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Utilizing Bacteria for Crude Oil-Contaminated Soil Bioremediation and Monitoring Through Tomato Plant Growth

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ABSTRACT

This paper provides an in-depth analysis of the process of cleaning up crude oil-contaminated soil by using a carefully selected combination of bacteria that are capable of hydrocarbon breakdown. We assessed this bioremediation approach's efficacy by evaluating tomato plant growth and vigour as indications of soil recovery. According to our research, adding hydrocarbon-degrading bacteria significantly enhanced the crude oil's ability to break down in contaminated soil. Over time, the amount of petroleum hydrocarbons in the soil decreased significantly as a result of the bacterial consortium's effective hydrocarbon metabolism. It became out that this bioremediation method was both economically and environmentally viable. Furthermore, we noticed significant improvements in the general health and growth of tomato plants grown in the bioremediated soil. These plants showed signs of excellent soil quality restoration, including higher biomass, enhanced root development, and less stress symptoms. This work highlights the possibility of bacteria-mediated bioremediation as a workable and long-term solution to soil pollution caused by crude oil. Additionally, incorporating plant growth monitoring highlights the ecological benefits of bioremediation as a remediation approach for repairing contaminated ecosystems and provides a useful way to assess the efficacy of bioremediation operations. The findings showed a substantial decrease in petroleum hydrocarbons and enhanced tomato plant growth in treated soils, demonstrating effective ecosystem restoration. By using bioremediation to treat soil contamination caused by crude oil, this research supports the conservation and sustainable use of terrestrial ecosystems, which is in line with Sustainable Development Goal 15: Life on Land.

Key Words	Bioaugmentation, Biostimulation, Bacillus spp., Pseudomonas spp., Petroleum hydrocarbons, Soil contamination, Mitigation
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1. INTRODUCTION

Crude oil pollution of soil is a serious environmental problem that frequently results from industrial processes, tank breaches, or unintentional spills. Ecosystems are upset by this pollution, which also endangers public health and lowers agricultural output (Udeh et al. 2013). Excavation and disposal are two costly conventional methods of cleaning up crude oil-contaminated soil that might worsen environmental harm. As a result, eco-friendly and sustainable bioremediation has gained popularity. This method uses microorganisms, specifically bacteria that break down hydrocarbons, to break down petroleum hydrocarbons in soil, producing safe byproducts in the process and improving

the quality of the soil. It is still essential to assess how well bioremediation methods work and how they affect plant growth and soil health. This publication provides a comprehensive study of the use of a carefully chosen consortia of hydrocarbon-degrading bacteria in the bioremediation of soil contaminated by crude oil (Sundravel et al. 2021). While previous study has mostly concentrated on chemical analyses, our work adds a new dimension by observing the development and health of tomato plants grown in the treated soil. Tomato plants were specifically chosen as indicators because they are sensitive to changes in soil quality and provide important information about how well the rehabilitation process is going. With this project, we hope to show that bacteria-mediated bioremediation can improve soil health and plant growth in addition to lowering crude oil contamination (Silva et al. 2020). Our research demonstrates the method's ecological and practical benefits, offering a viable way to restore soil contaminated by crude oil while fostering environmentally friendly farming practices.

1.1 Global Effects

The problem of oil spill-related soil contamination is one of the most urgent issues facing the world today. Petroleum-contaminated soil poses serious health concerns to people, pollutes groundwater organically, limiting its use, causes economic hardship, degrades the environment, and reduces the productivity of agricultural soil (Rowland et al. 2014). There is a wealth of evidence demonstrating the detrimental effects of petroleum hydrocarbons on human populations, flora, animals, and microbes. The main sources of contamination are spills from factories and refineries, accidents involving oil tankers, and events that occur while transporting oil. Either marine tankers or land pipelines that are prone to spills and accidents are used to transfer crude oil across great distances (Zhu et al. 2020). A considerable amount of the oil pollution problem arises from the gap between major oil producing countries and major oil consumers, which forces petroleum to be transported on a massive scale from production centres to consuming areas.

Handling contaminated soil requires the application of a variety of techniques, including physical, chemical, and biological ones. Since biological remediation usually takes longer than physical or chemical interventions, many strategies have been developed to accelerate the breakdown of petroleum products in soil. Biological methods are frequently chosen because of their cost-effectiveness and effectiveness when compared to their counterparts in the chemical and physical fields. In the field of biological methods, microorganism-based bioremediation stands out as particularly effective (Algaifi et al. 2021).

