



Research Article

Evaluation of Soil Moisture Content, pH, and Dominant Microorganisms in Crude Oil-contaminated Soil Treated with Cow Dung, Indole-3-Acetic Acid (IAA), and Simulated Microgravity

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Abstract

Crude oil negatively affects soil physicochemical properties and microbial activity, thereby hindering plant growth and posing notable environmental and agricultural issues. The combined effects of cow dung, indole-3-acetic acid (IAA), and simulated microgravity on the recovery of soil degraded by crude oil were explored using *Zea mays* as the test plant. Soil was contaminated with crude oil at different concentrations (0%, 1%, 3%, and 5% v/w), with each concentration comprising eight treatments: cow dung, *Zea mays* seeds exposed to microgravity, and IAA alone or in combination. Changes in plant height, soil pH, soil moisture content, and soil microbial diversity were assessed. Crude oil contamination decreased plant height and soil moisture, reduced pH, and suppressed microbial diversity. However, the various treatments significantly enhanced the soil parameters both individually and in combination. Notably, T7 (cow dung + IAA + simulated microgravity) had a plant height of 60.14 ± 9.8 cm at 12 weeks, and the soil pH and moisture content at the 5% concentration during the 3rd month were 6.45 ± 0.02 and $25.25 \pm 2.35\%$, respectively. Microbial analysis revealed the presence of the hydrocarbon-associated taxa *Fusarium oxysporum*, *Pseudomonas aeruginosa*, and *Bacillus cereus* and the nutrient cycling taxa *Alcaligenes faecalis* and *Bacillus cereus*. Microbial diversity was highest in T7 (3.18) and lowest in T2 (maize alone) and T5 (maize+IAA), both of which had a diversity index of 2.94. Their occurrence suggests a potential contribution to soil recovery, although their functional roles were not directly assessed in this study. This research demonstrated the synergistic potential of integrating bioremediation strategies to mitigate the negative effects of crude oil contamination and to restore the ability of soil to support plant growth. The results offer a framework for developing sustainable and effective restoration practices, with implications for enhancing soil health and agricultural productivity in crude oil-polluted areas.

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Introduction

The contamination of the environment by crude oil has remained a persistent problem because of its complex and recalcitrant nature. It affects soil physicochemical properties, ecological function, and microbial equilibrium (Yang et al., 2023). Healthy soil is necessary

for optimal plant growth; it serves as a pool of nutrients and water. However, oil pollution compromises these functions by blocking soil pores, impeding aeration and water penetration, and eventually decreasing permeability (Saikia et al., 2023). Furthermore, crude oil can alter soil pH, leading to acidification that adversely inhibits

soil microbial activity (Huang et al., 2023). These changes in soil pH reduce the availability of essential nutrients such as nitrogen, phosphorus, and magnesium while increasing the bioavailability of toxic metals such as aluminum, iron, and manganese (Saikia et al., 2023). A reduction in nutrient uptake hinders the growth and productivity of plants (Ogbeide et al., 2023). Njoku et al. (2012) reported that soil acidity increases with increasing crude oil concentration, but with the application of cow dung as an organic amendment, acidic soil improved. Egobueze et al. (2019) reported that cow dung improves soil physicochemical properties and supports plant growth in crude oil-contaminated soils, although these properties differ across different soil types.

Microbes play important roles in biogeochemical cycling and pollutant degradation, which aid in soil recovery (Yang et al., 2023). The interaction between plant roots and rhizospheric bacteria increases plant resistance to hydrocarbon toxicity (Hussain et al., 2023), with moisture content and pH influencing the activity and variety of microbes in soil (Hussain et al., 2023; Yang et al., 2023). Plant roots and rhizospheric bacteria have a mutually beneficial connection; root exudates increase the metabolism of microorganisms, and microbes respond by stimulating root exudation (Njoku et al., 2022). This interaction increases microbial abundance and activity, enhances soil characteristics, and promotes plant growth. However, plants may not be primarily responsible for rebuilding degraded soil microbial populations. The addition of cow dung (organic matter) may help to improve the microbial structure and function of soil undergoing crude oil degradation (Njoku et al., 2022). Indole-3-acetic acid (IAA) improves plant morphological growth during shoot and root development (Sosnowski et al., 2023). Adequate soil water content optimizes plant growth and microbial functions (Hussain et al., 2023; Koch et al., 2018). Previous research has revealed that microbial activity peaks at approximately 60% of the water holding capacity; however, the results of moisture responses in oil-contaminated soils remain unclear (Barua et al., 2011; Okenmor et al., 2022).

