soil**

Parameter	Value
Ph	7.11
Conductivity (mS/ cm)	0.57
Nitrate (NO <sub>3</sub> ) (µg/ kg)	16.50
Phosphate (PO <sub>4</sub> ) (mg/ kg)	12.09
Total organic carbon (% TOC)	3.90
Total organic matter (% TOM)	6.72
Total residual oil content (mg/ kg)	740.44

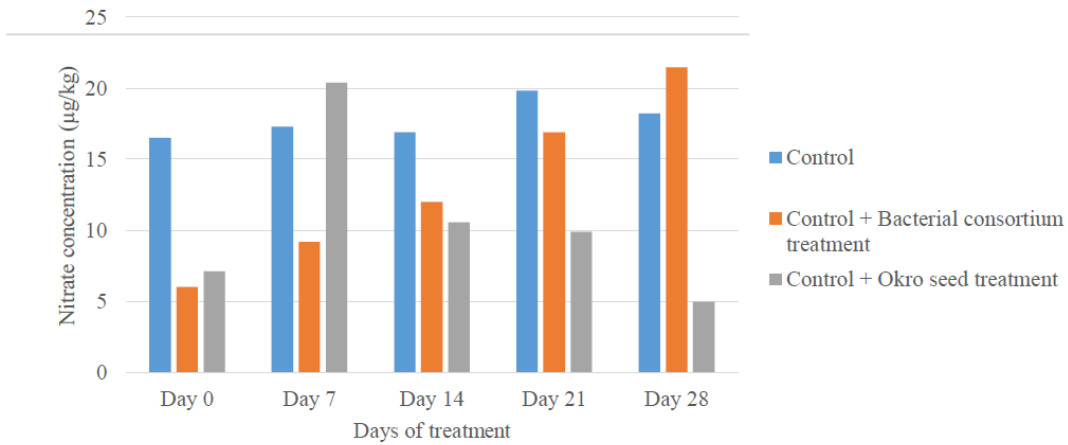
### 3.3 Bioremediation Profile



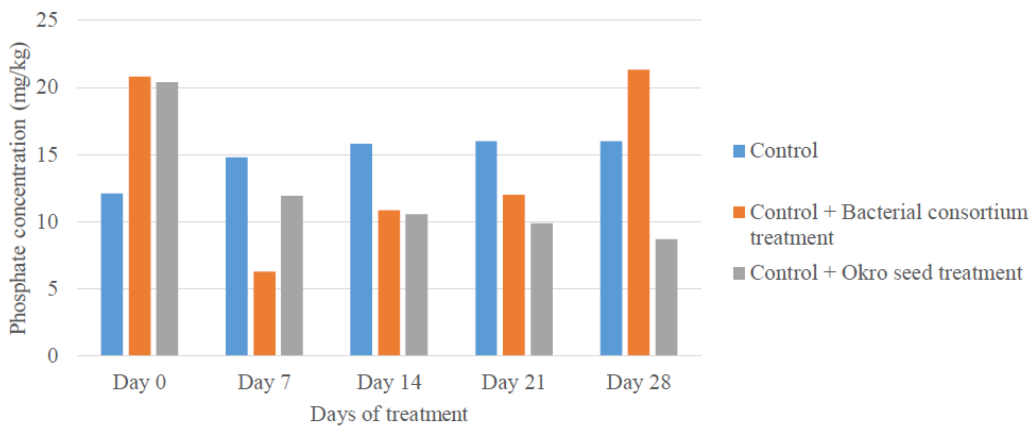
**Chart 1. pH variations during bioremediation treatment of petrol contaminated soil**



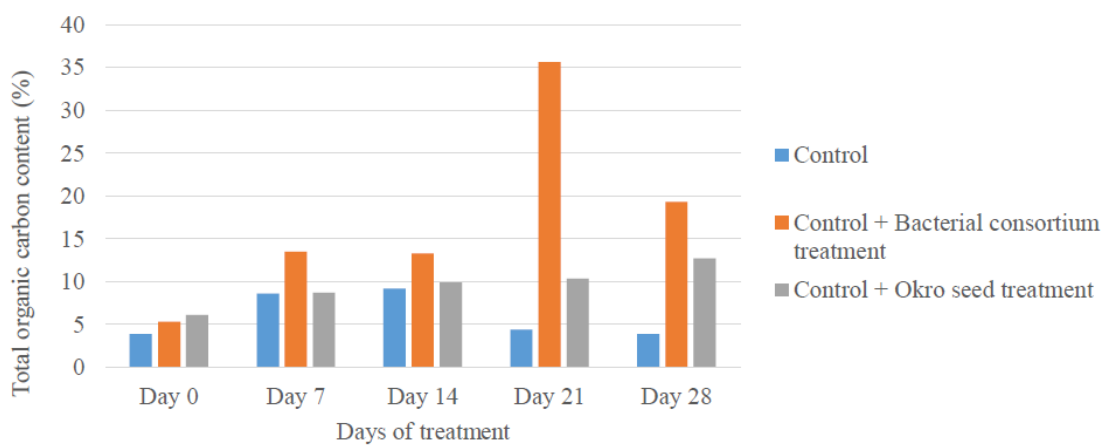
**Chart 2. Conductivity variations during bioremediation treatment of petrol contaminated soil**