1.2 Bioremediation Mechanism:

Some microbes have the amazing capacity to decompose petroleum hydrocarbons and use them as a source of energy and carbon. The accuracy of this breakdown process is closely linked to the microbe's genetic composition, which allows it to add molecular oxygen to hydrocarbons and produce intermediates that can then be incorporated into the cell's larger energy-producing metabolic pathways (Agamuthu et al. 2013). Certain bacteria are mobile and have a chemotactic response, meaning they will move in the direction of the contaminant when they detect its presence. On the other hand, in the close vicinity of the contamination, fungus and other bacteria may develop in the form of filaments.

1.3 Principle of Bioremediation:

Crude oil is a complex mixture of thousands of different chemical components, each with a different composition (Vermeer et al. 2021). In light of this variability, a variety of strategies are used,

including microbial and floral therapies, to remediate oil contamination. Bioremediation can happen naturally or be aided by the addition of fertilisers and bacteria. First, bio-surfactants and bio-emulsifiers are used by the bacteria in the soil to identify the oil and its constituent parts. After that, they adhere to the oil and use its hydrocarbons as a carbon and energy source. Microorganisms are unable to reach high molecular weight hydrocarbons due to their low solubility and adsorption (Muonye et al. 2024). By adding biosurfactants, the solubility and removal of these pollutants are improved, which increases the rates at which oil biodegrades. The volatile, soluble, and biodegradation-prone characteristics of oil components vary greatly. While some substances break down quickly, others show resistance, and yet others are not biodegradable. Because certain microbial species prefer certain petroleum compounds over others, distinct petroleum compounds biodegrade concurrently but at different speeds. As a result, over time, the various components of petroleum steadily dwindle. When carbon sources are present, microorganisms release enzymes that are essential for attacking hydrocarbon molecules (Su et al. 2021). The breakdown of hydrocarbons in petroleum involves a wide range of enzymes and metabolic processes. On the other hand, an insufficient amount of the right enzymes may prevent the attack or prevent total hydrocarbon breakdown.

1.3.1 Bacteria:

While many microorganisms may break down crude oil in soil, using mixed cultures in bioremediation has shown beneficial due to the synergistic interactions they exhibit rather than pure cultures.

1.3.2 Nutrient:

Although polluted soil may naturally harbour microorganisms, their numbers may not grow to the point where the location can be effectively bioremediated (Oyedeji et al. 2024). It is therefore essential to encourage their development and activity. The essential nutrient needed by all living things is carbon. To guarantee the effective breakdown of oil, bacteria also require macronutrients like nitrogen and phosphorus in addition to carbon. The ideal ratio of carbon to nitrogen to phosphorus, which is essential for hydrocarbon cleanup, is sometimes expressed as 100:10:4. Effective bioremediation typically requires a minimum concentration of 1 ppm of ammonium nitrogen and 0.4 ppm of orthophosphate (Yang et al. 2024).

1.3.3 Electron Acceptor / Oxygen:

Even though it's not usually the limiting factor, oxygen is nonetheless one of the most important components in the microbial breakdown of hydrocarbons (Mostafa et al. 2010). The first breakdown of hydrocarbons depends critically on oxygen, and further processes may also require oxygen. The complete deterioration of oil is dependent on oxygen availability. But when significant amounts of oil are present, the soil's oxygen content can quickly drop, resulting in anaerobic conditions. Bacteria turn to different electron acceptors as nitrate, iron, or sulphate when living in such anaerobic environments (Koshlaf et al. 2017). In contrast to oxygen, microorganisms that use these substitute acceptors have a reduced energy output. As a result, anaerobic bacteria degrade at slower rates, which lengthens the remediation time.

1.3.4 Detergent:

Oils have a low accessibility to bacteria due to their hydrophobic nature, which causes a long breakdown process. Detergent is added to oil-contaminated soil to aid in the hydrocarbons'

desorption, which speeds up the cleanup procedure (Xu et al. 2020). Detergents produced by microbes, including rhamnolipids, are typically used for this purpose. The presence of rhamnose moieties and a fatty acid tail set rhamnolipids apart.