In addition to organic amendments, emerging strategies for enhancing plant–microbe interactions under environmental stress include the application of plant growth regulators and exposure to simulated microgravity. Indole-3-acetic acid, a key auxin, regulates root architecture, stimulates root exudation, and promotes microbial recruitment in the rhizosphere, thereby strengthening plant–microbe interactions under stress conditions (Sosnowski et al., 2023). Simulated microgravity exposure at the germination stage has been shown to alter plant physiological and cellular responses by creating thinner, less rigid cell walls, which promote cell expansion and growth; increase

nutrient and water uptake; and facilitate root exudates, which in turn increase root growth patterns and influence microbial behavior (Nie et al., 2025; Ovenseri et al., 2024; United Nations Office for Outer Space Affairs, 2013). While the individual benefits of cow dung, IAA, and simulated microgravity on improving plant growth have been demonstrated, their synergistic effects on rhizosphere dynamics under crude oil stress remain unexplored. This study addresses this gap by evaluating a novel integrated remediation approach. We specifically investigated the combined application of cow dung amendment, IAA seed treatment, and simulated microgravity exposure at the germination stage across varying crude oil concentrations (0%, 1%, 3%, and 5% v/w). The primary outcomes evaluated included plant growth height, soil pH, moisture retention, and microbial community diversity (Shannon index) over 12 weeks. *Zea mays* was selected because of its well-documented ability to tolerate and remediate oil-polluted soils and its extensive use in microgravity studies (Njoku et al., 2022; Ovenseri et al., 2023; 2024). Its C4 physiology and production of phosphoenolpyruvate carboxykinase (PEPCK) also increase its biomass and contaminant degradation efficiency (Sonowal et al., 2018). This study proposes a novel method for understanding how multiple approaches (cowdung, IAA, and microgravity) restore the role of the plant's rhizosphere in crude oil-degraded soil. The outcomes are expected to enhance the understanding of soil remediation techniques and inform sustainable methods for restoring soil health and improving plant productivity in oil-contaminated soil. How the treatments (cow dung, *Zea mays* treated with IAA, and exposed to simulated microgravity) impact soil pH, moisture content, and rhizospheric microbial diversity in crude oil-degraded soil should be investigated.

Materials and methods

1) Study location

Zea mays were exposed to simulated microgravity at the Space-Earth Environment Research Laboratory, University of Benin, which is managed by the National Space Research and Development Agency (NASRDA). Following this exposure, pot experiments were conducted in a screen house built within the botanical garden of the Department of Plant Biology and Biotechnology, University of Benin (Latitude 6.3971492, Longitude 5.6149716) from October to December 2023.

2) Preparation of contaminated soil and cow dung

Bonny light crude oil from the Emreke-Kokori flow station in Ughelli, Delta State, Nigeria, was used to contaminate the soil. Soil samples weighing approximately 150 kg (0–15 cm and 15–30 cm depths) were collected from the Department of Agriculture, University of Benin, Nigeria (Latitude 6.3971492, Longitude 5.6149716), thoroughly mixed, and characterized in the

laboratory. Afterward, 5000 g of sun-dried and sieved soil from each sample was mixed with crude oil (5%, 3%, 1%, and 0% v/w). The percentage of crude oil in the soil was calculated using Eq. 1.

After contamination, the soil was placed into experimental pots with diameters of approximately 25 cm and depths of 15 cm, labeled according to the different treatments. Each treatment was replicated three times. The contaminated soil was then left in the dark for 7 days to acclimatize (Ovenseri et al., 2023; 2024; United Nations Office for Outer Space Affairs, 2013). Fifty grams of sun-dried cow dung ground into a fine powder was mixed with portions of soil in pots containing 5%, 3%, and 1% v/w crude oil (Egobueze et al., 2019). The GC–MS specifications (Hewlett Packard 5890 Series 2 as the chromatographic equipment with an Agilent 7683B injector (Agilent Technology, Santa Clara, CA, USA)) were used to analyze residual total petroleum hydrocarbons and polyaromatic hydrocarbons.

% of crude oil

$$= (\text{Volume of crude oil (cm}^3\text{)} / \text{Weight of soil(g)}) \times 100$$

(Eq.1)

3) Microgravity simulation, constitution of agar substrate and preparation of IAA

To simulate microgravity for seeds, a one-axis clinostat with a horizontal rotational axis positioned perpendicular to the ground's gravity vector was employed. By rotating at 0.5 rotations per minute (rpm), the device equalized the gravity vector to provide the simulated conditions. Agar (1.5 g) was dissolved in 100 mL of boiling distilled water with constant stirring. After they had cooled for 10 minutes in 33.33 mL Petri dishes, the seeds were half-implanted onto the agar. IAA solution (150 ppm) was prepared by dissolving 0.15 g of powdered IAA in 1,000 mL of distilled water and mixing it via vigorous shaking. After confirming seed viability, a set of seeds was soaked in the solution for 90 minutes before being inoculated onto agar plates for 5 days to achieve germination. Germinated seeds in Petri dishes under simulated microgravity (0.5 rpm) or gravity control conditions were introduced to the nursery. After two weeks in the nursery, the seedlings were transplanted into the contaminated soil pots in the screen house at a temperature range of 25–26°C. The plants were watered every 2 days (Ovenseri et al., 2024).

4) Experimental design and treatments

T1 = microgravity-exposed *Zea mays* seeds, T2 = nonmicrogravity-exposed *Zea mays* seeds (No treatment), T3 = microgravity-exposed *Zea mays* seeds with augmented IAA growth hormone and cow dung, T4 = nonmicrogravity-exposed *Zea mays* seeds augmented with cow dung, T5 = microgravity-exposed *Zea mays*

seeds with increased IAA growth hormone, T6 = non-microgravity-exposed *Zea mays* seeds with increased IAA growth hormone, T7 = microgravity-exposed *Zea mays* seeds with increased IAA growth hormone and cow dung, T8 = nonmicrogravity-exposed *Zea mays* seeds with increased IAA growth hormone and augmented cow dung.