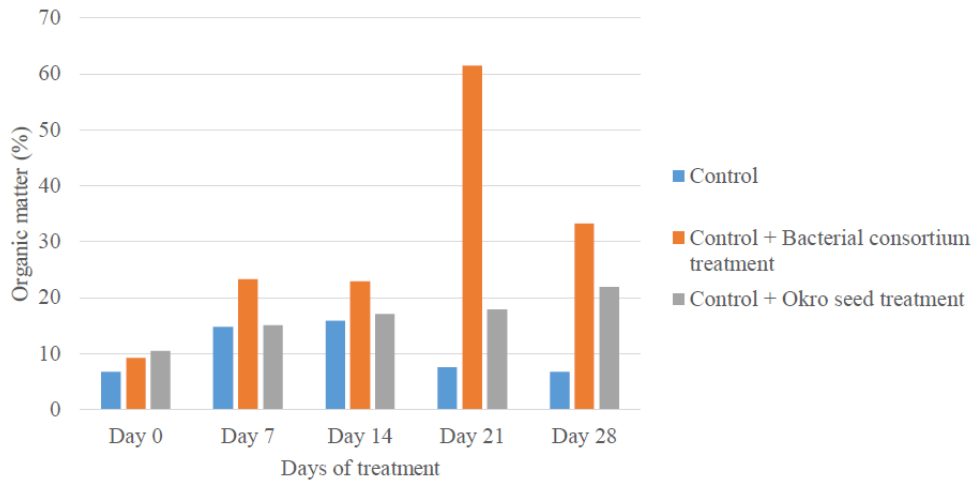
**Chart 3. Nitrate variations during bioremediation treatment of petrol contaminated soil**



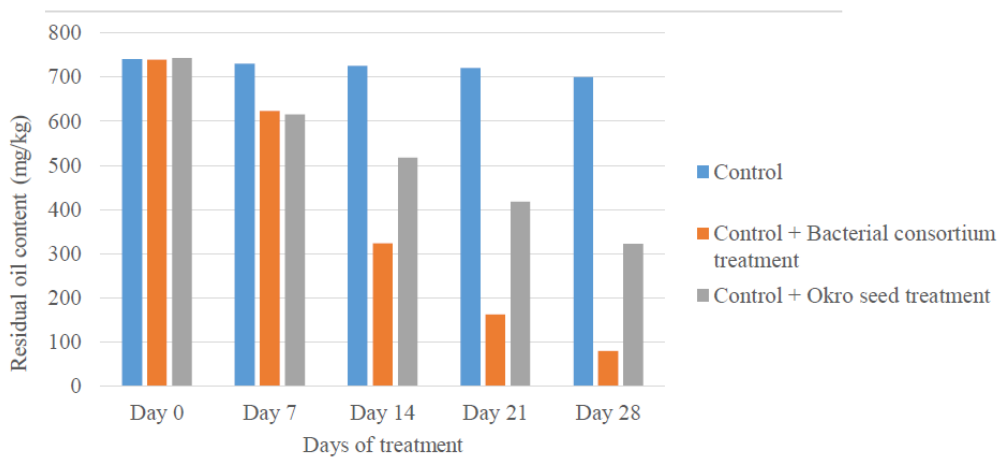
**Chart 4. Phosphate variations during bioremediation treatment of petrol contaminated soil**



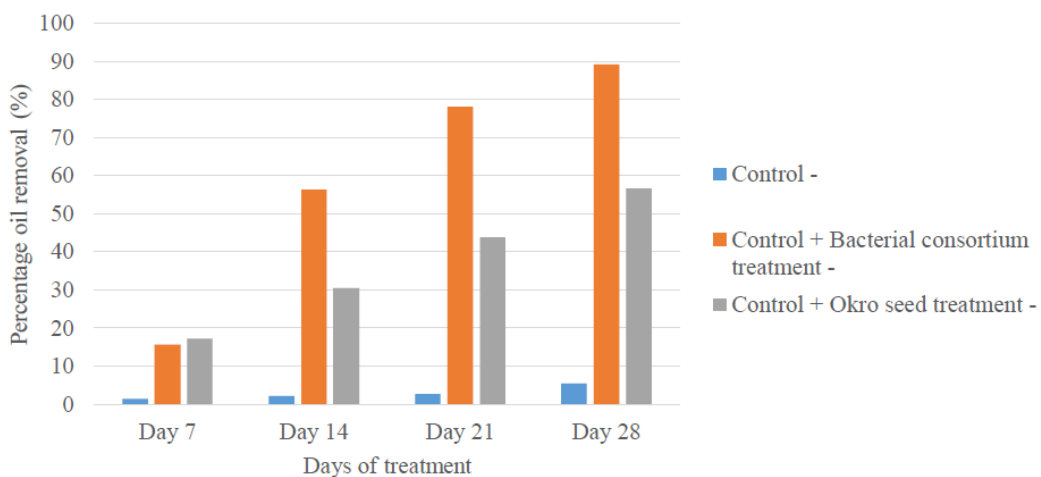
**Chart 5. Total organic carbon variations during bioremediation treatment of petrol contaminated soil**



**Chart 6. Organic matter variations during bioremediation treatment of petrol contaminated soil**



**Chart 7. Residual oil content variations during bioremediation treatment of petrol contaminated soil**



**Chart 8. Percentage oil removal during bioremediation treatment of petrol contaminated soil**



## 4. 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 gotten from this project is considering various Elements, including 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.

## 5. 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.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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