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Synergistic bioaugmentation and biostimulation enhance peanut (*Arachis hypogaea*) productivity in diesel- and waste-oil-contaminated soils

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ABSTRACT

This study aimed to improve the productivity of peanut (*Arachis hypogaea*) cultivated in soils polluted with diesel and engine oils, a type of contamination known to disrupt soil structure, reduce fertility, and limit crop yields. To address this challenge, indigenous microflora were isolated from a previously contaminated experimental plot and used to develop biodegradation treatments capable of enhancing both pollutant removal and plant performance. The experimental approach involved applying individual and combined strategies of bioaugmentation, using *Rhodococcus erythropolis* or an indigenous bacterial consortium, and biostimulation, using poultry manure or *Bacillus amyloliquefaciens*. Pollutant degradation, along with peanut growth, physiological responses, and yield attributes, was systematically monitored. The results identified a competent indigenous microflora composed of strains affiliated with *Micrococcus*, *Lactobacillus*, *Planococcus*, *Achromobacter*, and *Serratia*. Combined treatments produced the most substantial benefits, significantly improving plant vigor and yield while accelerating petroleum hydrocarbon removal. The highest degradation rates were achieved with Consortium + poultry manure (76.2%), R. erythropolis + poultry manure (76.9%), Consortium + *B. amyloliquefaciens* (84.38%), and *R. erythropolis* + B. amyloliquefaciens (98.57%). These synergistic combinations consistently outperformed single-factor treatments. The findings demonstrate that integrating bioaugmentation and biostimulation is an effective approach for restoring the health and fertility of petroleum-polluted soils. Such combined strategies not only enhance the biodegradation of diesel and engine oils but also substantially improve peanut growth and productivity, offering a sustainable and applicable solution for managing contaminated agricultural lands.

Contribution/Originality: This study is one of the few to combine indigenous microflora with targeted bioaugmentation and biostimulation, simultaneously enhancing hydrocarbon degradation and peanut productivity. Its primary contribution is demonstrating that specific microbe–amendment synergies significantly improve both soil restoration and crop performance in petroleum-contaminated soils.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous crop native to Latin America [1] and is now widely cultivated throughout the intertropical zone. It holds significant nutritional and economic importance, ranking as the sixth most

important oilseed crop globally [2]. Groundnut seeds contain 48–50% oil, 26–28% protein, and are rich in fiber, minerals, and vitamins [2]. The crop is cultivated in more than 100 countries, covering approximately 26.4 million hectares, with an average yield of 1.4 t/ha [3].

Despite its potential, groundnut production faces several constraints, including fungal and bacterial diseases, insect pests, adverse climatic conditions, and, more critically, soil fertility issues. These soil-related challenges manifest as compaction, nutrient and organic matter deficiencies, excessive acidity, salinity, and reduced biological activity [4, 5]. In some cases, soil fertility decline is further exacerbated by pollution resulting either from the excessive use of pesticides and chemical fertilizers or from the disposal of industrial waste into agricultural lands [6, 7].

Soil contamination by untreated industrial effluents remains a major environmental concern [8]. Such effluents may contain dyes, paints, and their derivatives, volatile organic compounds, as well as combustion residues such as fly ash. Among these pollutants, petroleum hydrocarbons including crude oil, gasoline, diesel, and lubricating oils represent a dominant category, accounting for nearly 80% of soil pollution cases. Their impacts extend to soil quality, natural resources, vegetation, soil microflora and microfauna, and even human health [9].

Petroleum hydrocarbons and lubricating oils, being insoluble in water, disrupt the physical structure of soils, reduce water-holding capacity, and increase surface runoff. They can also eliminate beneficial soil microorganisms and microfauna, which play essential roles in nitrogen fixation, and phosphorus and potassium solubilization, ultimately leading to a progressive decline in soil fertility [10, 11].

Petroleum contamination of agricultural soils thus poses a growing threat to food crop production, particularly in areas located near extraction sites, storage facilities, or fuel transportation corridors [12]. Groundnut, a strategic crop for human nutrition, vegetable oil production, and household income, is especially sensitive to soil disturbances caused by hydrocarbon pollution [13]. In the context of increasing demand, it is therefore imperative to develop agronomic, biological, and technological approaches capable of mitigating the harmful effects of petroleum hydrocarbons and restoring soil productivity.

Several remediation strategies have been proposed. Biostimulation involves the addition of organic matter and essential nutrients (N and P) to enhance the activity of indigenous hydrocarbon-degrading microorganisms [14]. Bioaugmentation involves inoculating specialized bacterial and/or fungal strains (e.g., *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, ligninolytic fungi) [15].

Rhizodegradation and phytoremediation exploit the synergistic interaction between plant roots and microorganisms [16]. Other methods include the application of stabilizing amendments such as biochar or activated clays, and complementary agronomic practices tailored to groundnut cultivation, such as rhizobial inoculation, plant growth-promoting rhizobacteria (PGPR) application, or mycorrhization [17-19].

The general objective of this study is to contribute to the improvement of groundnut productivity in soils contaminated with diesel and lubricating oils. Specifically, the study aims to (i) characterize the competent microflora present in the soil, (ii) assess the effects of diesel and lubricating oils on groundnut growth parameters, and (iii) evaluate the effectiveness of different soil treatments.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study included groundnut seeds, commercial diesel fuel, engine oils, bacterial strains, and poultry manure.

- The groundnut variety selected was the local cultivar *Ngonibayon*, chosen for its relatively short growth cycle of 90 days and its multiple agronomic, nutritional, economic, and health-related benefits, as well as its adaptability to saline soils.

- Commercial diesel and mineral engine oil were employed as soil pollutants. Both were purchased from a local automobile fuel station. Commercial diesel consists of approximately 75% aliphatic hydrocarbons with carbon chain lengths ranging from C_{11} to C_{24}, and 24.7% aromatic hydrocarbons. Mineral engine oil, derived from crude petroleum, is primarily composed of hydrocarbons with traces of sulfur- and nitrogen-containing compounds.
- Three categories of microorganisms were used in this study: two exogenous bacterial strains (*Rhodococcus erythropolis* and *Bacillus amyloliquefaciens*) and one indigenous bacterial consortium.
- Rhodococcus erythropolis was obtained from the Bioindustry Laboratory of Gembloux Agro-Bio Tech, University of Liège (Belgium). The genus Rhodococcus comprises highly versatile bacteria capable of degrading a wide range of organic compounds, including recalcitrant and toxic molecules [20]. They can metabolize aliphatic, branched, cyclic, polycyclic aromatic, sulfur-containing, and chlorinated hydrocarbons, and tolerate environmental stresses such as extreme temperatures, water scarcity, high or low pH, salinity, nutrient limitation, and the presence of toxic solvents or pollutants [21, 22]. In this study, Rhodococcus erythropolis was used as a reference strain to compare degradation efficiency with the indigenous bacterial consortium.
- *Bacillus amyloliquefaciens*, also obtained from the same laboratory, is a plant growth-promoting rhizobacterium (PGPR). It produces antifungal and antibacterial metabolites, acts as an antagonist to soilborne pathogens, and stimulates plant defense mechanisms. This bacterium, naturally associated with plant rhizospheres, is resistant to various biotic and abiotic stresses.
- The indigenous bacterial consortium was isolated from polluted soil sediments at the experimental site, developed as a bacterial starter capable of hydrocarbon degradation, and characterized in this study.
- Poultry manure was collected from a local poultry farm. Derived from both solid and liquid excreta of chickens, poultry manure is among the most nutrient-rich organic fertilizers, particularly in nitrogen and phosphorus content. Its composition varies depending on production system (intensive vs. extensive), litter type (wood shavings, straw), and poultry diet. On average, dried poultry manure contains 2–4% nitrogen, 2–4% phosphorus (P_2O_5), 1.5–3% potassium (K_2O), 4–8% calcium, and 60–75% organic matter [23].