5) Determination of plant height and soil pH

The plant height was measured and recorded biweekly for 12 weeks. A ruler was used to measure from the top of the visible soil to the peak of the plant (Ovenseri et al., 2024). The method described by Sims and Eckerts (2011) was employed to measure the pH of soil contaminated with crude oil. First, the soil samples were left to air-dry, after which 5 g of the dried soil was combined with 5 mL of distilled water and mixed thoroughly. The mixture was then allowed to rest for 20 min before the resulting slurry was poured into a test tube. Finally, the electrode of a pH meter was placed into the slurry to obtain the pH measurement

6) Moisture content determination

The moisture content of the contaminated soil (Eq. 2) was determined as follows: A crucible was dried in an oven at 105°C for one hour to ensure that it was completely dry. The crucible was then transferred to a desiccator and allowed to cool for approximately 30 min. The weight of the crucible was measured using an electronic balance and recorded as W1.

Next, 1 g of the soil sample was weighed into a pre-weighed crucible (W2). The crucible and its contents were oven-dried at 105°C for 3 hours. After drying, the crucible was placed in a desiccator for approximately 10 min to cool and then reweighed. This process was repeated until a constant weight (W3) was achieved.

Moisture content (M%)

$$= ((W2 - W3) / (W3 - W1)) \times 100$$

(Eq. 2)

7) Microbial determination

One gram of soil was placed into sterile test tubes, followed by the addition of 9 mL of sterile distilled water. The mixture was then vortexed for 5 min and left to settle for another 5 min. An eightfold serial dilution was subsequently performed, and approximately 0.1 mL was subsequently plated onto nutrient agar and potato dextrose agar (PDA). Fungal identification was primarily based on morphological characteristics observed through lactophenol cotton blue staining and slide culture techniques as described by Barnett and Hunter (1987). The bacterial isolates were identified following the Bergey's manual. (Whitman, 2011). Identification was not based on a single test but rather on a combination of morphological, physiological, and biochemical analyses.

Key tests include Gram staining, motility and spore formation assays, as well as biochemical tests such as catalase, oxidase, and citrate utilization tests, which collectively enable differentiation at the species level.

8) Data analysis

Plant height, soil moisture content, soil pH, and microbial diversity were subjected to descriptive and inferential statistics using R for Windows (Version 4.3.1) and paleontological statistics (PAST 4.03). Boxplots, bar charts and line graphs were used to visualize the data, while analysis of variance (ANOVA) and Tukey's HSD tests were used to determine whether significant differences were detected across the various treatments and times (sampling months) for parametric data, whereas the Kruskal–Wallis test and Dunn post hoc test were used for nonparametric data.

Results and discussion

1) Plant height

The plant height generally tended to increase across treatments, with minimal differences observed between T1 and T2, followed by moderate fluctuations at T3 and T4. Pronounced growth was recorded from T5 onward, with a maximum plant height at T7 (Figure 1). T7 strongly positively affected plant height, although the effect was not significantly different from that of T8. The synergistic interaction of the treatments is responsible for the observed increase in plant height. While cow dung improves soil structure and promotes microbial-mediated nutrient cycling, simulated microgravity increases plant water uptake efficiency. Simultaneously, IAA-induced lateral root proliferation increases the root surface area, which makes it easier for plants to absorb water and vital nutrients. These mechanisms work together to increase plant growth (Ovenseri et al., 2024).

2) Soil moisture content

The contamination of crude oil affects the moisture content of the soil in adverse ways; it makes the soil hydrophobic, reduces pore connectivity, and affects the structure of the soil, thereby restricting water infiltration and movement and hence creating physiological drought. The soil moisture content was evaluated across all treatments (T1–T8) at different crude oil contamination levels (0%, 1%, 3%, and 5% v/w) over three sampling months. The results revealed low moisture in T1 and T2; improved moisture in T3; and reduced moisture in T4, T5 and T6 before a spike in T7 and T8 at 5% crude oil contamination (Figure 2A). The soil moisture content was lowest in the 1st month, particularly in the treatments without cow dung (T1, T2, T5, and T6).

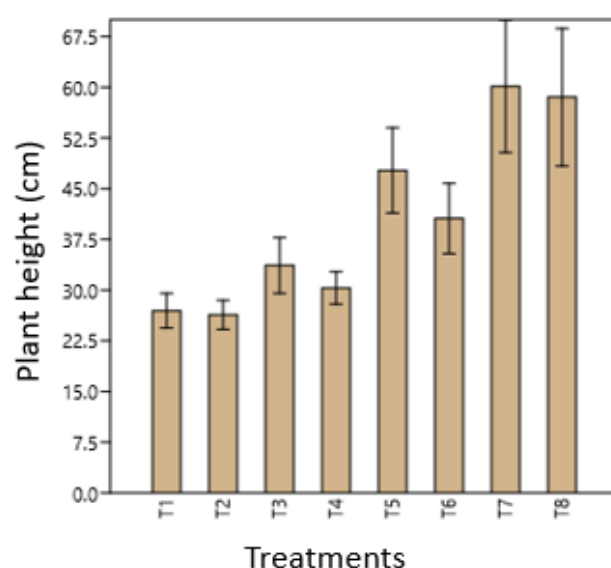


Figure 1 Plant height measurements after 12 weeks. The bars represent the means $\pm$ SEs. Different letters indicate significant differences among treatments (Dunn's post hoc test, $p < 0.05$). The x-axis represents the treatments, and the y-axis represents the mean plant height (cm).