1.4 Bioremediation Strategies:

1.4.1 Biostimulation:

Biostimulation becomes essential at contaminated sites where microorganisms exist but need to be stimulated for cleanup to be effective. In order to promote the growth of the bacteria already present in bioremediation, biostimulation entails the supply of nutrients, oxygen, and electron acceptors. The repair site's environmental conditions are optimised by this technique. Usually, injection wells are used to introduce additives into the subsurface. When creating a biostimulation system, a number of subsurface factors, including lithology, hydraulic conductivity, and groundwater velocity, are important considerations. Pollutant breakdown is primarily carried out by native microorganisms found in the soil; however, bioaugmentation can improve biostimulation. Enhancing the efficacy of remediation by adding particular microbial strains to the contaminated site, known as bioaugmentation, replenishes the native microbial community (Jakubovskis et al. 2020).

1.4.2 Bioaugmentation:

To aid in the cleanup of contaminated soil, bioaugmentation involves introducing a group of naturally occurring microbial strains or genetically modified bacteria. When natural microorganisms are either not present in the soil or do not have the metabolic capacity to carry out the restoration process, this approach works especially well (Jinlan et al. 2014).

1.4.3 Anaerobic degradation:

Oil-spill bioremediation commonly uses urea and ammonia-based fertilisers, which may create an oxygen demand due to biological ammonia oxidation, even though many bioremediation strategies focus on increasing oxygen levels in contaminated sites and assume aerobic respiration as the primary means of hydrocarbon elimination (Sundravel et al. 2015). Under some conditions, oxygen mass transfer could not be enough to restore the oxygen that microbiological metabolism has used up. This situation is most clear when there is limited oil infiltration into deeper sediment layers, especially in fine sediments. Anaerobic hydrocarbon degradation becomes relevant in these situations. When oxygen is scarce, anaerobic degradation pathways might kick in, forcing microbial communities to use other electron acceptors like nitrate, iron, or sulphate to break down hydrocarbons. Therefore, anaerobic hydrocarbon degradation pathways may play a major role in bioremediation efforts in situations with restricted oxygen availability.

1.4.4 Land Farming:

As a remediation approach, land farming spreads contaminated soil across a bed that has been prepared, sometimes with fertilisers and sometimes without. By encouraging bacterial activity, this technique improves the breakdown of oil pollutants in the soil (Sidiq et al. 2020). A few parameters need to be kept in mind when choosing a suitable location for land farming. Among them is the requirement to keep the ground surface and the seasonal high groundwater table at least three feet apart in order to avoid contaminating groundwater. In addition, a land slope of no more than 8% is necessary to ensure sufficient soil stability and stop erosion. Following these guidelines helps

minimise possible negative effects on the environment while ensuring the effectiveness of the land farming operation.

1.4.5 Composting:

The process of composting involves piling polluted soil next to organic resources such as manure or agricultural waste (Khan et al. 2021). The addition of organic materials raises the pile's temperature and encourages the growth of a diverse microbial community. This elevated temperature, in conjunction with the promotion of microbial proliferation via nutrient addition, enables the effective biodegradation of pollutants in a comparatively short amount of time.

2 ENVIRONMENTAL REQUIREMENTS

2.1 Optimum Environmental Conditions:

Table 1 below outlines the ideal environmental conditions required for contaminant degradation.

Table 1 Environmental conditions affecting degradation

Parameters	Criteria for microbial activity	Optimal circumstances for the degradation of oil.
Oxygen content	Conditions favoring aerobic degradation, with at least 10% air-filled pore space.	10–42%
Contaminants	Non-toxic conditions	Hydrocarbons making up 6–12% of the soil's dry weight.
Temperature (°C)	17–43	22–34
Soil moisture	26–29% of water retention capacity	31–92%
Soil pH	6–9	6.5–8.0
Nutrient content	Nutrient concentrations essential for supporting microbial growth.	C:N:P = 100:10:1
Type of soil	Reduced clay or silt presence.	

2.2 Strategies to Uphold Ideal Environmental Parameters:

Temperature, humidity, and pH all have a big impact on the growth and function of microorganisms (Singh & Gupta 2020). Although microorganisms can survive in harsh environments, most of them do well in a limited range. Thus, in order to guarantee their efficacy in bioremediation procedures, it is essential to create and preserve optimal circumstances.