2.2. Methods

2.2.1. Characterization of Hydrocarbon-Degrading Microflora

2.2.1.1. Artificial Soil Pollution

Following the method of Tam and Wong [24] with modifications, soil ridges were artificially contaminated with a mixture of commercial diesel, used engine oil, and hexane. Soil was collected into heaps and irrigated with the pollutant mixture using a watering can. Homogenization was achieved by repeated shoveling after each pollutant application (Figure 9). The contaminated soil was reconstituted into ridges and left to stabilize for two weeks, allowing for complete hexane evaporation and hydrocarbon adsorption to soil particles before sowing and treatment application.

2.2.1.2. Isolation of Bacterial Colonies

The aim was to obtain a competent bacterial consortium capable of degrading diesel and engine oil using the serial dilution technique.

Microbial enumeration. One gram of soil was added to 9 ml of sterile peptone water (Peptone 10 g, NaCl 5 g, Na_2HPO_4 9 g, K_2HPO_4 1.5 g; pH 7.0 ± 0.2) to obtain a 10^{-1} dilution. Successive dilutions were prepared up to 10^{-8}. From 10^{-3}, 100 µl aliquots were plated onto (i) a nutrient-rich medium (glucose 20 g, peptone 10 g, yeast extract 10 g, agar 15 g, pH 7.0 ± 0.2) for total microflora and (ii) a selective medium (diesel at 10 ppm as the sole carbon source,

supplemented with mineral salts) for hydrocarbon-degrading microflora. All media were sterilized at 120 °C for 20 min and poured into Petri dishes.

Each dilution was plated in triplicate and incubated at 35 °C for 48–72 h. Colony counts were performed on plates containing 30–300 colonies. Results were expressed as CFU/ml using the formula.$N = \frac{n}{d \times v}$

Where n = number of colonies, d = dilution factor, and v = inoculated volume.

Bacterial consortium production. A mother suspension was prepared by inoculating 1 g of polluted sediment into 9 ml of sterile peptone water, followed by serial dilutions up to 10^{-7}. Aliquots (100 µl, in triplicate) were spread on mineral medium supplemented with 10 ppm diesel. Hydrocarbonoclastic colonies were harvested, transferred into 100 ml of nutrient broth, and incubated for 48 hours under agitation. Cultures were subsequently scaled up into 1 L flasks, incubated at 35 °C for 96 hours under agitation, centrifuged at 3,500 rpm for 10 minutes, and the bacterial biomass obtained was preserved with 2.5% glycerol as a cryoprotectant.

2.2.1.3. Consortium Characterization

Morphological traits. Macroscopic colony morphology (shape, elevation, margin, pigmentation) was visually assessed. Microscopic examination was conducted after Gram staining (crystal violet, iodine, alcohol decolorization, fuchsin), observed under oil immersion (×100 objective) to determine shape, size, and cell arrangement.

Biochemical traits. Catalase and oxidase tests were performed following [25]. Catalase activity was detected by bubble formation upon addition of hydrogen peroxide to fresh cultures. Oxidase activity was determined by the color change to violet on oxidase reagent–impregnated filter paper.

Bacterial identification was further carried out using the API 20E system [26], which characterizes biochemical profiles of Enterobacteriaceae and non-fastidious Gram-negative bacilli. Microtubes were inoculated with bacterial suspensions, incubated at 37°C for 24 hours, and interpreted according to the manufacturer's codebook.

2.2.2. Assessment of Diesel and Engine Oil Effects on Groundnut Growth

2.2.2.1. Experimental Design

A partially randomized block design was established on a 920 × 410 cm field plot comprising ridges previously contaminated with the diesel–oil mixture (see section 2.2.1.1). Each ridge (2.4 m², 50 cm spacing) received one of ten treatments.

- T0: Polluted soil (Positive control).
- T1: Non-polluted soil (Negative control).
- T2: Polluted soil + *R. erythropolis* (10^7 CFU/g soil).
- T3: Polluted soil + *B. amyloliquefaciens* (10^7 CFU/g).
- T4: Polluted soil + 864 g poultry manure.
- T5: Polluted soil + indigenous bacterial consortium (10^7 CFU/g).
- T6: Polluted soil + *R. erythropolis* + *B. amyloliquefaciens* (10^7 CFU/g each).
- T7: Polluted soil + consortium + *B. amyloliquefaciens* (10^7 CFU/g each).
- T8: Polluted soil + 864 g poultry manure + *R. erythropolis* (10^7 CFU/g).
- T9: Polluted soil + 864 g poultry manure + consortium (10^7 CFU/g).

2.2.2.2. Sowing

Sowing was conducted two weeks after soil contamination. Two seeds were sown per hole, spaced 40 cm between rows and 15 cm within rows, totaling three rows of seven lines per ridge. Irrigation with tap water was carried out twice daily.

2.2.2.3. Growth Parameters

Measurements were taken at 2, 4, 6, and 10 weeks after germination on five plants per treatment.

- Leaf number: Counted manually [27] and averaged per plant [27]:

  $Nf = \frac{Na}{Np}$

  where N_a = total leaves counted on sampled plants, N_p = number of sampled plants.
- Leaf area: Calculated from the third leaf on the main stem following [28].

  $S = L \times l \times 0{,}80 \times N \times 0{,}662$ (cm²)

  Where L = leaf length, l = maximum width, N = total leaves per plant; 0.80 and 0.662 are proportionality factors [29].
- Plant height: Measured from the collar to the main apex using a measuring tape.

2.2.2.4. Chlorophyll and Carotenoid Content

At flowering, 0.80 g of fresh leaves were collected, washed, ground with 10 ml of 80% acetone, $CaCO_3$, and sterile sand, then centrifuged at 3,500 rpm for 5 minutes. Supernatant absorbances were read at 663, 645, and 470 nm using a BIOBASE BK UV-1900 spectrophotometer. Concentrations (mg/L) were calculated according to Billot [30].

$Chl\ a = 12{,}5\ (DO_{663}) - 2{,}79\ (DO_{645})$

$Chl\ b = 21{,}5\ (DO_{645}) - 5{,}1\ (DO_{663})$

$Chl\ (a+b) = 7{,}15\ (DO_{663}) + 18{,}71\ (DO_{645})$

$$Caroténoïdes = \frac{1000\ xDO_{470} - (1{,}82\ xChla + 85{,}02\ xChlb)}{198}$$

2.2.2.5. Fresh Pod Weight and Seed Dry Weight

At maturity (3 months), 100 pods per treatment were harvested and weighed. Seeds were shelled, dried at 70°C for 72 hours, and weighed to a precision of 0.01 g.

2.2.3. Evaluation of Treatment Effectiveness

2.2.3.1. Soil pH

Measured at 1, 3, and 6 weeks. Ten grams of dried, sieved soil were mixed with 50 ml of distilled water or KCl, equilibrated for 10 minutes, and measured using a pH meter.