However, an improvement was observed at the 3rd month, especially in T3, T7, and T8, where cow dung was combined with microgravity. These treatments resulted in high water retention capacity, which could be linked to the addition of cow dung enhancing the porosity and water-holding capacity of the soil. T1 (microgravity exposure) and T2 (no treatment) consistently had the lowest moisture values, indicating that exposure of *Zea mays* at the germination stage to microgravity alone was not sufficient to improve soil moisture. A significant difference was evident from the 1st month to the 3rd month ($p = 0.009$), indicating the influence of time and plant growth (Sonowal et al., 2018). In the 3% crude oil-contaminated soil (Figure 2B), the trend was consistent with the 5% contamination results. In treatments T3, T4, T7, and T8, the moisture content significantly increased from the 1st month to the 3rd month ($p = 5.2e-07$). The progressive increase in moisture content was most evident in T7 and T8. Although all the treatments improved somewhat over time, T3, T4, T7, and T8 consistently yielded better results. In the uncontaminated control soil (0% crude oil) (Figure 2D), the treatments resulted in the highest moisture content across all the contamination levels. T8, with the combination of cow dung and IAA, recorded the highest value (~35%) by the 3rd month, although it was not significantly different from that of T7 (cowdung, IAA and microgravity). The addition of cow dung played an important role in improving the moisture content of the contaminated soil. Egobueze et al. (2019) reported improved physicochemical parameters, including the moisture content in cow dung-augmented crude oil-

degraded soil. The effect of the cow dung resulted in a weak positive ($p = 0.2944$) correlation between the moisture content and concentration of crude oil in the soil, indicating a weak relationship between the moisture content and crude oil concentration. Other factors, such as the uniform gram of cow dung applied across all concentrations, might have influenced the outcome. Egobueze et al. (2019) reported that for optimal performance, the ratio of cow dung to contaminated soil varies according to the concentration of crude oil in the contaminated soil. Cow dung is a rich source of organic matter, and when added to crude oil-contaminated soil, it helps create a crumbly structure with large pores, and these pores allow for better water infiltration and storage while also improving drainage (Hussain et al., 2023). The decrease in soil moisture content with increasing crude oil concentration is in agreement with the findings of other reports (Akpokodje and Uguru, 2019; Barua et al., 2011; Ighovie and Ikechukwu, 2014). The moisture content was highest in the 0% crude oil-contaminated sample and lowest in the 5% crude oil-contaminated sample. The decrease in the moisture content with increasing crude oil concentration is attributed to the hydrophobic nature of crude oil, which causes low infiltration of water by coating the soil surface (Akpokodje and Uguru, 2019; Ovenseri et al., 2024). The roots of plants create a path that allows air into the soil and releases exudates, which then activate aerobic microbes to act on the crude material by breaking it down and reducing its hydrophobicity (Njoku et al., 2022). The result of 0% crude oil contamination highlights that even in clean soils, the synergistic effect of cow dung, IAA, and microgravity improves moisture retention, making these treatments beneficial beyond remediation purposes. All the treatments significantly differed across months (5% $p = 0.00995$, 3% $p = 5.2e-07$, 1% $p = 1.72e-13$). A significant difference was consistently observed between the 1st and 3rd months across the various crude oil concentrations.

3) Soil pH

The variations in soil pH across the different treatments (T1–T8) and crude oil contamination levels (0%, 1%, 3%, and 5% v/w) over the three sampling months are shown in Figure 3. The results show that both crude oil concentration and treatment combination significantly influenced the soil pH pattern over time. The soil pH remained relatively stable, ranging between 6.11 and 6.65 across all the treatments and months in the control, indicating that the soil was slightly acidic. The lowest pH values were recorded in the 5% crude oil-contaminated soil during the 1st month, with T1, T2, and T5 having pH values as low as 5.34–5.61, indicating soil acidity. However, during the 3rd month, substantial recovery was observed in T7 and T8, where the soil pH increased to 6.45 and 6.65, respectively, and approached

