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Enhancement of anthracene and chrysene biodegradation in contaminated agricultural soil through bacterial bioaugmentation and organic amendments

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Abstract

The contamination of agricultural soils with polycyclic aromatic hydrocarbons (PAHs) represents a major environmental concern due to their persistence and toxicity. This study evaluated the effectiveness of bioaugmentation, biostimulation, and their combination in the biodegradation of anthracene and chrysene in sterile and non-sterile agricultural soils. Soils artificially contaminated with each of the two PAHs were treated for ten weeks using a bacterial consortium, *Pseudomonas fluorescens*, compost, and poultry manure, either individually or in combination. Soil physicochemical properties, pH, the growth kinetics of the competent indigenous microflora, and biodegradation rates were assessed and subsequently analyzed using ANOVA ($p < 0.05$). The treatments significantly enhanced the degradation of both PAHs. In sterile soils, biodegradation rates ranged from 1.5 to 43.6% for anthracene and from 0.4 to 37.1% for chrysene. In non-sterile soils, higher biodegradation rates were observed, reaching 74.8% for anthracene and 56.4% for chrysene. The treatment combining poultry manure and the bacterial consortium consistently showed the highest degradation performance, whereas the control treatments exhibited the lowest efficiencies. Combined treatments also promoted a substantial increase in the competent microflora and a decrease in soil pH, indicating enhanced microbial activity. Furthermore, anthracene was more readily biodegraded than chrysene, owing to its greater bioavailability and less complex molecular structure. These findings demonstrate that the combined application of bioaugmentation and biostimulation, particularly through the use of poultry manure and a bacterial consortium, represents an effective and sustainable strategy for accelerating the bioremediation of PAH-contaminated agricultural soils.

Keywords: Biofertilizers, biodegradation, hydrocarbons, microorganisms, pollution, poultry manure.

1. Introduction

The contamination of agricultural soils by polycyclic aromatic hydrocarbons (PAHs) represents a major environmental and agronomic concern, particularly in regions experiencing intense anthropogenic and industrial pressure. Petroleum hydrocarbons comprise aliphatic, cyclic, monocyclic aromatic, and polycyclic aromatic compounds (Baker et al., 1994a). Among these, PAHs such as anthracene, phenanthrene, fluoranthene, fluorene, naphthalene, chrysene, pyrene, and benzo(a)pyrene are considered priority organic pollutants because of their persistence, ubiquity, and toxicity in the environment (Tam & Wong, 2008; Lu et al., 2011; Eze et al., 2021). They originate mainly from the incomplete

combustion of organic matter and fossil fuels, as well as from accidental hydrocarbon spills and industrial emissions (Marini & Frapiccini, 2013; Semboung et al., 2014). Their polycyclic molecular structure confers low biodegradability, high hydrophobicity, and a strong affinity for soil organic matter, thereby promoting their accumulation and persistence. This contamination poses a threat to soil microorganisms, cultivated plants, and human health through the food chain (Barathi & Vasudevan, 2001).

In agricultural soils, PAHs alter soil physicochemical and biological properties by reducing microbial activity, the availability of essential nutrients, and biological processes that are critical to the functioning of agroecosystems (Costes & Druelle, 1997). These disturbances result in reduced soil fertility, decreased agricultural productivity, and long-term deterioration of environmental quality. Among the various PAHs, anthracene, phenanthrene, and chrysene are among the compounds most frequently detected in contaminated soils and are widely used as indicators of aromatic pollution because of their persistence and relative toxicity (Chen et al., 2015).

In Cameroon, the rapid expansion of urban areas, industrial activities, and petroleum exploitation is increasing the risk of agricultural soil contamination, particularly in the vicinity of oil-producing basins, road infrastructure, and industrial areas. However, available information on PAH contamination in agricultural soils remains very limited. Studies conducted in several sub-Saharan African countries have nevertheless reported concerning concentrations of aromatic compounds in agricultural soils located near industrial and petroleum-producing areas, with adverse effects on soil quality and agricultural production (Chen et al., 2015). This lack of data represents a significant scientific gap that limits the development of management and remediation strategies adapted to tropical conditions.

In response to this issue, bioremediation is currently regarded as one of the most promising approaches for the sustainable restoration of contaminated soils. This technology relies on the use of microorganisms capable of transforming or mineralizing organic pollutants, a process whose efficiency can be enhanced through the application of organic and microbial biofertilizers (Eze et al., 2022). Organic amendments, such as compost and poultry manure, improve soil structure, increase organic matter content, and supply essential nutrients, particularly nitrogen and phosphorus, thereby promoting the growth of pollutant-degrading microorganisms (Wu et al., 2020). Several studies have shown that combining these amendments with specialized microorganisms significantly enhances PAH biodegradation compared with either treatment applied alone or with unamended soils (Wu et al., 2020).

Despite these advances, the synergistic effects of organic biofertilizers and microbial inoculation on the biodegradation of high-molecular-weight PAHs remain insufficiently documented, particularly in tropical agricultural soils. Few studies have comprehensively evaluated their influence on the biotreatability of soils contaminated with compounds such as anthracene and chrysene under the agroecological conditions of tropical Africa. A better understanding of these interactions is nevertheless essential for optimizing bioremediation strategies and facilitating their application in agricultural systems affected by petroleum-related pollution.

Against this background, the present study aimed to evaluate the effectiveness of the combined application of organic biofertilizers (compost and poultry manure) and microbial biofertilizers in enhancing the biotreatability of agricultural soils contaminated with anthracene and chrysene. Specifically, the objectives were to: (i) assess the effect of targeted microbial inoculation on PAH biodegradation; (ii) compare the effectiveness of treatments combining organic and microbial amendments with that of the individual amendments; and (iii) quantify the influence of compost and poultry manure on the biodegradation of anthracene and chrysene. The expected results will contribute to a better understanding of the biological mechanisms involved in PAH degradation and provide a scientific basis for developing sustainable bioremediation strategies suited to contaminated agricultural soils in tropical regions.