2.2.3.2. Residual Hydrocarbons

Residual hydrocarbons were quantified following MA.415-HGT. Five grams of dried soil were extracted with hexane in the presence of silica, filtered, evaporated, and weighed. Hydrocarbon mass was calculated as.

$$m = mf - mi$$

Where mf = Mass of beaker after extraction, mi = Tare mass.

2.2.4. Statistical Analysis

Data were processed in Microsoft Excel (means, standard deviations, graphs) and analyzed using one-way ANOVA (Minitab 21, Fisher's LSD test, $\alpha = 0.05$).

3. RESULTANTS

3.1. Isolation of Hydrocarbonoclastic Bacteria

3.1.1. Determination of Total and Competent Microflora

Microbiological counts were performed on soil samples to quantify both the total microflora and the hydrocarbonoclastic (competent) microflora.

- Total microflora: defined as the overall bacterial population present in 1 g of uncontaminated soil. Enumeration was performed on a nutrient-rich medium containing glucose as a carbon source.
- Competent microflora (Hydrocarbonoclastic bacteria): refers to bacteria capable of mineralizing carbon from hydrocarbons. This group was quantified using a minimal mineral medium.

After incubation and colony counting, the total microflora in uncontaminated soil was estimated at 8.1×10^9 CFU·g^{-1}, while the hydrocarbonoclastic microflora reached 1.02×10^3 CFU·g^{-1} on minimal medium.

3.1.2. Characterization of Competent Bacterial Isolates

The isolated bacterial strains were characterized at three levels: macroscopic, microscopic, and biochemical.

Table 1. Macroscopic examination of bacterial colonies.

Colony	Shape	Color	Margin	Opacity	Elevation	Surface
CB1	Circular	Orange	Entire	+++	Convex	Smooth
CB2	Circular	Pale yellow	Undulate	--	Flat	Smooth
CB3	Circular	Beige	Undulate	++	Convex	Rough
CB4	Circular	Beige	Undulate	++	Crateriform	Smooth
CB5	Circular	Pink	Entire	+++	Convex	Smooth

3.1.2.1. Macroscopic Characteristics

Macroscopic examination revealed five distinct colony morphotypes (CB1–CB5) (Table 1). All colonies were circular in shape but differed in pigmentation, margin type, opacity, elevation, and surface texture.

Table 2. Microscopic characteristics of bacterial isolates.

Colony	Motility	Gram	Shape	Arrangement
CB1	Motile	+	Coccus	Single
CB2	Motile	+	Coccobacillus	Single
CB3	Motile	+	Coccus	Single
CB4	Motile	–	Bacillus	Single
CB5	Motile	–	Bacillus	Single

Microscopic observations revealed notable morphological diversity among the five bacterial isolates (Table 2). Three isolates (CB1, CB2, and CB3) were Gram-positive, whereas isolates CB4 and CB5 were Gram-negative. Cell shapes ranged from cocci (CB1, CB3) to coccobacilli (CB2) and bacilli (CB4, CB5).

All isolates exhibited active motility, indicating a high colonization potential. The cells were mainly observed as single arrangements, suggesting non-aggregated growth.

The characteristics of CB4 and CB5, both mobile Gram-negative bacilli, suggest a possible affiliation with genera known for their role in biodegradation.

3.1.2.2. Microscopic Characteristics

All isolates were motile and appeared as single cells. CB1, CB2, and CB3 were Gram-positive cocci, whereas CB4 and CB5 were Gram-negative bacilli.

Table 3. Biochemical characteristics of endogenous bacterial isolates.

Test/Property			CB1	CB2	CB3	CB4	CB5
Energy metabolism	Oxidase		+	-	+	+	-
	Catalase		+	-	+	+	+
	Respiration		Aerobic	Anaerobic	Aerobic	Aerobic	Aerobic
Carbohydrate metabolism	TSI	Glucose	+	+	+	-	-
		Lactose/sucrose	+	+	+	+	+
		H_2S	-	-	-	-	-
		Gelatinase	+	+	+	-	+
	Arabinose		-	-	-	-	-
	ONPG		-	-	-	-	-
	Citrate perméase		-	-	-	-	+
	Mannitol/ Mobility	Mobility	+	+	+	+	+
		Mannitol	-	-	-	-	+
	Clark and Lub medium	VP	+	+	-	-	-
Proteinmetabolism	Moeller medium	ADH	-	-	-	-	-
		LDC	-	-	-	-	-
		ODC	-	-	-	-	-
	Urease/Indole medium	Urease	-	-	-	-	-
		Indole	-	-	-	-	-
		TDA	-	-	-	-	-

3.1.2.3. Biochemical Characteristics

Biochemical tests were performed using the API 20E gallery (Table 3).

- Energy metabolism: Catalase and oxidase activities indicated aerobic respiration in CB1, CB3, CB4, and CB5, whereas CB2 was anaerobic.
- Carbohydrate metabolism: Glucose was metabolized by CB1, CB2, and CB3; lactose/sucrose by all isolates. None produced H_2S. Gelatinase activity was detected in all strains except CB4. Arabinose and ONPG utilization were absent. Citrate and mannitol were only used by CB5.
- Protein metabolism: All isolates were negative for ADH, LDC, ODC, urease, indole, and TDA, indicating a lack of proteolytic, deaminative, or hydrolytic activity.

3.2. Agronomic Parameters of Peanut Plants

3.2.1. Leaf Number Dynamics

The progression of leaf number in *Arachis hypogaea* varied with treatment and developmental stage (Figure 1).

During the first two weeks after sowing (WAS), no significant differences were observed among treatments T0, T2, T5, T6, T8, and T9, while T9 exhibited significantly more leaves than T1, T3, T4, and T7. At 4 WAS, only T1 showed significantly fewer leaves compared to T2 and T5. At 6 WAS, T4 displayed the highest leaf number, significantly exceeding all other treatments except T3. At 8 WAS, T3 and T4 had significantly higher values than T0, T1, T2, and T9. At 10 WAS, T3 and T5 exhibited the maximum values, significantly higher than those of T0, T1, T2, and T9.