neutrality. Overall, soil pH showed a time-dependent recovery trend, increasing toward neutrality across treatments. The pH value of all the treatments increased toward neutrality as the duration increased. A significant difference ($p = 0.001$) was observed between the soil pH obtained during different months of sampling, suggesting that the period affected the soil pH, but the correlation between the soil pH and the sampling period was low, suggesting that the observed changes might have been influenced by other factors. These results are in agreement with those of Njoku et al. (2008), who reported a weak correlation between the number of days of sampling and soil pH. Slight changes in soil pH were recorded from month to month. Soil pH affects the growth of plants chiefly by impacting the availability of nutrients. Relatively high or low pH leads to deficiencies in essential plant nutrients that are needed for the proper growth of plants (Oyem and Oyem, 2013). Essential plant elements such as calcium, magnesium and phosphorus are deficient in highly acidic soil, resulting in crop retardation (Oyem and Oyem, 2013). Soil pH also affects the soil microflora. It can promote or hinder the growth of useful microbes that help breakdown organic matter in soil (Oyem and Oyem, 2013). The suitable pH value for most microbes is between 6 and 8, with its optimal functionality being 7 (Wang et al., 2019). Cowdung has a liming effect that helps increase soil pH and decrease acidity because of its calcium and magnesium contents (Jidere et al., 2012; Njoku et al., 2012), whereas simulated microgravity and IAA stimulate the plant to release flavanols through its roots (Mehmood et al., 2018; Takahashi et al., 2021). The flavanols signal rhizobacteria, and the rhizobacteria breakdown and cycle nutrients, which helps to stabilize soil pH (Nie et al., 2025; Takahashi et al., 2021).

4) Soil microbial diversity

The isolated microbial species found in the soil samples were subjected to different crude oil contamination levels and treatment combinations (Table 1). The presence (+) or absence (-) of bacterial and fungal species across treatments resulted in changes in microbial abundance in response to crude oil pollution. The microbial mass observed in the 0% crude oil (control) included *Enterobacter cloacae*, *Micrococcus luteus*, *Penicillium chrysogenum*, and *Aspergillus niger*. Additionally, treatments involving cow dung (CD), indole-3-acetic acid (IAA), and simulated microgravity (MG), such as T3 and T7, resulted in increased detection of microbial species, including beneficial fungi (*Aspergillus niger*, *Penicillium chrysogenum*, *Debaryomyces hansenii*) and bacteria (*Pseudomonas aeruginosa*). These treatments promoted microbial diversity even under 5% crude oil stress, suggesting their role in enhancing microbial resilience and bioremediation.