2. Materials and Methods

2.1. Materials

Agricultural soil

The soil used in this study was collected from experimental agricultural plots. Prior to the experiment, the soil was characterized to determine its main physicochemical properties.

Organic biofertilizers

Two organic amendments were used as biostimulation agents: dehydrated and granulated poultry manure and commercial compost. Poultry manure was applied at a rate of 1.5 g kg⁻¹ dry soil. Its chemical composition was not determined; according to Li et al. (2022), poultry manure generally contains 2–6% nitrogen, 1–3% phosphorus, and 2–4% potassium on a dry matter basis.

Compost was incorporated at a rate of 4 g kg⁻¹ dry soil. Although its composition was not analyzed, compost of plant or animal origin typically contains 1–3% nitrogen, 0.5–1.5% P₂O₅, and 0.5–2% K₂O (FAO, 2005). The application rates were selected to improve nutrient availability and stimulate the activity of hydrocarbon-degrading microorganisms (Nikolopoulou et al., 2013).

Microorganisms

Two types of inocula were used. The first consisted of an indigenous bacterial consortium isolated from the agricultural soils used in the experiment. The second was a pure bacterial strain of *Pseudomonas fluorescens*, provided by the Biology Laboratory of the University of Mons (Belgium).

Polycyclic aromatic hydrocarbons

The two PAHs investigated were anthracene (Ant) and chrysene (Chr), purchased from UCB (Belgium) with a purity of >97%. These two compounds were selected because of their frequent occurrence in hydrocarbon-contaminated soils, their strong affinity for soil organic matter, and their persistence in the environment.

2.2. Methods

2.2.1. Artificial contamination of soils

The soils were artificially contaminated following the method described by Tam et al. (2002), with some modifications. Anthracene and chrysene were separately dissolved in acetone and then incorporated into the soil to obtain a final concentration of approximately 3,367.33 mg kg⁻¹ dry matter. After homogenization, the mixtures were left exposed to air for 48 h to allow acetone evaporation and adsorption of the PAHs onto the soil organic matter. Soil moisture was subsequently adjusted using distilled water.

2.2.2. Isolation and production of the bacterial consortium

The indigenous bacterial consortium was obtained according to the method described by Weekers et al. (1999), with modifications adapted to the present study. Following serial decimal dilutions, microorganisms were isolated on a selective mineral medium containing diesel as the sole carbon source and incubated at 30 °C for 72 h.

The resulting colonies were subsequently cultured through two successive precultures in a rich medium before being transferred to a 5-L bioreactor containing 4 L of culture medium supplemented with diesel. Bacterial growth was monitored by measuring optical density at 600 nm for 96 h. The resulting biomass was harvested by centrifugation and stored at 0 °C in a solution containing 2.5% glycerol and 10% maltodextrin as cryoprotective agents.

Medium compositions:

- **Peptone water (g L⁻¹):** casein peptone, 10; NaCl, 5; Na₂HPO₄, 9; K₂HPO₄, 1.5; pH 7.0 ± 0.2.
- **Rich medium (g L⁻¹):** glucose, 20; casein peptone, 10; yeast extract, 10; agar, 15; pH 7.0 ± 0.2.
- **Selective mineral medium (mg L⁻¹):** (NH₄)₂SO₄, 1000; K₂HPO₄, 800; KH₂PO₄, 200; MgSO₄·7H₂O, 200; CaCl₂·2H₂O, 100; FeSO₄·7H₂O, 12; MnSO₄·7H₂O, 3; ZnSO₄·7H₂O, 3; CoSO₄·7H₂O, 1; (NH₄)₆Mo₇O₂₄·4H₂O, 1; NaCl, 4; agar, 15 g; diesel, 10 ppm; pH 7.0 ± 0.2. Diesel served as the sole carbon source.

2.2.3. Characterization and identification of bacteria

The bacterial isolates were characterized by Gram staining and catalase and oxidase tests. Biochemical identification was performed using the API 20E identification system according to the manufacturer's instructions. The resulting biochemical profiles were used to identify the different bacterial species comprising the consortium.

2.2.4. Assessment of PAH biodegradation

Two independent experiments were conducted, one using anthracene and the other using chrysene as the sole contaminant. The experiments were performed under sterile and non-sterile conditions, with each treatment consisting of three microcosms containing 3 kg of soil contaminated at 3,367.33 mg kg⁻¹ dry matter.

Under sterile conditions (Fig. 1), the microcosms were autoclaved prior to inoculation. The control treatment (T0) consisted of sterilized soil without inoculation. The other treatments consisted of inoculation with either the indigenous bacterial consortium or *Pseudomonas fluorescens* at a concentration of 10⁷ CFU g⁻¹ dry matter, either alone or in combination with compost (4 g kg⁻¹) or poultry manure (1.5 g kg⁻¹).

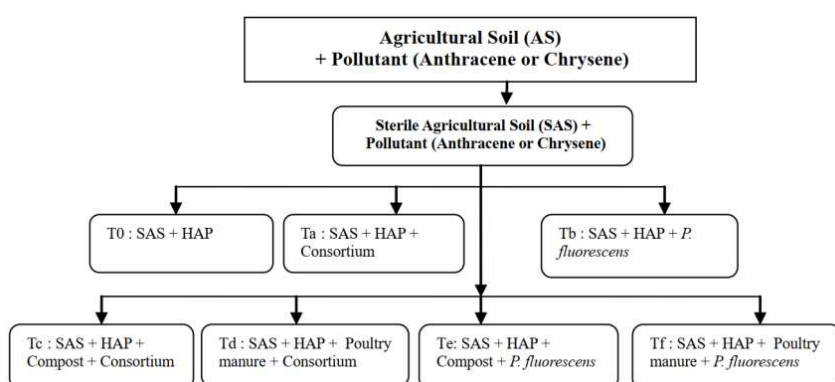


Figure 1. Experimental design of the different treatments under sterile conditions.