2.2.1 Soil:

High levels of alkalinity or acidity in soils can hinder the processes involved in biodegradation. However, pH can be adjusted by adding lime, which will neutralise the soil and create an environment that will support biodegradation.

2.2.2 Temperature:

Biochemical reaction rates are highly dependent on temperature; several reactions double in speed for every 10°C increase in temperature (Reddy & Ravitheja 2019). However, going over a

certain temperature threshold might cause cells to die or become dormant. Plastic covering can be used to raise temperatures in order to efficiently capture solar heat, especially in the late spring, summer and autumn.

2.2.3 Water Content:

All living things depend on water to survive, and irrigation is key to achieving the ideal moisture content required for efficient bioremediation procedures.

2.2.4 Oxygen:

Whether an aerobic or anaerobic system runs depends on the availability of oxygen. Anaerobic conditions are mostly used for the breakdown of chlorinated chemicals, whereas aerobic circumstances are more effective in the breakdown of hydrocarbons. You can use techniques like tilling or air sparging to increase the amount of oxygen in the soil. Furthermore, to increase oxygen levels in the environment, magnesium or hydrogen peroxide may occasionally be added.

2.2.5 Soil Structure:

The efficient transportation of air, water, and nutrients is significantly impacted by the composition of the soil. Gypsum and organic matter are two examples of elements that can be applied to improve soil structure. Poor permeability soils, on the other hand, are not appropriate for in situ cleanup methods because they can impede the flow of water, nutrients, and oxygen.

3 EXPERIMENTAL PROCEDURES

3.1 Bacteria Isolation:

The bacteria used in this study were isolated from the sandy surface layer, which is identified by the presence of little silt and clay (Sundravel et al. 2023). This soil sample was taken from a car workshop in the Namakkal district of Tiruchengode that had been purposefully contaminated with crude oil before. The tests that were performed to identify the bacteria present in the oil-contaminated soil are displayed in Figure 1.

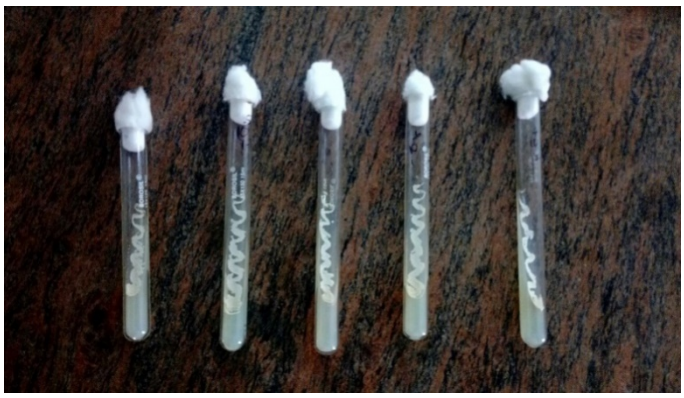


Fig .1 Tests for identification of bacteria

On agar plates, the microbial population was evaluated by means of the serial dilution method under 26°C incubation. For the purpose of counting all bacteria, nutrient agar was used. First, one gramme of soil was combined with one hundred millilitres of water. Next, one millilitre of the tainted water was diluted one at a time, reaching volumes of 106, 107, and 108 millilitres. The experiment's temperature shouldn't be higher than room temperature. In a different method, 20 millilitres of sterile nutritional broth were mixed with four grammes of contaminated soil. The process of autoclaving at 121°C for 25 minutes was used to achieve sterilisation. A mixer was used to mix the soup for an hour. The electrical mixer used to continuously agitate the broth culture over a lengthy period of time is shown in Figure 2.

Fig 2 Mixer

Figure 3 Depicts the preliminary tests conducted for the isolation of bacteria within the broth culture.

Fig 3 Laboratory tests for bacterial isolation

The samples were sterilised and then incubated for 24 hours at 37°C. Solid nutritional agar was streaked over petri dishes, which were subsequently incubated at 37°C for eighteen hours. Until pure colonies were achieved, organisms exhibiting defining traits of a colony were isolated and subsequently subcultured on sterile nutrient agar plates. These plates were then incubated at 37°C for a further 18 hours. The progression of bacterial colony growth in petri dishes subjected to autoclaving conditions is illustrated in Figure 4.