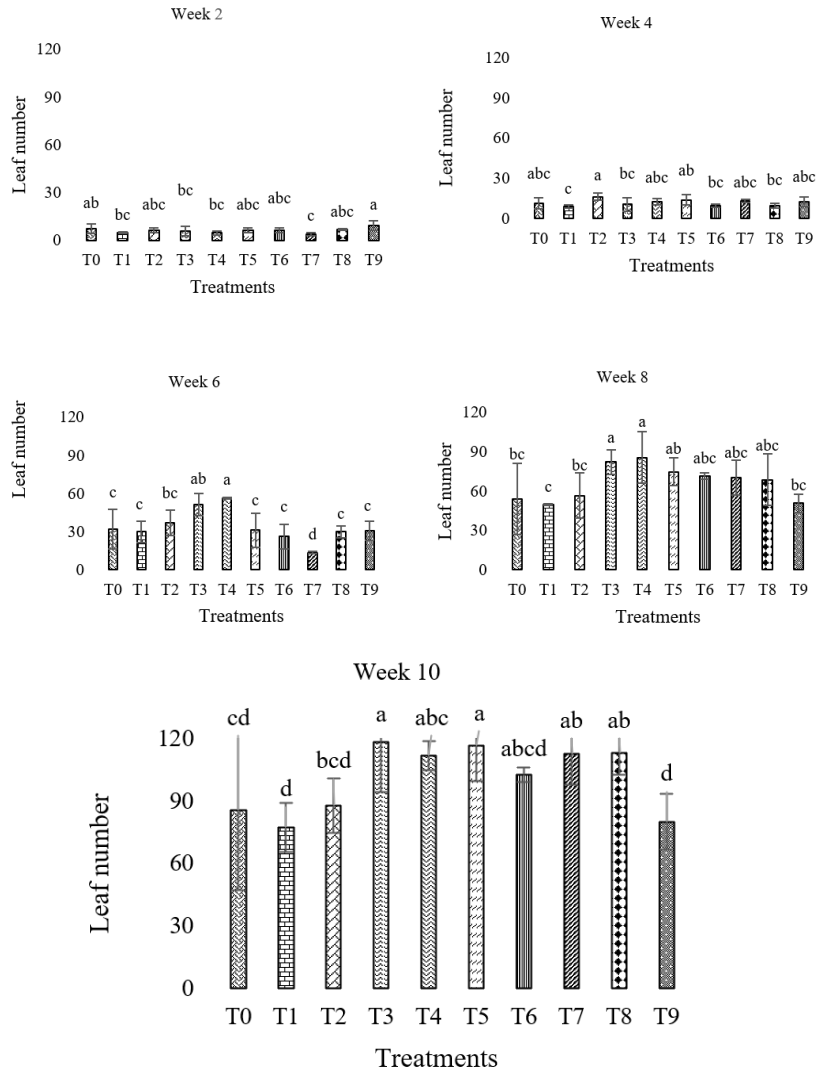
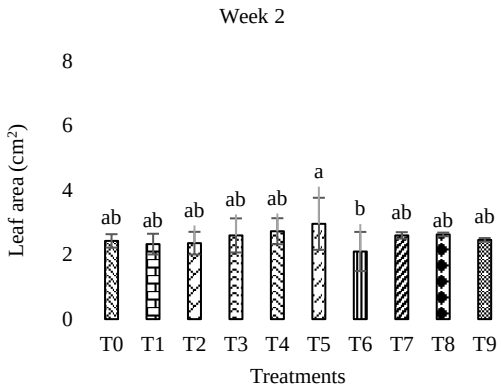


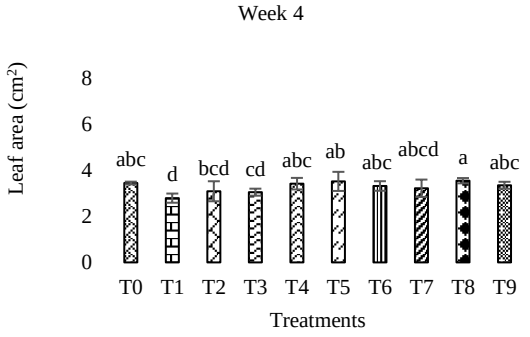
Figure 1. Evolution of the number of leaves in *Arachis hypogaea* plants.

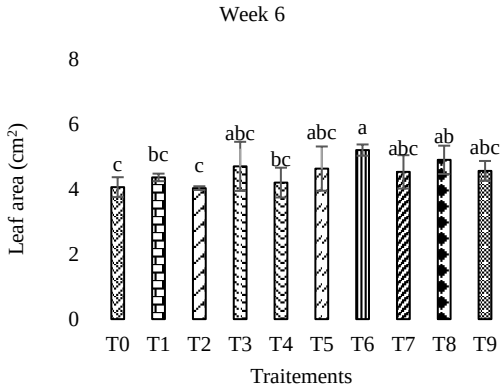
Note: T0: Polluted soil (Positive control)
T1: Non-polluted soil (Negative control)
T2: Polluted soil + R. erythropolis (10^7 CFU/g soil)
T3: Polluted soil + B. amyloliquefaciens (10^7 CFU/g)
T4: Polluted soil + 864 g poultry manure
T5: Polluted soil + indigenous bacterial consortium (10^7 CFU/g)
T6: Polluted soil + R. erythropolis + B. amyloliquefaciens (10^7 CFU/g each)
T7: Polluted soil + consortium + B. amyloliquefaciens (10^7 CFU/g each)
T8: Polluted soil + 864 g poultry manure + R. erythropolis (10^7 CFU/g)
T9: Polluted soil + 864 g poultry manure + consortium (10^7 CFU/g)

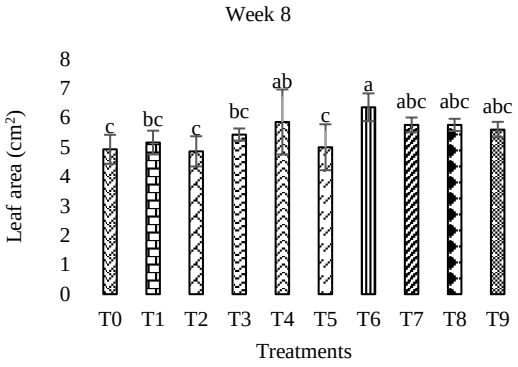
3.2.2. Evolution of Leaf Area

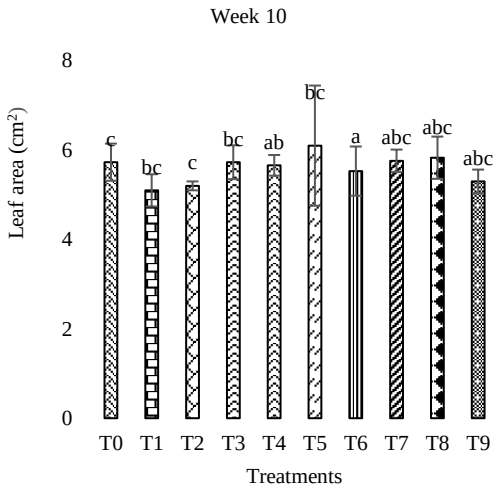
The dynamics of leaf area in *Arachis hypogaea* varied according to both treatment and growth stage (Figure 2). At 2 weeks after sowing (WAS), no significant differences were observed, except for T5, which exhibited a larger leaf area than T6. At 4 WAS, T8 displayed a significantly higher value compared to T1, T2, and T3. At 6 WAS, T6 showed the largest leaf area, significantly exceeding those of T0, T1, T2, and T4. At 8 WAS, T6 maintained this advantage over T0, T1, T2, T3, and T5. Finally, at 10 WAS, the only significant difference was recorded between T5 (higher) and T6, while no notable variation was observed among the other treatments.