Under non-sterile conditions (Fig. 2), the treatments included natural attenuation, biostimulation (compost or poultry manure), bioaugmentation (bacterial consortium or *P. fluorescens*), as well as combinations of bioaugmentation and biostimulation. The microcosms were incubated at room temperature for 10 weeks.

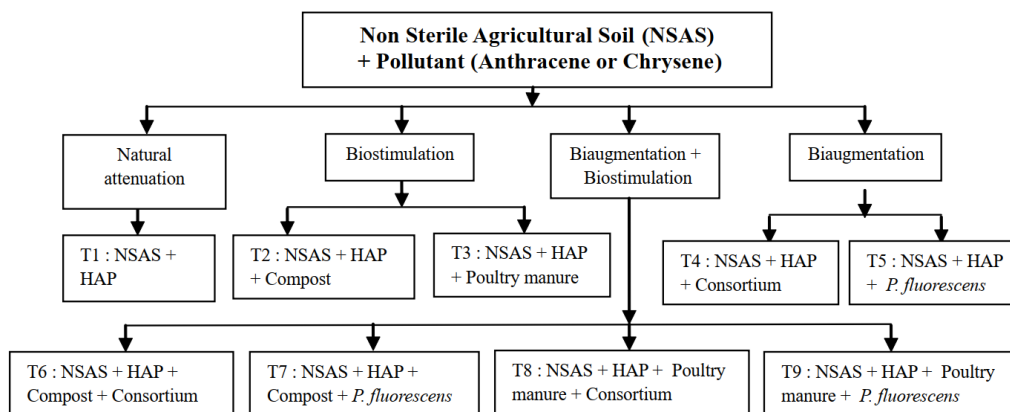


Figure 2. Experimental design of the different treatments under non-sterile conditions.

2.2.5. Experimental Monitoring and Chemical Analyses

The pH of the different treatments was measured weekly throughout the experimental period using a HANNA HI 207 pH meter.

The residual concentrations of anthracene and chrysene were determined every two weeks according to the method described by Wannoussa et al. (2015), with slight modifications. Following extraction with hexane, concentration of the extract, and reconstitution in methanol, the samples were analyzed by high-performance liquid chromatography (HPLC)

using an Agilent 1100 system equipped with a C18 LiChroCART® Purospher® STAR RP-18 column maintained at 30 °C.

2.2.6. Statistical Analysis

The results are expressed as means $\pm$ standard deviations. Differences among treatments were assessed by analysis of variance (ANOVA) at a significance level of $p \leq 0.05$, using MINITAB® 17 statistical software.

3. Results

3.1. Physicochemical Analyses of Soil Samples

Table 1. Physicochemical properties of the soil

Parameters	Identification criteria	Results	Parameters	Identification criteria	Results
Depth (cm)		20-40	Pb		4
	Clay	46,5	Cd		0,03
Texture (%)	Silt	7	Cu		7,7
Textural class	Sand	47	Ni		4
	Loam	Loam	Zn		19,8
	pH-H ₂ O	5,7	Cr		28,2
Soil reaction	pH-KCl	5,1	As		1,15
	Δ pH	0,6	Hg		0,02
	OC (%)	1,33	Co		2,9
Organic matter	OM (%)	2,30	CN ⁻		< 0,01
	Total N (%)	0,12	F ⁻		41,4
	C/N	11	MAH's		< 0,06
	Calcium	2	Halogen solvents		< 0,2
	Magnesium	0,8	HAP's		
Exchangeable cations (meq/100 g)	Potassium	0,04	Mixing Borneff		< 0,07
	Sodium	0,08	Total		< 0,18
	Sum of bases	3	Total petroleum		
			Hydrocarbon		< 100
Cation exchange capacity (meq/100 g)	CEC pH 7	5	C ₉ – C ₄₀		3,28
Base saturation (%)		58	PCB's		< 0,015
Available phosphorus	Bray II (mg kg ⁻¹)	31,85	Pesticides chlorinated		< 0,02
Dry matter (%)		80.7			

The physicochemical properties of the soil are presented in Table 1. The soil exhibited a loamy texture, consisting of 47% sand and 46.5% clay, which is favorable for aeration and water retention. It was slightly acidic (pH-H₂O = 5.7; pH-KCl = 5.1) and contained 2.30% organic matter, with a C/N ratio of 11, indicating favorable mineralization activity. The cation exchange capacity was low (5 meq/100 g), despite a base saturation of 58%, while available phosphorus was relatively high (31.85 mg kg⁻¹). The concentrations of trace metals (Pb, Cd, Cu, Ni, Zn, Cr, As, and Hg) and organic pollutants (total petroleum hydrocarbons, PAHs, PCBs, halogenated solvents, and organochlorine pesticides) were low or negligible. Overall, the soil exhibited moderate fertility, low contamination levels, and environmental quality compatible with agricultural use, although its low cation exchange capacity may limit long-term soil fertility.