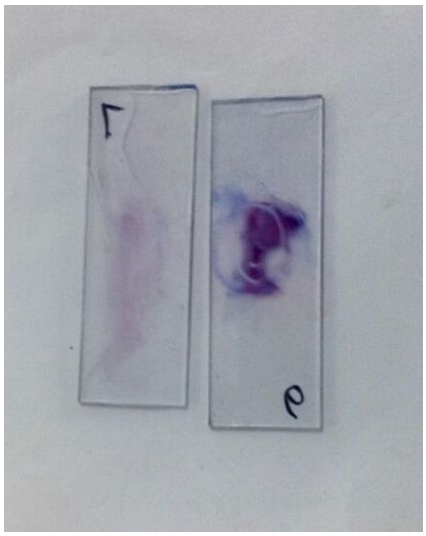
Figure 4 illustrates the formation of bacterial colonies.

Figure 5 depicts the incubator used in the experiment.

3.2 Biochemical Tests

One millilitre of crude oil was introduced to four millilitres of synthetic medium, with the crude oil acting as the only carbon source, in order to evaluate the isolates for hydrocarbon utilisation. One litre of deionized water was used to dissolve 1% NaCl, 0.042% MgSO4.7H2O, 0.042% NaNO3, 0.029% KCl, 0.083% KH2PO4, and 0.125% NaHPO4 to create the synthetic medium. The medium's pH was brought to 7.4. The cultures were then cultured for five days at 27°C, and the synthesis of biosurfactants was monitored every day. The streaking test used to identify the bacteria is shown in Figure 6.

Fig 6 Streaking method for bacterial identification



3.3 Characterisation and Identification

Two of the eight distinct strains showed evidence of using hydrocarbons. Following that, these two isolates underwent processes for characterisation and identification (Jamuna et al. 2020). For characterisation, morphological inspection, staining reactions, microscopic investigation, and biochemical testing were carried out. The two species were later determined to be Pseudomonas sp. and Bacillus sp. The culture method used in the Bijou bottles, set up for the incubation phase, is shown in Figure 7.

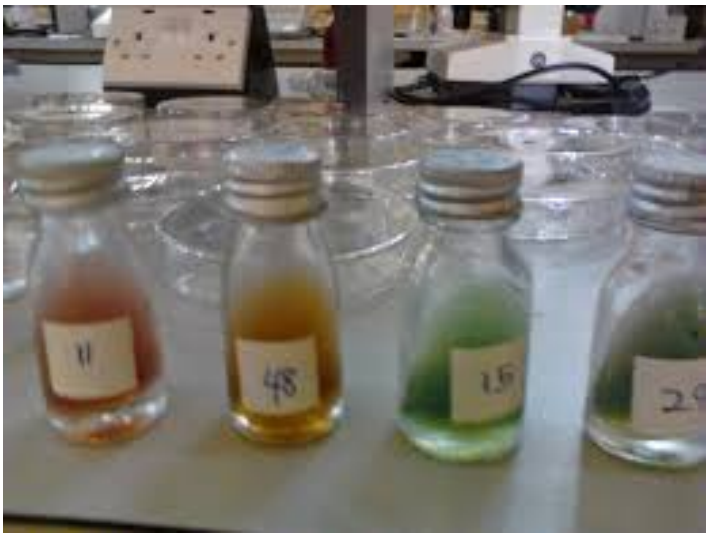


Fig 7 Bacteria Culture for Various bacteria

The outcomes of biochemical analyses and observations of colony features that were used to identify the bacterial isolates are shown in Table 2.

Table 2 The isolates were characterised and identified using the Gramme staining technique.

Test	**Result**	
Trehalose	+	-
Motility	+	+
Glucose	+	+
Endospore	+	-
Gram stain	Gram positive	Gram negative
Sucrose	+	-
L-Rhamnose	+	-
Colony Characteristics	The colony has a mucoid texture, is elevated, spherical, and appears white.	The colony has smooth edges, an uneven form, and a yellow-orange colouring.
D-Xylose	-	+
D-Ribose	-	-
Oxidase	+	+
Catalase	+	+
Possible identification	Bacillus	pseudomonas

3.4 Inoculum Build Up:

Bijou bottles with 5 ml of nutritive broth were filled with Pseudomonas and incubated at 37°C. A spectronic 20 spectrophotometer was used to measure absorbance at a wavelength of 560 nm in order to count the number of bacteria (Pattharaphon et al. 2020). This process was repeated until a cell concentration of 1.5 X 108 colony forming units (CFU/ml) was reached. Then, a 1-liter culture of nutrient broth was added to the 5-milliliter culture. Pseudomonas was replaced with Bacillus for the rerun of this procedure. Equal amounts (500 ml) of the culture from each isolate were combined with the previously indicated cell concentration to create a bacterial consortium. We used these cultures in our fieldwork.