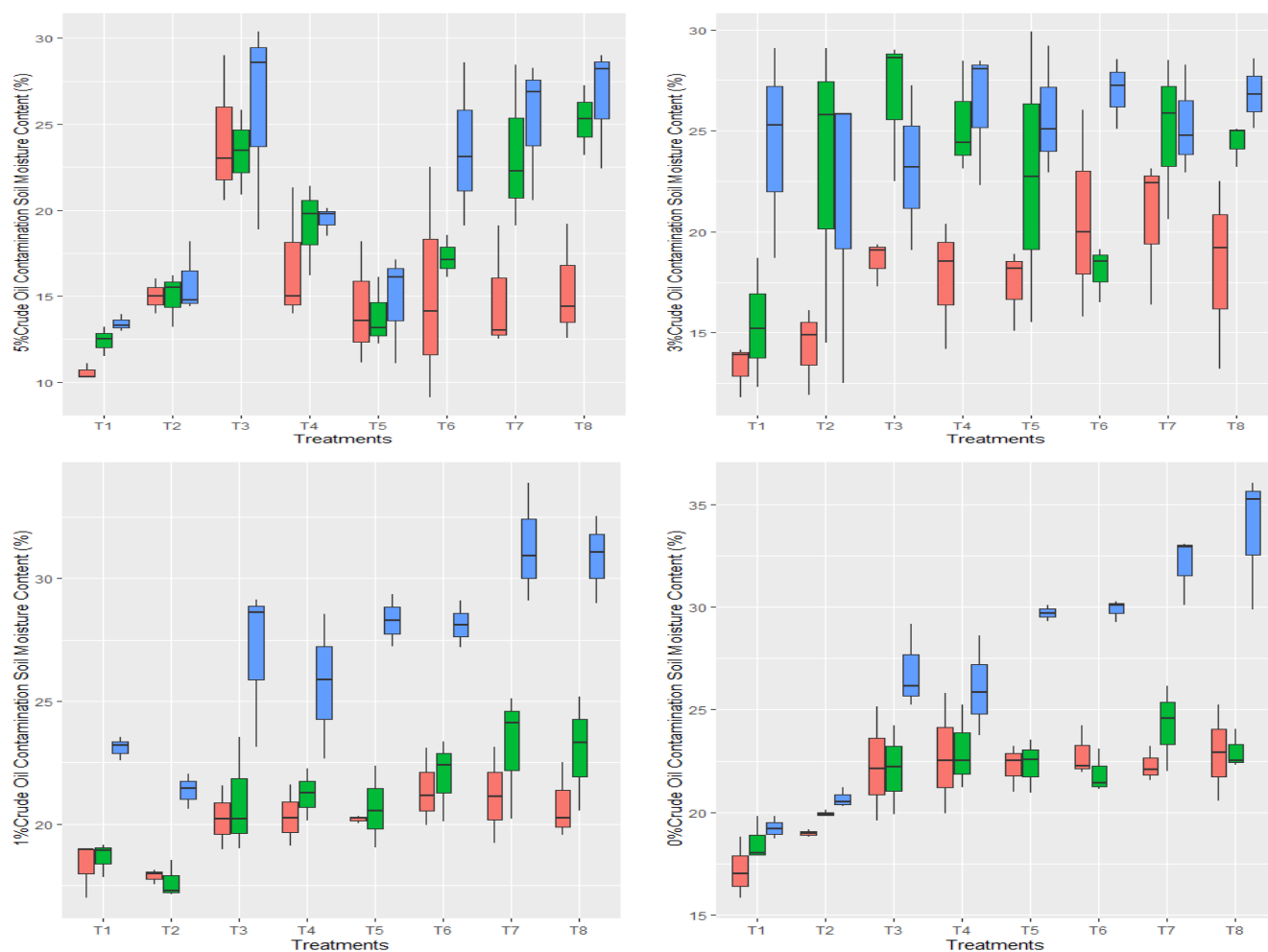


Figure 2 A-D: Influence of the treatments on the moisture content (%) of 5% - 0% crude oil-contaminated soil. The x-axis represents the treatments, and the y-axis represents the measured soil moisture content (%). Red, green, and blue plots represent the 1st, 2nd, and 3rd months of study, respectively.

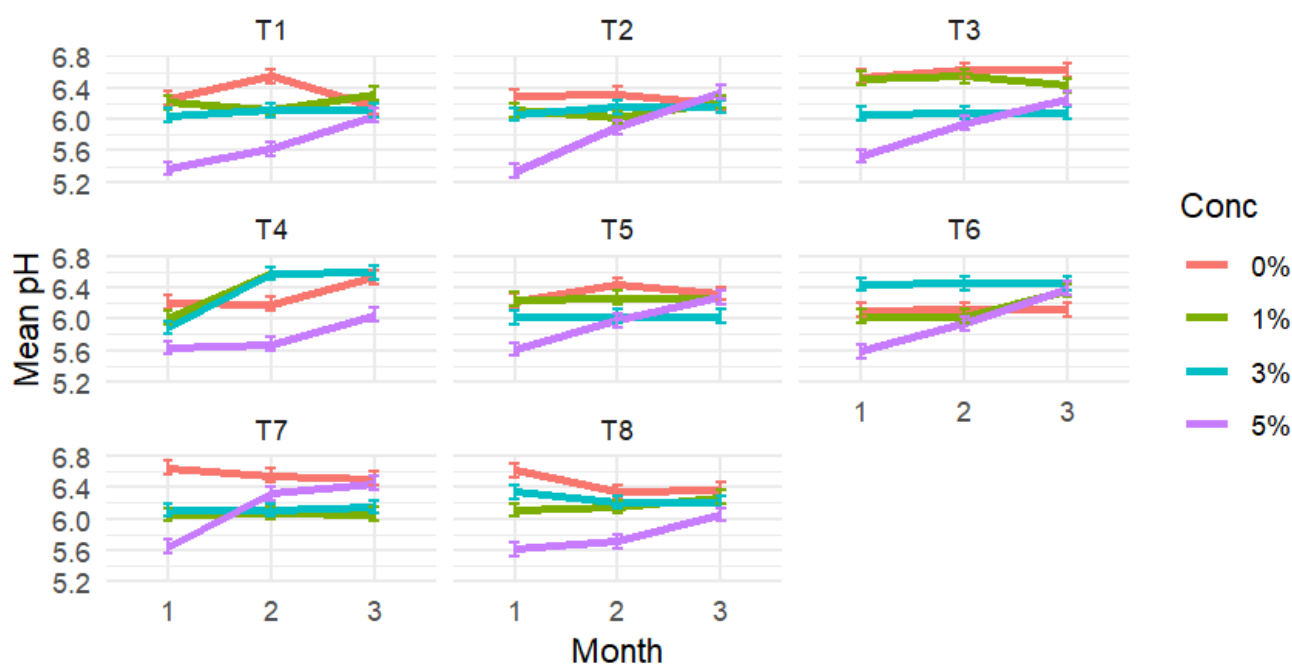


Figure 3 Monthly changes in soil pH under the different treatments and crude oil concentrations. The x-axis represents the sampled months, and the y-axis represents the mean soil pH (T1–T8 = treatments).