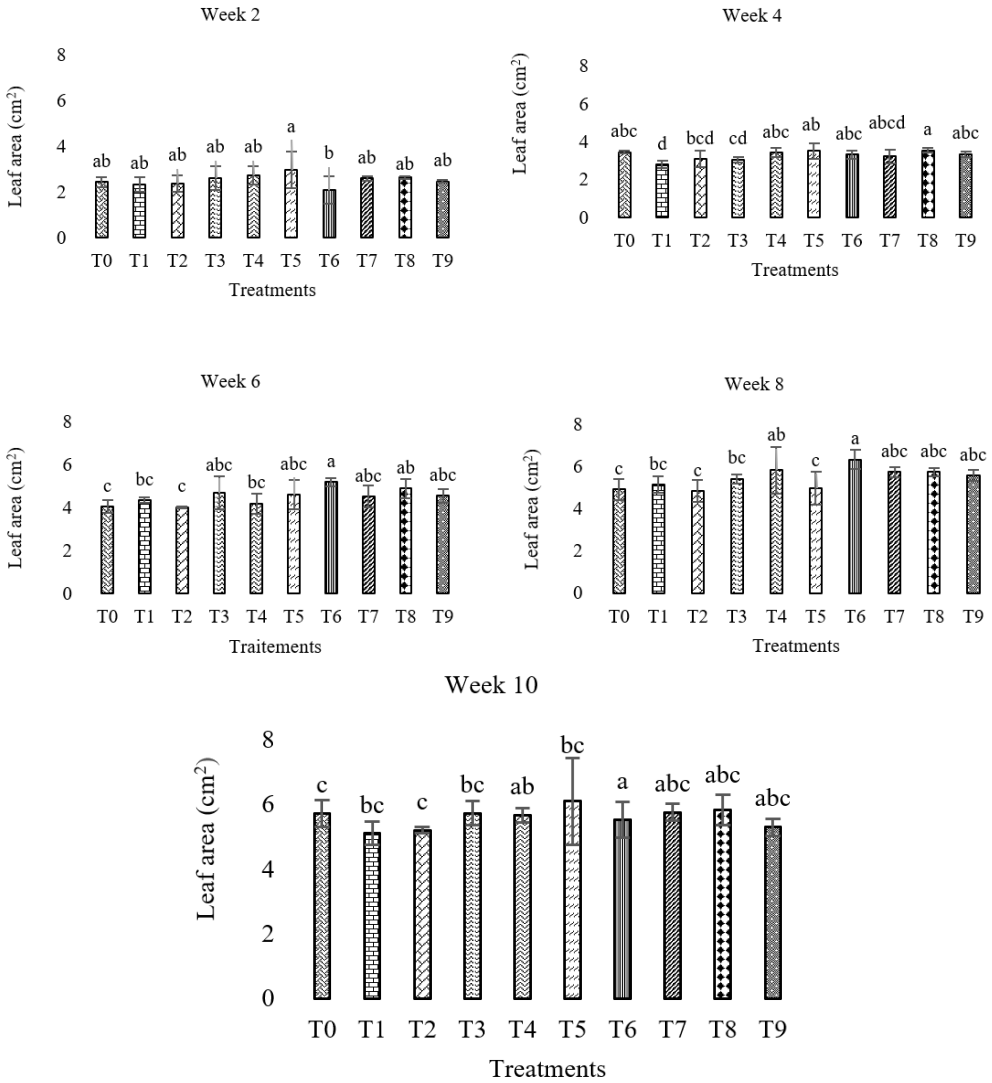
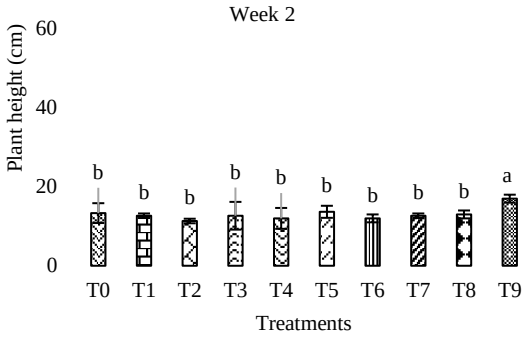


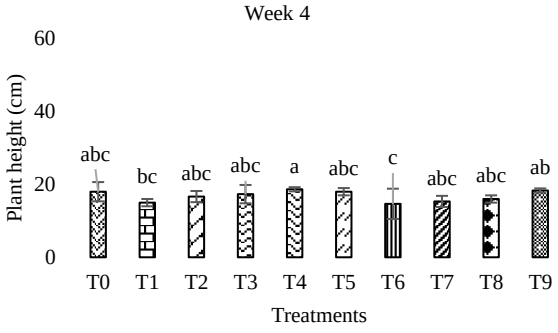
Figure 2. Evolution of the leaf area in *Arachis hypogaea* plants.

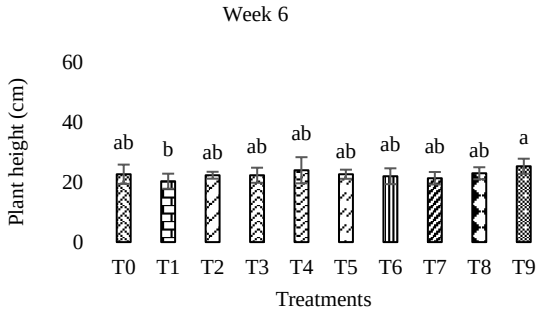
Note : T0:Pollutedsoil(Positive control)
T1: Non-polluted soil (Negative control)
T2:Polluted soil + R. erythropolis (10^7 CFU/g soil)
T3:Polluted soil + B. amyloliquefaciens (10^7 CFU/g)
T4:Polluted soil + 864 g poultrymanure
T5:Polluted soil + indigenousbacterial consortium (10^7 CFU/g)
T6:Polluted soil + R. erythropolis + B. amyloliquefaciens (10^7 CFU/g each)
T7:Polluted soil + consortium + B. amyloliquefaciens (10^7 CFU/g each)
T8:Polluted soil + 864 g poultrymanure + R. erythropolis (10^7 CFU/g)
T9:Polluted soil + 864 g poultrymanure + consortium (10^7 CFU/g)

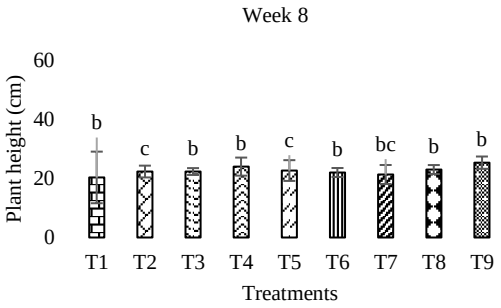
3.2.3. Evolution of Plant Height

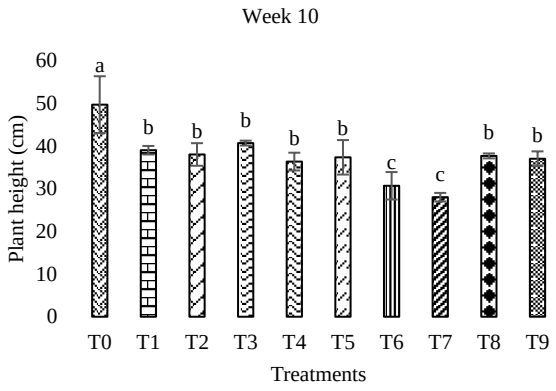
The growth dynamics of *Arachis hypogaea* plants revealed significant variations in height depending on both treatment and developmental stage (Figure 3). At 2 weeks after sowing (WAS), T9 exhibited a significantly greater height than all other treatments. At 4 WAS, T4 showed higher values compared to T6. At 6 WAS, T9 significantly exceeded T1. At 8 WAS, the lowest plant heights were recorded in T2 and T5. Finally, at 10 WAS, the control (T0) displayed the greatest height, significantly surpassing all other treatments, among which T6 and T7 showed the lowest values.