4 FIELD WORK

4.1 Preparation of Samples:

We used loamy topsoil with a depth of 0–15 cm to fill polythene bags. Since tomato plants grow well in acidic soil, the pH of the soil was slightly raised to an acidic level. Eight kilos made up each soil bag. Every dirt bag—aside from the control—was sprayed with 200 ml of crude oil, sometimes known as engine oil. We next infected one of the soil bags contaminated with crude oil with a one-liter culture of Pseudomonas cells at a concentration of 1.5 X 108. Consortium, Cow manure, and Bacillus were used to duplicate this procedure (Arunvivek et al. 2019). Furthermore, one soil bag was not treated in any way and was contaminated with crude oil (control). Two bags per treatment were observed during the experiment, and each bag was manually mixed. Throughout the trial, the bags were kept in the best possible environmental conditions by being exposed to sunlight and receiving regular irrigations.

4.2 Transplanting Tomato Seedlings:

After 30 days, the seedlings were carefully transferred from their original spot and placed inside polybags. After that, soil was added to the clay pots to provide the polybags support. The pot holes were plugged to stop water loss during the experiment. Water was initially maintained for the first nine days after transplantation at a level that was 1 cm above the ground. The next goal was to keep the soil at its ideal moisture content without flooding. Water levels were regularly checked and topped off as necessary. Documentation of observations was done for various soil samples and treatment settings. Figure 8 shows the germination of tomato seedlings on the ninth day after uprooting.

Fig 8 Germination of tomato seedling at 9th day

4.3 Study on the Growth of Plants:

On the thirtyth day, each plant's germination count, shoot height, leaf area, root-shoot ratio, dry weight, and root length were measured. At the 30-day point, the maximum height and leaf area were recorded. Furthermore, notes were collected about colour shifts in the plants as well as any deaths. On the ninth day, the shoot development in soil treated with cow dung is depicted in Fig 9

Fig 9. Germination of shoots in soil treated with cow dung

4.3.1 Leaf Area

On the thirty day following treatment, the leaf area was measured with a leaf area metre. The formula for calculating leaf area was as follows: length multiplied by maximum width multiplied by 0.75.

4.3.2 Specific leaf weight (SLW)

The weight of a leaf per unit area is known as specific leaf weight (SLW), and it is commonly stated in grammes per square metre (g/m^2).

4.3.3 Specific leaf area (SLA)

The leaf area per unit leaf dry weight is known as specific leaf area (SLA), and it is commonly stated in square metres per kilogramme (m2/kg). It shows how effectively the leaf is absorbing light and transferring gases to and from the atmosphere.

4.3.4 Dry weight

The weight of a material after all moisture has been removed is referred to as its "dry weight," and it is usually found by drying the sample in an oven until a steady weight is reached.

4.3.5 Root shoot ratio

A measure of a plant's biomass distribution—that is, the percentage of biomass allotted to roots as opposed to above-ground shoots—is the root-to-shoot ratio. It is computed by dividing the branches' dry weight by the roots' dry weight.

4.3.6 Root length

Thirty days following treatment application, the root length was measured. Watering the plants thoroughly helped to speed up the process. The plant's polybag was then gently sliced in half, making it possible to easily reach the roots without compromising the integrity of the plant. Next, the length of the root was measured.

5 COMPARISON AND ANALYSIS OF VEGETATIVE PARAMETERS

5.1 Non Destructive vegetative parameters:

Non-destructive vegetative parameters are those that are studied without causing any damage to the plant body (Arunvivek et al. 2022). The tomato plant in crude oil soil at days nine and thirty is shown in Figure.10. Plants cannot survive in an environment where air and water circulation into the soil is inhibited by crude oil. Leaf loss and yellowing of the leaves, which started on the ninth day, are among the symptoms. Figure 11 illustrates the variation in plant growth between polluted soil and treated soil.