The results of the study revealed the presence of microbes in all the treatments, including the control; however, the diversity and richness of the microbes differed across the treatments, with T7 having the highest microbial diversity index (3.18) (Figure 4). When soil is degraded by crude oil, the amount of nutrients available in the rhizosphere of plants decreases, and the oleophilic organisms inhabiting the rhizosphere breakdown contaminants in the soil and derive energy from the contaminant to perform other activities, such as recycling nutrients for plants (Santhos et al., 2018; Wang et al., 2019). The following bacteria and fungi were isolated in this study: *Enterobacter cloacae*, *Micrococcus luteus*, *Penicillium chrysogenum*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Fusarium oxysporum*, *Debaryomyces hansenii*, *Alcaligenes faecalis*, and *Proteus vulgaris* (Table 1). Some of these organisms have been previously identified as rhizospheric microbes in crude oil-contaminated soil (Santhos and Maranho, 2018; Singh and Goodwin, 2022; Yang et al., 2023). Our findings revealed an increase in species diversity and richness in 5% crude oil-degraded soil with a combination of MG, IAA and CD (T7) treatment compared with those in the control with no treatment (T2) (Figure 4). These findings highlight the contributory impact of the treatments on improving the soil physicochemical properties and microbial diversity, which in turn improved the growth and productivity of plants (Ovenseri et al., 2024). While not directly measured, crude oil causes phosphate stress in soil; the components of crude oil bind with phosphate, reducing its bioavailability for plant uptake (Singh and Goodwin, 2022). On the basis of the established literature, the microbes *Alcaligenes faecalis* and *Bacillus cereus* are notable phosphorus mobilizers and solubilizers, whereas *Pseudomonas* plays a specific role in soil organic phosphate mineralization (Singh and Goodwin, 2022). The literature suggests that these organisms improve biogeochemical nutrient cycling, which plants need for proper functioning (Yang et al., 2023). The roots of *Zea mays* have been reported to release secondary metabolites such as ben-zoxazinoids (BX) and dolabrallexins, which influence rhizosphere microbial community structure and microbiome traits [20]. The metabolite kauralexins, released by the roots of *Zea mays*, strongly inhibits the growth of the fungal pathogen *Fusarium oxysporum*, which is also a crude oil-degrading fungus. When plants are under stress, they are vulnerable to pathogens. The absence of the fungus (*Fusarium oxysporum*) in the control and in 5% crude oil (T7) suggested that the release of the metabolite kauralexins from the plant roots was not impacted by the stress posed by the contaminant. Toxic stress has been reported to affect the rate at which a metabolite is released (Murphy et al., 2021).

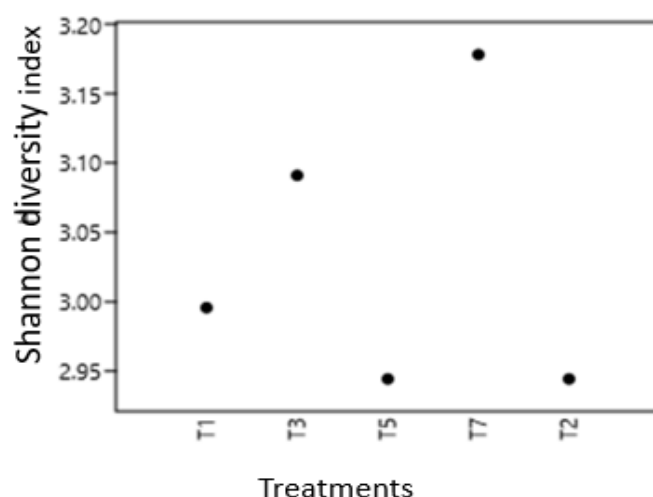


Figure 4 Shannon microbial diversity index of the treatment group.

The coliform count data (Figure 5) further support this observation. Across all the treatments, T7 (5% crude oil + MG + IAA + CD) resulted in the highest bacterial colony count, approaching $160 \text{ cfu per g} \times 10^{-3}$, which was significantly greater than that in all the other treatments. These findings indicate a robust microbial response when all three interventions were combined. Similarly, fungal counts were also highest in the CD-containing treatments, although they were lower overall than bacterial populations were. In contrast, the microbial counts were lowest in the untreated soils, especially at higher contamination levels (5%), reflecting the inhibitory effects of crude oil on microbial survival and proliferation. Moderate microbial recovery was observed in the treatments with individual amendments (e.g., T1 and T5), but the greatest improvement was consistently recorded in the integrated treatments (T7). The bacterial coliform count in the 5% crude oil-degraded soil across all the treatments was relatively higher than the fungal coliform count. This could be attributed to the bacterial advantages of having a wide range of enzymes and metabolic pathways that enable them to breakdown petroleum hydrocarbons. Additionally, they can form biofilms that serve as protective supplements against environmental stress. The sensitivity of fungi to varied soil pH and the inability to form biofilms such as bacteria could explain the low count.

Conclusion

In this study, the effects of cow dung, IAA, and simulated microgravity treatment on the physicochemical properties and microbial diversity of crude oil-degraded soil were evaluated. Crude oil contamination negatively affects plant height, soil moisture content, soil pH, and microbial diversity. However, the combined treatments of simulated microgravity exposure, cow dung, and IAA significantly improved soil physicochemical parameters, enhanced microbial diversity, and promoted overall plant growth in the degraded soil. During the 3-month

pot experiment, the most effective treatment (T7: microgravity, IAA, and cow dung) optimized the soil pH and moisture content and increased the microbial diversity. It significantly improved the soil moisture content at 5% crude oil contamination. This synergistic approach emphasizes the potential of integrating cow dung, IAA, and simulated microgravity for restoring contaminated soils. It also highlights the critical role of innovative soil restoration techniques in mitigating environmental degradation caused by crude oil pollution. Future research should focus on overcoming the limitations of this research by scaling these findings to

field conditions, employing a molecular approach for microbial identification, and optimizing the treatment ratios for varying levels of contamination. In addition, functional validation of microbial activity, including enzyme assays, hydrocarbon degradation analyses, and functional gene profiling, should be incorporated to clarify the specific roles of microbial communities in soil recovery processes. Such integrative approaches will provide deeper mechanistic insights into plant–microbe–soil interactions and enhance the applicability of these strategies in diverse environmental settings.