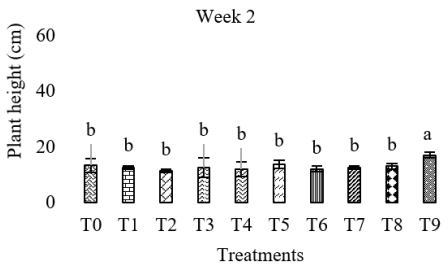


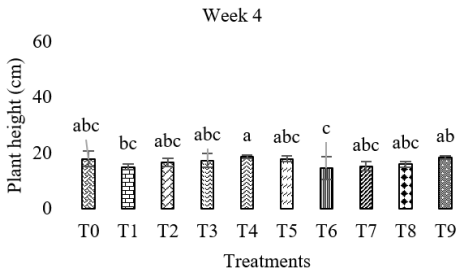


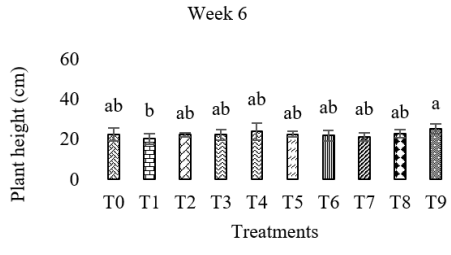


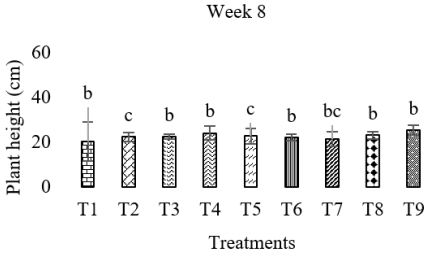












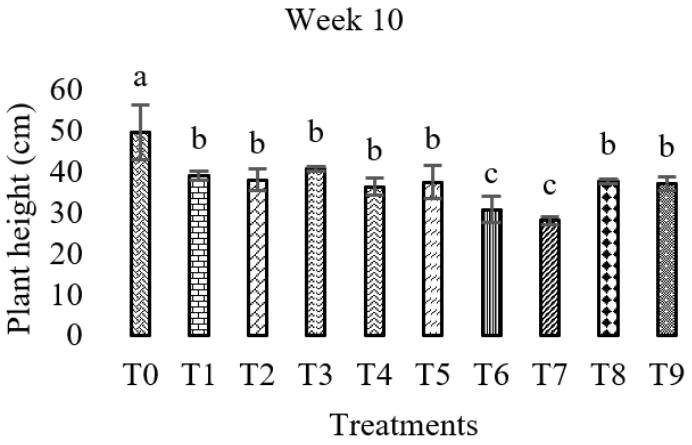


Figure 3. Evolution of plant height in *Arachis hypogaea*.

Note: T0: Polluted soil (Positive control).
T1: Non-polluted soil (Negative control).
T2: Polluted soil + *R. erythropolis* (10^7 CFU/g soil).
T3: Polluted soil + *B. amyloliquefaciens* (10^7 CFU/g).
T4: Polluted soil + 864 g poultry manure.
T5: Polluted soil + indigenous bacterial consortium (10^7 CFU/g)
T6: Polluted soil + *R. erythropolis* + *B. amyloliquefaciens* (10^7 CFU/g each).
T7: Polluted soil + consortium + *B. amyloliquefaciens* (10^7 CFU/g each).
T8: Polluted soil + 864 g poultry manure + *R. erythropolis* (10^7 CFU/g).
T9: Polluted soil + 864 g poultry manure + consortium (10^7 CFU/g).

3.2.4. Evaluation of Chlorophyll **a** *and* **b** *Contents*

The results indicate that chlorophyll *a* and *b* contents varied significantly across treatments (Figure 4). Treatment T8 (polluted soil + poultry manure + *R. erythropolis*) exhibited values comparable to the non-polluted control (T0) and were significantly higher than all other treatments. Except for T8, no significant differences were observed among treatments for chlorophyll *a*. Similarly, chlorophyll *b* content reached its maximum in T8, showing a significant difference ($p < 0.05$, Duncan's test) compared to all other treatments, which did not differ significantly from one another.

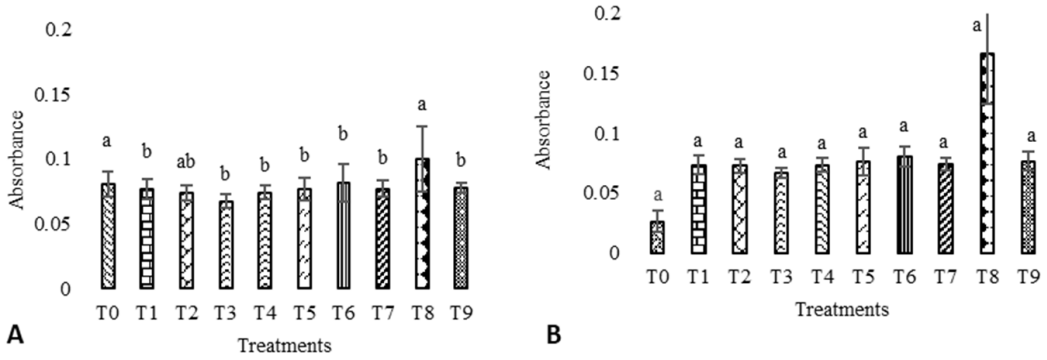


Figure 4. Chlorophyll *a* and *b* contents in *Arachis hypogaea* plants under different treatments. A: Chlorophyll a content; B: Chlorophyll b content.

Note: T0: Polluted soil (Positive control) ; T1: Non-polluted soil (Negative control) ; T2: Polluted soil + *R. erythropolis* (10^7 CFU/g soil) ; T3: Polluted soil + *B. amyloliquefaciens* (10^7 CFU/g) ; T4: Polluted soil + 864 g poultry manure ; T5: Polluted soil + indigenous bacterial consortium (10^7 CFU/g) ; T6: Polluted soil + *R. erythropolis* + *B. amyloliquefaciens* (10^7 CFU/g each) ; T7: Polluted soil + consortium + *B. amyloliquefaciens* (10^7 CFU/g each) ; T8: Polluted soil + 864 g poultry manure + *R. erythropolis* (10^7 CFU/g) ; T9: Polluted soil + 864 g poultry manure + consortium (10^7 CFU/g)

3.2.5. Evaluation of Carotenoid Content

Carotenoid contents showed slight variations among the treatments, with the highest value recorded in T8 (0.63 mol·L^{-1}), followed by T9 (0.49 mol·L^{-1}) and T1 (0.42 mol·L^{-1}) (Figure 5). Although overall differences were modest, T8 exhibited significantly higher carotenoid content compared to all other treatments. Treatments sharing the same letter, according to the applied statistical test, did not differ significantly.

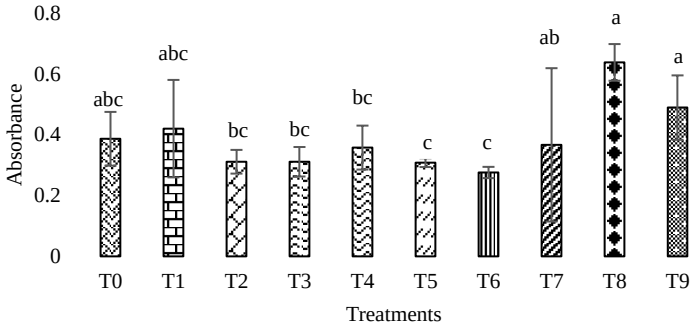


Figure 5. Evaluation of carotenoid content.