Fig 10 The growth of tomato plants on the 30th day was assessed.

Fig 11 Disparity in plant growth between polluted soil and treated soil.

The vegetative characteristics of tomato plant growth in crude oil-polluted soil treated with cow dung and bacterial culture are shown in Table 3.

Table 3 Vegetative characteristics of tomato plant development

Treatments	**Plant height(cm)**	**Shoot height(cm)**	**Leaf colour**
Control	28.5	16.5	The green colour of the leaf signifies the health of the plant.
Consortium	30.7	19.5	Leaf green
Bacillus	24.4	13.5	The older leaves' fading and their pale green hue point to a possible nutrient shortage or stress in the plant.
Pseudomonas	22.6	11.4	The plant's health may be impacted by a nutrient shortage or

			environmental stress as shown by the pale green colour, backward curling of leaves, and fading of older leaves.
Cowdung	20.3	9.3	The leaf's green hue suggests that it is in good health.
oil only	13.9	4.9	Blackish yellow

The plant destructive growth parameters of soil treated with different treatments are shown in Table 4.

Table 4 Plant destructive Growth Studies

Treatment	**Leaf dry weight (g/plant)**	**Root dry weight (g/plant)**	**Total dry weight (g/plant)**	**Root shoot ratio**	**Root length(cm)**
Control	0.922	0.092	6.900	0.011	19.03
Consortium	1.330	0.075	7.870	0.012	18.75
Bacillus	0.857	0.067	5.320	0.010	16.45
Pseudomonas	0.756	0.045	4.870	0.008	13.32
Cowdung	0.655	0.030	3.883	0.007	11.78
oil only	0.240	0.018	2.187	0.00096	4.37

5.2 Result

The microbes that could use hydrocarbons were Bacillus and Pseudomonas, according to the results of their characterisation and identification. In terms of vegetative metrics, the treatments containing Pseudomonas and Bacillus alone, the consortium (a mixture of Pseudomonas and Bacillus), and the control group displayed the greatest mean values for plant height, root-shoot ratio, leaf count, and maximum root height. On the other hand, the treatments with just crude oil and cow dung showed the lowest values for these vegetative metrics. Within two to three weeks of germination, tomato plants planted in soil treated exclusively with crude oil showed yellowing of the leaves with a black tinge at the corners, whereas plants cultivated in other treatments displayed leaf fading about four weeks after germination.

6 COMPARISON AND ANALYSIS OF SOIL PROPERTIES

Examining and contrasting different aspects of soil samples from diverse treatments or locations is part of the process of comparing and analysing soil properties. Parameters like soil pH, organic matter content, nutrient levels (such as nitrogen, phosphorous, and potassium), soil texture, and microbial activity are often included in this analysis. Significant variations between soil samples may be found using statistical tests, and data visualisation can be aided by using graphical representations like bar charts or scatter plots (Arunvivek et al. 2015). To further comprehend the connection between soil health and plant performance, correlations between soil characteristics and plant growth metrics may be investigated. In general, the comparison and analysis of soil parameters offer insightful information on how environmental factors and treatments affect plant productivity and soil quality.

6.1 Soil Nutrient Composition:

The use of hydrocarbons by higher bacterial populations is responsible for the decrease in oil and grease content. Nutrients including potassium, phosphorus, and nitrogen are abundant in cow manure. Air and water cannot flow freely through contaminated soil, especially in soils that include a lot of silt and clay. The nutrients found in the soil for each of the treatments are shown in Table 5.

Table 5 Analysis of Soil properties in Oil, Cow dung and Consortium treatment

Soil Properties	**Before The Experiment**	**After the Experiment**		
		Oil treatment	**Oil + Cow dung**	**Oil+ Consortium**
%Total nitrogen	0.15	0.07	0.53	0.7
%Organic carbon	1.8	2.3	1.98	1.9
%silt	2.0	2.7	3.1	2.9
Cation exchange capacity	9.2	9.1	9.1	9.0
%Fine sand	51.3	50.6	50.3	50.9
%clay	16.72	16.64	16.20	16.67
%Coarse sand	29.98	29.86	29.89	29.92
Calcium(ppm)	3.4	3.2	3.3	3.2
Potassium(ppm)	0.05	0.09	0.06	0.07
%Organic matter	2.3	2.1	2	2.2
Sodium(ppm)	0.36	0.38	0.38	0.41
pH	6.4	4.4	5.3	6.1
Phosphorus(ppm)	0.29	0.18	0.21	0.23
Magnesium(ppm)	1.4	1.3	1.2	1.4