3.2. Characterization of the Indigenous Competent Microflora

3.2.1. Morphological and Microscopic Characteristics of Bacterial Isolates

Table 2. Morphological and microscopic characteristics of bacterial isolates

Isolate	Colony shape	Color	Margin	Opacity	Elevation	Surface	Motility	Gram	Cell shape	Arrangement
Isolate 1	Circular	Orange	Entire	+++	Convex	Smooth	Motile	+	Cocci	Single
Isolate 2	Circular	Pale yellow	Undulate	--	Flat	Smooth	Motile	+	Coccobacillus	Single
Isolate 3	Circular	Beige	Undulate	++	Convex	Rough	Motile	+	Cocci	Single
Isolate 4	Circular	Beige	Undulate	++	Crateriform	Smooth	Motile	–	Rod	Single
Isolate 5	Circular	Pink	Entire	+++	Convex	Smooth	Motile	–	Rod	Single
Isolate 6	Circular	Milky white	Undulate	ND	Convex	Rough	Motile	–	Cocci	Single

The morphological analysis of the bacterial isolates (Table 2) revealed considerable phenotypic diversity. All colonies were circular but differed in color (orange, milky white, pale yellow, beige, and pink), margin (predominantly undulate), elevation (mostly convex, with one crateriform isolate), surface texture (smooth or rough), and opacity, indicating marked morphological heterogeneity among the isolates. Microscopic examination showed that all isolates were motile and occurred as cocci, coccobacilli, or rods. Gram staining revealed the presence of Gram-positive bacteria (isolates 1–3) and Gram-negative bacteria (isolates 4–6), while all cells were observed singly, with no chains or clusters.

Overall, this morphological and microscopic diversity indicates substantial taxonomic and functional heterogeneity within the bacterial consortium, which may promote metabolic complementarity among the isolates and enhance its biodegradation and bioremediation potential.

3.2.2. Biochemical and Metabolic Characteristics of Bacterial Isolates

Table 3. Enzymatic and metabolic activities of bacterial isolates

Reactions	Characteristics		Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Energy metabolism	Oxidase		+	-	+	+	-	+
	Catalase		+	-	+	+	+	+
	Respiratory type		Aerobic	Anaerobic	Aerobic	Aerobic	Aerobic	Aerobic
Carbohydrate metabolism	TSI	Glu	+	+	+	-	-	-
		Lac/suc	+	+	+	+	+	-
		H ₂ S	-	-	-	-	-	-
		GEL	+	+	+	-	+	-
	ARA		-	-	-	-	-	-
	ONPG		-	-	-	-	-	-
	Citrate permease		-	-	-	-	+	-
	Mannitol/Mobility	Mobility	+	+	+	+	+	+
		Mannitol	-	-	-	-	+	-
	Clark and Lubs medium	VP	+	+	-	-	-	-
Protein metabolism	Moeller medium	ADH	-	-	-	-	-	-
		LDC	-	-	-	-	-	-
		ODC	-	-	-	-	-	-
	Urea/indole medium	UREASE	-	-	-	-	-	-
		INDOLE	-	-	-	-	-	-
		TDA	-	-	-	-	-	-

The biochemical test results for the six bacterial isolates (Table 3) revealed considerable functional diversity within the consortium. Oxidase tests were positive for isolates 1, 3, 4, and 6, whereas isolates 2 and 5 were negative. Catalase activity was detected in all isolates except isolate 2. Most strains (isolates 1, 3, 4, 5, and 6) exhibited an aerobic metabolism, whereas isolate 2 was anaerobic.

The TSI tests revealed glucose fermentation by isolates 1–3 and lactose/sucrose fermentation by all isolates except isolate 6. None of the isolates produced H₂S. Gelatin hydrolysis was positive for isolates 1, 2, 3, and 5, whereas ARA and ONPG tests were negative for all isolates. Citrate was used as the sole carbon source only by isolate 5.

All isolates were motile. Mannitol fermentation was negative for all isolates except isolate 5. The Voges–Proskauer test was positive only for isolates 1 and 2, whereas ADH, LDC, ODC, urease, indole, and TDA tests were negative for all strains, indicating limited protein metabolism.

Overall, these results highlight substantial metabolic diversity, characterized by a predominance of aerobic bacteria, limited fermentative capacities, and complementary enzymatic profiles. This functional complementarity may represent an important advantage for PAH biodegradation processes and, more broadly, for environmental bioremediation applications.

3.3. Monitoring of Anthracene and Chrysene Biodegradation

Monitoring the biodegradation of anthracene and chrysene in contaminated agricultural soil allowed simultaneous assessment of several parameters, including pH, the indigenous competent microflora, and the removal rates of the target hydrocarbons.

3.3.1. Changes in pH under Sterile and Non-Sterile Conditions

In sterile soils contaminated with anthracene and chrysene, the pH of the uninoculated control (T0) remained relatively stable, ranging from 5.5 to 5.9 and from 5.6 to 6.0, respectively, throughout the incubation period. In contrast, all inoculated treatments exhibited a progressive decrease in pH during the first weeks. In anthracene-contaminated soil (Fig. 3A), the most pronounced acidification was observed in treatments Td (poultry manure + bacterial consortium), Tf (poultry manure + *Pseudomonas fluorescens*), and Tc (compost + bacterial consortium), with pH values reaching 3.3, 3.4, and 3.5, respectively, during the fourth week. In chrysene-contaminated soil (Fig. 3B), the decrease in pH was less pronounced, with the lowest value observed in treatment Tf, at approximately 3.6 from the eighth week onward. At the end of the experiment, pH values ranged from 3.8 to 5.9 for anthracene and from 3.6 to 5.8 for chrysene. The greater acidification observed in the bioaugmented and biostimulated treatments reflects increased metabolic activity of the degrading microorganisms. The more pronounced decrease in pH in anthracene-contaminated soils also suggests faster biodegradation of this compound compared with chrysene.