Note: T0: Polluted soil (positive control) ; T1: Non-polluted soil (negative control) ; T2: Polluted soil + *R. erythropolis* (10^7 CFU/g soil) ; T3: Polluted soil + *B. amyloliquefaciens* (10^7 CFU/g) ; T4: Polluted soil + 864 g poultry manure ; T5: Polluted soil + indigenous bacterial consortium (10^7 CFU/g) ; T6: Polluted soil + *R. erythropolis* + *B. amyloliquefaciens* (10^7 CFU/g each) ; T7: Polluted soil + consortium + *B. amyloliquefaciens* (10^7 CFU/g each) ; T8: Polluted soil + 864 g poultry manure + *R. erythropolis* (10^7 CFU/g) ; T9: Polluted soil + 864 g poultry manure + consortium (10^7 CFU/g)

3.2.6. Fresh Weight of 100 Pods and Dry Weight of 100 Seeds

The assessment of the fresh weight of 100 pods (Figure 6) showed that, except for T1 (120 ± 4 g), which was significantly lower than all other treatments, no significant differences were observed among T2 to T9 (150–190 g) and the control T0 (182 ± 3 g). Regarding the dry weight of 100 seeds, T1 (42 ± 3 g) exhibited a significantly lower value compared to T5 (64 ± 2 g). No notable differences were detected among the other treatments and the control T0 (66 ± 1.52 g).

3.2.7. Fresh Weight of 100 Pods and Dry Weight of 100 Seeds

The evaluation of the fresh weight of 100 pods (Figure 6) indicated that, except for T1 (120 ± 4 g), which was significantly lower than all other treatments, no significant differences were observed among T2 to T9 (150–190 g) and the control T0 (182 ± 3 g). For the dry weight of 100 seeds, T1 (42 ± 3 g) showed a significantly lower value compared to T5 (64 ± 2 g). No notable differences were detected among the other treatments and the control T0 (66 ± 1.52 g).

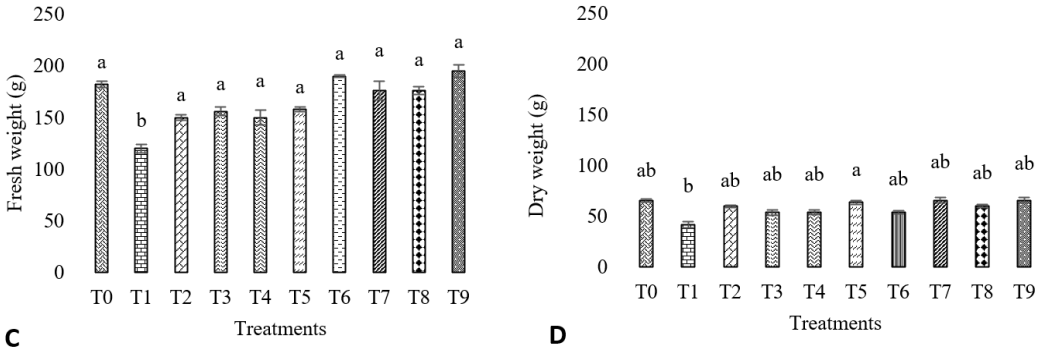


Figure 6. Evaluation of fresh pod weight and dry seed weight across treatments. C: Fresh weight of 100 pods; D: Dry weight of 100 seeds.

Note: T0: Polluted soil (positive control) ; T1: Non-polluted soil (negative control) ; T2: Polluted soil + *R. erythropolis* (10^7 CFU/g soil) ; T3: Polluted soil + *B. amyloliquefaciens* (10^7 CFU/g) ; T4: Polluted soil + 864 g poultry manure ; T5: Polluted soil + indigenous bacterial consortium (10^7 CFU/g) ; T6: Polluted soil + *R. erythropolis* + *B. amyloliquefaciens* (10^7 CFU/g each) ; T7: Polluted soil + consortium + *B. amyloliquefaciens* (10^7 CFU/g each) ; T8: Polluted soil + 864 g poultry manure + *R. erythropolis* (10^7 CFU/g) ; T9: Polluted soil + 864 g poultry manure + consortium (10^7 CFU/g)

3.3. Monitoring Hydrocarbon Biodegradation in Soil

3.3.1. pH Evolution

Weekly monitoring of soil pH over six weeks (Figure 7) revealed variations related to both time and treatment. Initially, pH values ranged from 7.7 to 9.2. In the control T0, pH remained generally stable (6.9–7.2), with a peak of

7.9 on day six. The treatment "polluted soil + bacterial consortium + *B. amyloliquefaciens*" also showed minimal variation (7.2–7.3). In "polluted soil + *R. erythropolis*," pH remained constant at 6.2. In contrast, combinations incorporating either the consortium or *R. erythropolis* with added nutrients showed a gradual decrease, from 7.2 to 6.87.

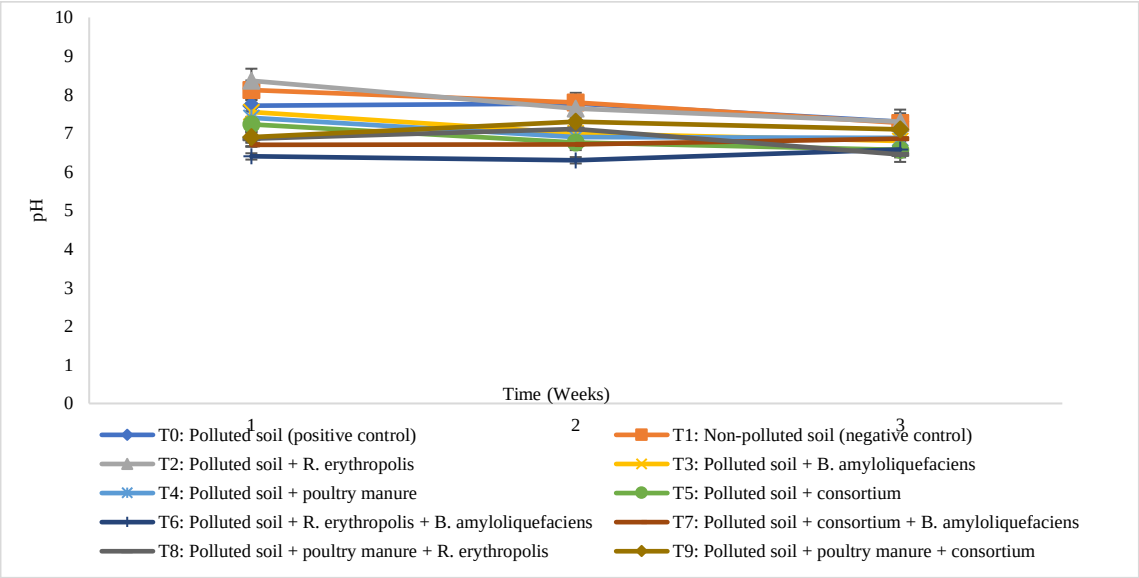


Figure 7. Soil pH variation across different treatments.

3.3.2. Hydrocarbon Degradation Rate

The degradation rate, representing the relative reduction in hydrocarbon concentration, was used to evaluate the effectiveness of the applied treatments (Figure 8). In the control (without bacterial addition), this rate reached 29.66%. Under bioaugmentation, values were 36% with the bacterial starter and 54% with *R. erythropolis*. The combination of bioaugmentation and biostimulation significantly enhanced these performances, reaching 66% (starter) and 62% (*R. erythropolis*).

Temporal monitoring showed a notable decrease in diesel concentration from the first week in several treatments. In the bioaugmentation–biostimulation combinations, degradation rates increased from 37.14% in the first week to 71.42% in the second week, peaking at 98.61% by the sixth week (T9).