6.2 Soil Physical Properties:

The treated samples show bonded granules, while the contaminated sample displays tiny grains. The polluted area's degraded soil is the reason for this differentiation. Soil's permeability, or its capacity to let water through, is determined by its aggregation, swelling, and textural characteristics. There is no longer any permeability in the contaminated area due to the loss of the coagulating and colloidal states of the soil. Table 6 provides more information on these findings.

Table 6 The treated and control soil samples' physical characteristics were contrasted.

Treatment	**Water permeability**	**Odour**	**Texture**	**Structure**	**Colour**
Control	Positive	Not distinct	Bound	Durable	Undefined
consortium	Positive	Not distinct	Bound	Durable	Undefined
Bacillus	Positive	Not distinct	Bound	Durable	Undefined
Pseudomonas	Positive`	Not distinct	Bound	Durable	Undefined

Cow dung	Positive	Not distinct	Bound	Durable	Black
oil only	Negative	Oil	Fine/ loose	Not durable	Black/ Brown

6.3 Result:

The results of the soil property analysis showed that the percentage of nitrogen in soils treated with cow dung (0.53%) and the consortium (0.7%) was higher than the initial nitrogen content (0.15%) prior to the experiment. On the other hand, the amount of nitrogen in the soil that was only given crude oil decreased (to 0.07%). Before the trial, the pH values were 6.4; for the consortium treatment, they were 6.1; for cow dung, they were 5.3; and for the control, they were 4.4. Furthermore, the soil treated with simply crude oil showed lower phosphorus levels, but the soils treated with cow dung and the consortium showed a relative rise in phosphorus levels.

7 CONCLUSION

The consortium's and the Pseudomonas and Bacillus treatments' significant bioremediation capacities were demonstrated in this study by their beneficial impacts on germination, plant height, root-shoot ratio, and root length. These findings imply that using bacteria for bioaugmentation can successfully reduce the pollution caused by crude oil. Although this study offers important insights, it is limited by the short duration of the experiment and its focus on only one plant species. Future studies should investigate the long-term effects and include a diverse range of plant species to better comprehend the broader ecological impacts of these bioremediation methods. It's possible that the bacteria created enzymes that could break down petroleum substrates by metabolising the hazardous elements of crude oil. Although cow dung helped mitigate the negative impacts of crude oil pollution, in terms of vegetative and reproductive parameters, its restorative benefits were not as strong as those of the consortium, Pseudomonas, and Bacillus treatments. Even while the yield of tomato plants treated with cow dung was higher than that of crude oil alone, it was still less than that of treatments with bacteria. This disparity in efficacy could be explained by the greater nutritional supply that cow dung offers, which is sometimes absent or insufficient in soil that has been contaminated by crude oil. Furthermore, by binding soil particles together, cow dung improves soil permeability and structure. The results highlight the potential of cow dung (biostimulation) and microorganisms (bioaugmentation) for cleaning up soil contaminated by crude oil. In instance, bioaugmentation using a consortium of Pseudomonas and Bacillus proven to be more successful than biostimulation alone. This bioremediation strategy presents considerable environmental advantages, such as decreased soil toxicity, enhanced soil fertility, and the potential for ecosystem recovery in oil-contaminated areas. These techniques could be especially beneficial in regions impacted by oil spills or prolonged petroleum industry activities. The microbial community that is metabolically active was essential to the hydrocarbon biodegradation process. The findings of this study have direct applications in environmental remediation projects. The combination of bacterial treatments and plant growth monitoring provides a practical and cost-effective approach for assessing and implementing soil restoration in oil-contaminated areas. Future studies will examine how well combination bioaugmentation and biostimulation techniques work. By supporting sustainable methods for resource and pollution management, this study supports Sustainable Development Goals 12 and 15, which centre on responsible production and consumption as well as the preservation and sustainable

use of terrestrial ecosystems. It deals specifically with soil remediation and contamination from crude oil.

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