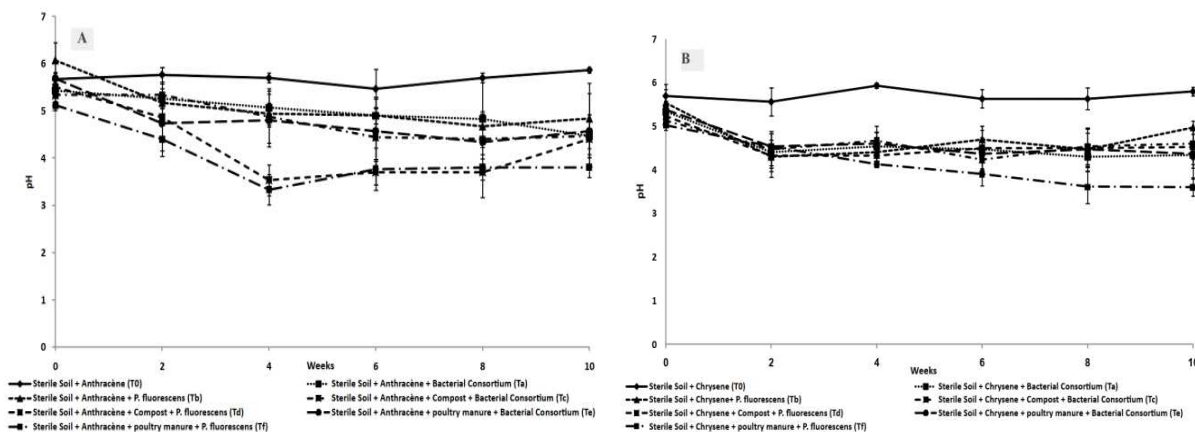


Figure 3. Evolution of pH in the different treatments of sterile soils contaminated with anthracene (A) and chrysene (B).

3.3.2 Evolution of the competent microflora in sterile agricultural soils contaminated with anthracene and chrysene

The results presented in Figure 4 show that the evolution of the microflora was significantly affected ($p < 0.05$) by both the treatment applied and the nature of the pollutant. The uninoculated control (T0) exhibited almost no microbial growth throughout the incubation period, confirming the inhibitory effect of PAHs in sterile soils.

In anthracene-contaminated soils (Figure 4C), all biological treatments resulted in a progressive increase in the microbial population, with the best performances observed in treatments combining organic amendments and microbial inocula. At

week 10, treatments Te (chicken manure + bacterial consortium) and Tf (chicken manure + *Pseudomonas fluorescens*) reached the highest microbial counts, with 8.80×10^7 and 8.95×10^7 CFU g⁻¹, respectively, followed by the compost-based treatments (Tc and Td). Treatments receiving only microbial inocula (Ta and Tb) exhibited lower microbial counts. In chrysene-contaminated soils (Figure 4D), microbial growth also increased, although the microbial counts remained lower than those observed in anthracene-contaminated soils. Treatment Tf exhibited the highest microbial population (3.37×10^7 CFU g⁻¹), followed by Te (1.40×10^7 CFU g⁻¹), whereas the compost-based treatments showed intermediate performance. Inoculation without organic amendment remained the least effective approach. Overall, the combination of bioaugmentation and biostimulation significantly enhanced microbial proliferation in PAH-contaminated soils. Chicken manure proved more effective than compost, probably because of its higher content of readily available nutrients. Furthermore, anthracene supported greater microbial growth than chrysene, reflecting its higher bioavailability and biodegradability.

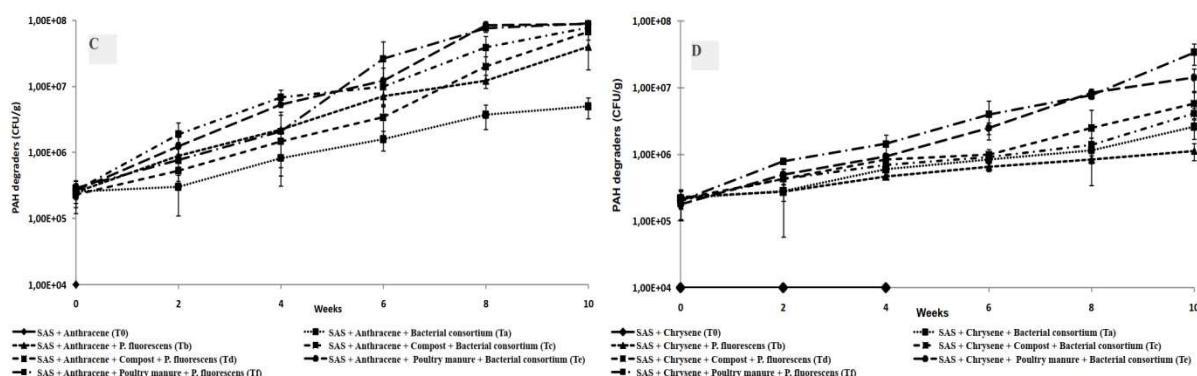


Figure 4. Evolution of the competent microflora in the different treatments of sterile soils contaminated with anthracene (C) and chrysene (D).

3.3.3 Hydrocarbon degradation rates under sterile conditions

Anthracene degradation rate in contaminated sterile soil

Figure 5 shows a progressive increase in the anthracene biodegradation rate in all biological treatments throughout the 10-week incubation period. In contrast, the sterile control (T0) exhibited very limited degradation (< 2%), confirming the near absence of abiotic degradation.

After two weeks, biodegradation rates remained below 10%, although treatments combining an organic amendment with a bacterial inoculum already showed the highest performances, particularly Te (chicken manure + bacterial consortium) and Tf (chicken manure + *Pseudomonas fluorescens*). From the fourth week onward, degradation rates increased significantly ($p < 0.05$) in all inoculated treatments, with the chicken manure-based treatments consistently exhibiting the highest performance.