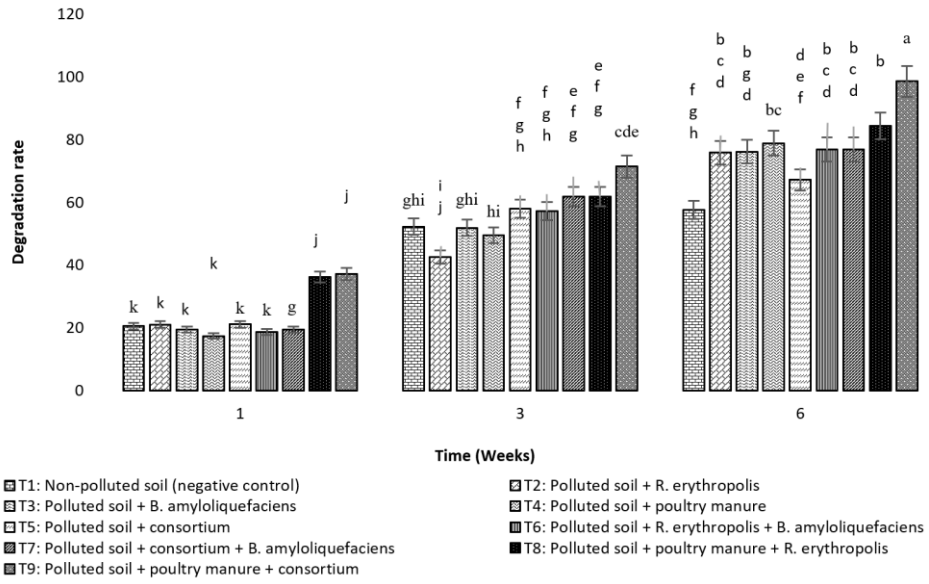


Figure 8. Total hydrocarbon degradation rate in polluted soil.

4. DISCUSSION

4.1. Characterization of the Competent Microflora

4.1.1. Hydrocarbonoclastic Bacterial Isolates

Analysis of the microflora in hydrocarbon-contaminated soils revealed notable bacterial diversity, although the density of competent microflora remained relatively low compared to the total microflora. The total microflora in uncontaminated soil was estimated at 8.1×10^9 CFU·g^{-1}, whereas the competent microflora in polluted soil reached only 1.02×10^3 CFU·g^{-1}. These results align with Benamara and Touati [31], who highlighted that competent microflora can accelerate hydrocarbon degradation while restoring a favorable microbial balance [31]. This confirms that the presence of specialized biodegrading bacteria, even at low density, represents an ecological asset for soil bioremediation.

4.1.2. Identification of the Competent Microflora

Identification of isolates CB1 to CB5 revealed taxonomic diversity adapted to hydrocarbon degradation.

- CB1: Micrococcus – Gram-positive cocci, catalase and oxidase positive, orange-pigmented colonies, consistent with the *Micrococcus* genus. This strain was isolated from hydrocarbon-contaminated soils in bioremediation studies [32, 33].
- CB2: Lactobacillus – Gram-positive bacillus, catalase negative, capable of fermenting glucose and complex sugars. This strain may indirectly contribute to biodegradation by enhancing nutrient availability in the soil and has also been isolated from hydrocarbon-contaminated soils [34-36].
- CB3: Planococcus – Gram-positive cocci, oxidase- and catalase-positive, lightly pigmented. Some *Planococcus* species produce biosurfactants that facilitate hydrocarbon solubilization [37, 38].
- CB4: Achromobacter – Gram-negative bacillus, strictly aerobic, oxidase positive, non-fermenting, capable of degrading hydrocarbons under aerobic conditions [39].
- CB5: Serratia – Gram-negative, motile bacillus, Lac/Sac positive, H_2S negative, urease negative, red-pink pigmented, capable of degrading various hydrocarbons in contaminated soils [40].

The bacterial consortium thus presents complementary potential for hydrocarbon degradation, combining biosurfactant-producing bacteria and specialized aerobic degraders.

4.2. Agronomic Parameters of Peanut Plants

4.2.1. Vegetative Growth

Treatments combining organic amendments and microorganisms significantly enhanced vegetative growth, particularly in leaf number and leaf area. Plants grown in polluted soil alone (T0) showed limited growth, confirming that hydrocarbons impair the uptake of essential minerals such as nitrogen, phosphorus, and potassium [41]. The addition of poultry manure and *B. amyloliquefaciens* (T1, T3) stimulated leaf development, while combined treatments (T4–T9) resulted in increased leaf area and plant height, highlighting the synergistic effect of microorganisms and biofertilizers. These observations are consistent with Mbouobda [41]and Konaté et al. [42], who demonstrated the importance of microbial biofertilizers in enhancing plant growth in polluted soils.

4.2.2. Physiological Parameters

Physiological measurements, such as chlorophyll and carotenoid contents, confirmed the improvement of plant physiological status under combined treatments. Treatment T8 (manure + consortium) exhibited high total chlorophyll levels, indicating efficient photosynthesis despite hydrocarbon stress. Moreover, carotenoids were strongly stimulated by poultry manure and *R. erythropolis*, contributing to oxidative stress protection and balanced tissue maturation [43, 44].

4.2.3. Pod and Seed Yield

Fresh and dry pod weights were significantly increased by combined treatments, particularly those including the bacterial consortium. These results support observations confirming that the introduction of endogenous strains and biofertilizers improves peanut plant productivity [41, 45].

4.3. Monitoring Biodegradation

4.3.1. Hydrocarbon Degradation Rate

Hydrocarbon degradation rates ranged from 56.15% to 98.57% depending on the treatment, with the highest value observed in "polluted soil + poultry manure + consortium" (98.57%). According to Lang et al. [46], treatments combining bioaugmentation and biostimulation exhibit superior efficiency, confirming that fertilizers enrich the soil with nutrients and promote the growth of specialized bacteria. Natural attenuation observed in the control (57.63%) underscores the importance of microbial intervention and biofertilizers to accelerate degradation [47, 48].

4.3.2. pH Monitoring

Initially, basic pH values (7.7–9.2) gradually decreased to a favorable range (6.2–7.5) for biodegradation [49]. This progression enables bacteria to efficiently mineralize hydrocarbons. Extremely acidic or alkaline pH limits microbial degradation [50].

4.3.3. Plant–Bacteria Symbiotic Interaction

The presence of peanut plants creates a favorable environment for bacteria through root exudates, while microorganisms supply essential nutrients to the plant. This synergistic interaction between biofertilizers, bacteria, and plants optimizes both vegetative growth and hydrocarbon degradation [51].

5. CONCLUSION

This study demonstrated that diesel- and motor oil-contaminated soils harbor competent microflora capable of hydrocarbon degradation. Five bacterial isolates were identified belonging to the genera *Micrococcus*, *Lactobacillus*, *Planococcus*, *Achromobacter*, and *Serratia*. Treatments combining organic biofertilizers (poultry manure) and endogenous or exogenous bacteria significantly improved peanut growth, physiology, and productivity, while achieving high hydrocarbon degradation rates (up to 98.57%). These findings highlight the effectiveness of integrated bioremediation approaches in restoring the fertility of contaminated soils and optimizing agricultural productivity under stress conditions. Treated plants achieved average pod yields of 30–40 g per plant, confirming the positive impact of combined treatments on agronomic performance.

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Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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