Table 1 Fungi and bacteria isolated from the various contaminated soils after the experimental trial

Treatment	<i>Enterobacter cloacea.</i>	<i>Micrococcus luteus</i>	<i>Penicillium chrysogenum</i>	<i>Aspergillus niger</i>	<i>Pseudomonas aeruginosa</i>
0% CO	+	+	+	+	-
1%CO +T1	+	-	+	+	+
3%CO +T1	+	-	+	+	+
5%CO +T1	+	-	-	-	+
1%CO +T2	+	-	+	+	+
3%CO +T2	+	-	+	+	+
5%CO +T2	+	-	+	+	-
1%CO +T3	-	-	+	+	-
3%CO +T3	-	-	+	+	+
5%CO +T3	-	-	+	+	+
1%CO +T5	+	-	+	+	+
3%CO +T5	-	-	-	+	+
5%CO +T5	-	-	+	-	+
1%CO +T7	+	+	-	+	+
3%CO +T7	+	+	-	+	+
5%CO +T7	+	+	+	+	+

Remark: *CO – Crude oil contamination

Table 1 Fungi and bacteria isolated from the various contaminated soils after the experimental trial (Continued)

Treatment	<i>Bacillus cereus</i>	<i>Fusarium oxysporum</i>	<i>Debaryomyces hansenii</i>	<i>Alcaligenes faecalis</i>	<i>Proteus vulgaris</i>
0% CO	-	-	-	-	-
1%CO +T1	+	+	-	-	-
3%CO +T1	-	+	-	-	-
5%CO +T1	+	+	+	+	-
1%CO +T2	-	+	-	-	-
3%CO +T2	-	-	+	-	-
5%CO +T2	+	-	+	+	-
1%CO +T3	+	+	-	-	-
3%CO +T3	+	+	-	-	-
5%CO +T3	+	+	-	-	+
1%CO +T5	-	+	-	-	-
3%CO +T5	+	+	-	-	-
5%CO +T5	+	+	+	+	+
1%CO +T7	-	-	-	-	-
3%CO +T7	-	-	-	+	-
5%CO +T7	+	-	+	+	-

Remark: *CO – Crude oil contamination

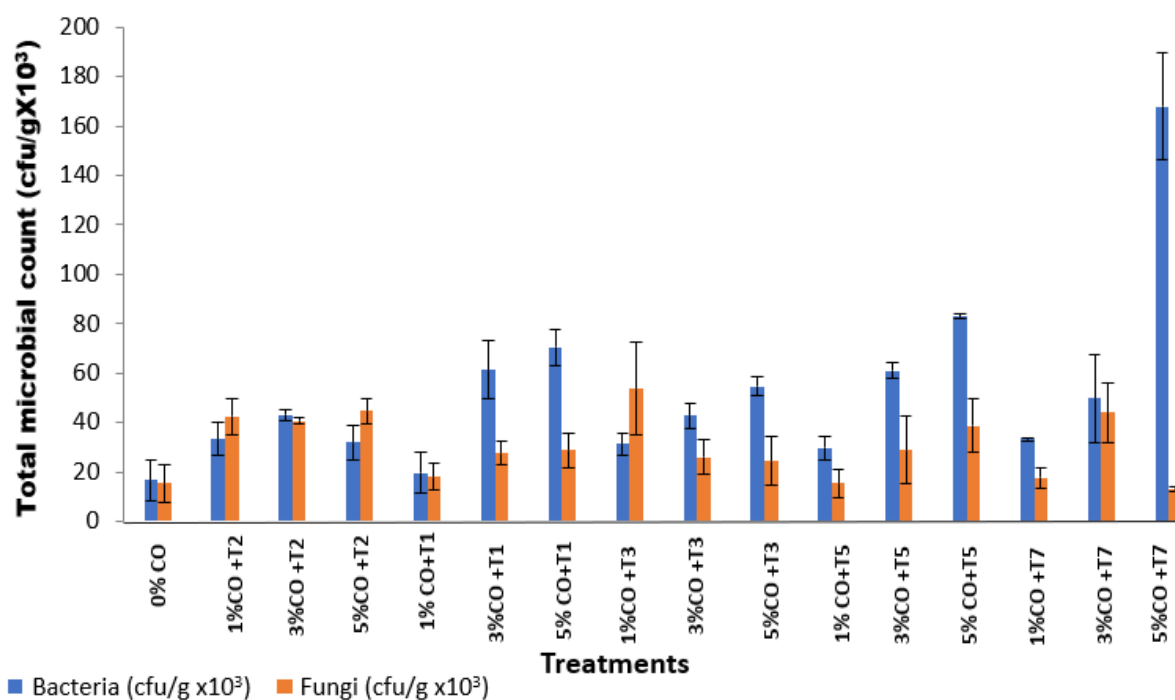


Figure 8 Total microbial count of bacteria and fungi in the rhizosphere of the different treated soils.

Remark: CO - Crude oil, where 0%, 1% 3% and 5% are the different contamination levels. The x-axis represents the treatments and contaminant concentrations, and the y-axis represents the microbial coliform count (cfu per g×10³).

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Data availability statement

Information and data used in the study will be disclosed upon request.

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Conflicts of interest

The authors declare that there are no conflicts of interest in competing financial or personal relationships that could have appeared to influence the work reported in this work.

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