By week 10, Te recorded the highest biodegradation rate ($\approx 44\%$), followed by Tf ($\approx 39\%$), Tc ($\approx 32\%$), and Td ($\approx 28\%$), whereas treatments receiving only microbial inocula (Ta and Tb) reached approximately 16% and 20%, respectively. The differences observed among treatments were statistically significant ($p < 0.05$).

Overall, the combination of an organic amendment with a bacterial inoculum significantly enhanced anthracene biodegradation under sterile conditions, with chicken manure proving more effective than compost in stimulating the activity of anthracene-degrading microorganisms.

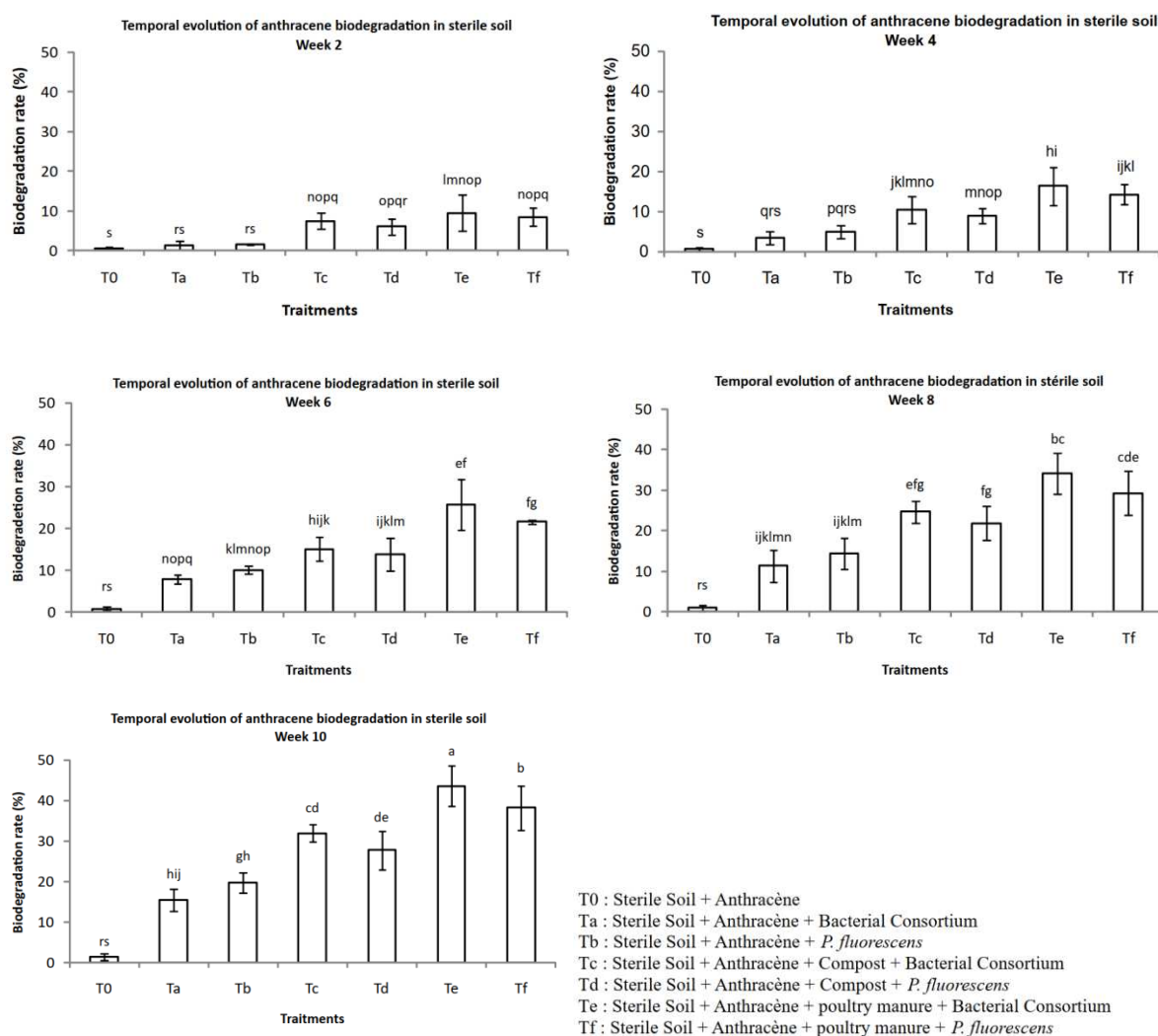


Figure 5. Anthracene degradation in sterile soils.

Chrysene degradation rate in contaminated sterile soil

Figure 6 shows a progressive increase in chrysene biodegradation in all biological treatments throughout the 10-week incubation period. In contrast, the sterile control (T0) exhibited negligible degradation, confirming that abiotic losses were minimal.

During the first two weeks, biodegradation rates remained below 10%, with treatments combining chicken manure and a bacterial inoculum (T5 and T6) showing the highest performance. From the fourth week onward, biodegradation increased significantly ($p < 0.05$) in all inoculated treatments, with T5 (chicken manure + bacterial consortium) and T6 (chicken manure + *Pseudomonas fluorescens*) consistently exhibiting the highest degradation rates.

By week 10, treatment T5 achieved the highest biodegradation rate ($\approx 37\%$), followed by T6 ($\approx 33\%$), T3 ($\approx 32\%$), and T4 ($\approx 29\%$), whereas treatments receiving only microbial inocula (T1 and T2) reached approximately 14% and 11%, respectively. The differences among treatments were statistically significant ($p < 0.05$).

Overall, the combination of an organic amendment and a bacterial inoculum significantly enhanced chrysene biodegradation under sterile conditions. Chicken manure was more effective than compost, although chrysene degradation rates remained lower than those obtained for anthracene, reflecting the greater recalcitrance of this high-molecular-weight PAH.

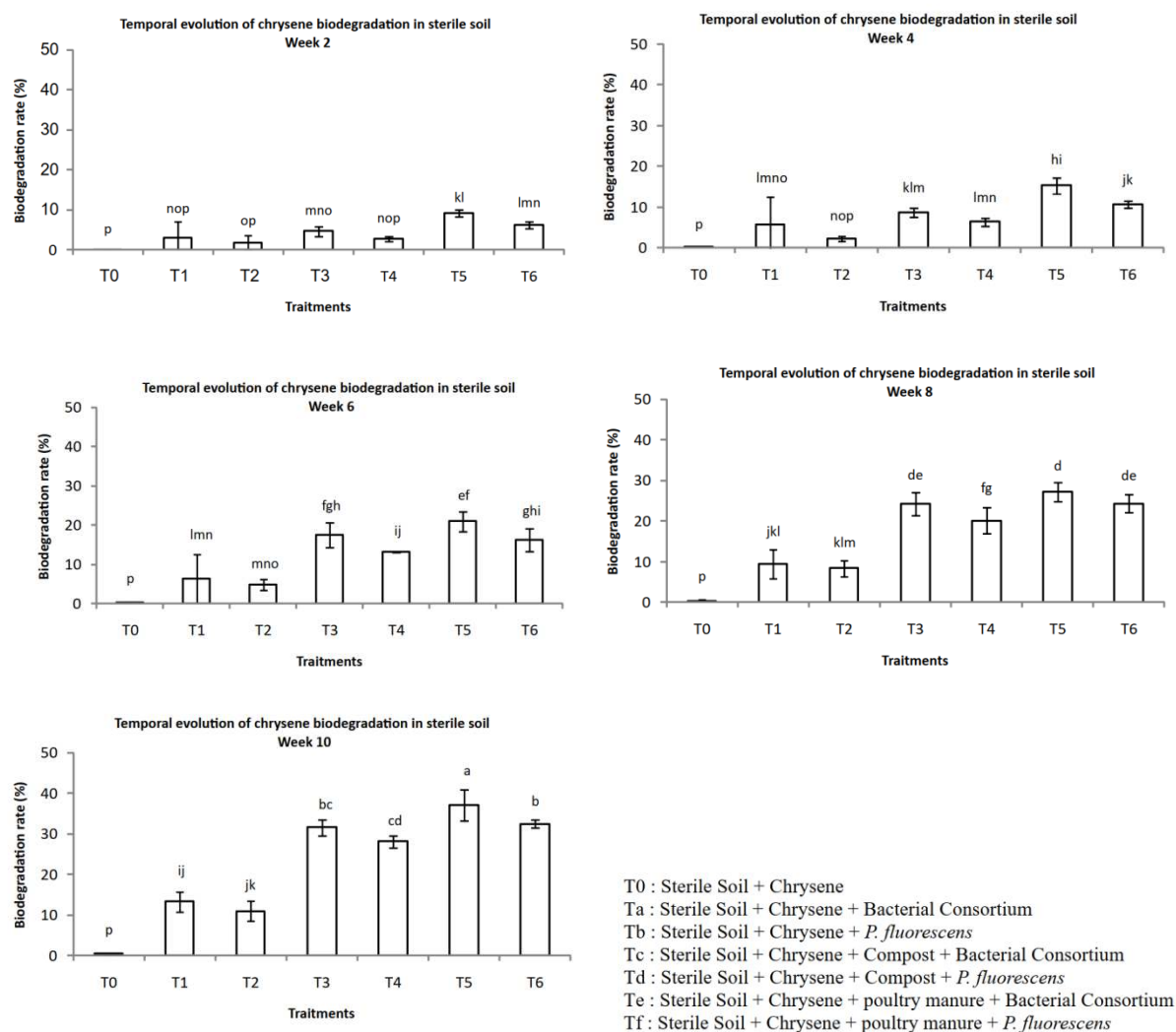


Figure 6. Chrysene degradation in sterile soils.

3.3.4 Hydrocarbon degradation rates under non-sterile conditions

Anthracene degradation rate in contaminated non-sterile soil

Figure 7 shows a progressive increase in the anthracene biodegradation rate across all treatments throughout the 10-week incubation period. Unlike in sterile soils, degradation was already observed by the second week, including in treatment T1, indicating the activity of the indigenous microflora, whereas the sterile control (T0) exhibited negligible degradation. By the second week, biodegradation rates ranged from 3% to 23%, with treatments combining chicken manure and a bacterial inoculum (T8 and T9) showing the highest performance. From the fourth week onward, biodegradation increased significantly ($p < 0.05$) in all treatments, with T8 (chicken manure + bacterial consortium) and T9 (chicken manure + *Pseudomonas fluorescens*) consistently exhibiting the highest degradation rates, followed by the compost-based treatments (T6 and T7).

By week 10, T8 achieved the highest biodegradation rate ($\approx 75\%$), followed by T9 ($\approx 70\%$) and T6 ($\approx 56\%$). Treatments T3, T4, T5, and T7 exhibited degradation rates ranging from 43% to 48%, whereas T1 and T2 reached approximately 30% and 35%, respectively. The differences among treatments were statistically significant ($p < 0.05$).

Overall, the results demonstrate that anthracene biodegradation was markedly higher under non-sterile conditions than under sterile conditions, highlighting the contribution of the indigenous microflora. The combination of bioaugmentation and biostimulation, particularly with chicken manure, was the most effective strategy for accelerating the degradation of this PAH.

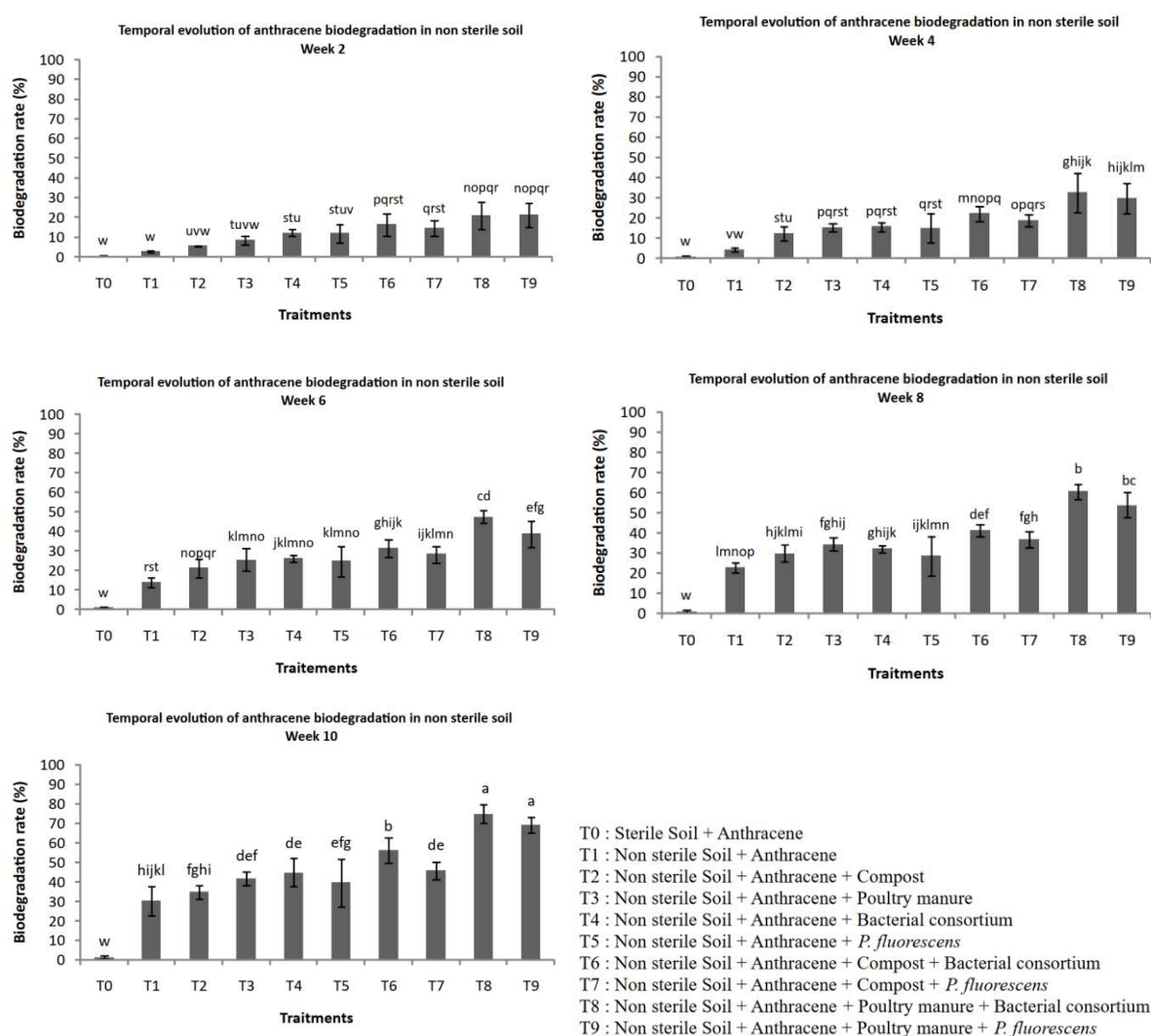


Figure 7. Anthracene degradation in non-sterile soils.

3.3.4 Chrysene degradation rate in contaminated non-sterile soils

Figure 8 shows a progressive increase in the chrysene biodegradation rate across all treatments throughout the 10-week incubation period, with significant differences among treatments ($p < 0.05$). The sterile control (T0) exhibited negligible degradation, confirming that abiotic losses were minimal. By the second week, biodegradation rates ranged from 4% to 18%, with treatments T8 (chicken manure + bacterial consortium) and T9 (chicken manure + *Pseudomonas fluorescens*) showing the highest performance. From the fourth week onward, biodegradation increased significantly in all treatments, with the highest values consistently observed for T8 and T9, followed by the compost-based treatments (T6 and T7). By week 10, T8 achieved the highest biodegradation rate ($\approx 58\%$), followed by T9 ($\approx 53\%$), T6 ($\approx 46\%$), and T7 ($\approx 42\%$). Treatments T3, T4, and T5 reached degradation rates ranging from 33% to 39%, whereas T1 and T2 showed more modest performance ($\approx 22\text{--}30\%$), although these values were significantly higher than those of the control ($p < 0.05$).

Overall, the combination of bioaugmentation and biostimulation, particularly with chicken manure, proved to be the most effective strategy for enhancing chrysene biodegradation under non-sterile conditions. The indigenous microflora also contributed to chrysene degradation, acting synergistically with the inoculated microorganisms and organic amendments. Compared with anthracene, chrysene consistently exhibited lower biodegradation ($\approx 58\%$ versus $\approx 75\%$), which can be attributed to its four fused aromatic rings, greater hydrophobicity, and stronger adsorption to soil particles, thereby reducing its bioavailability and biodegradability.

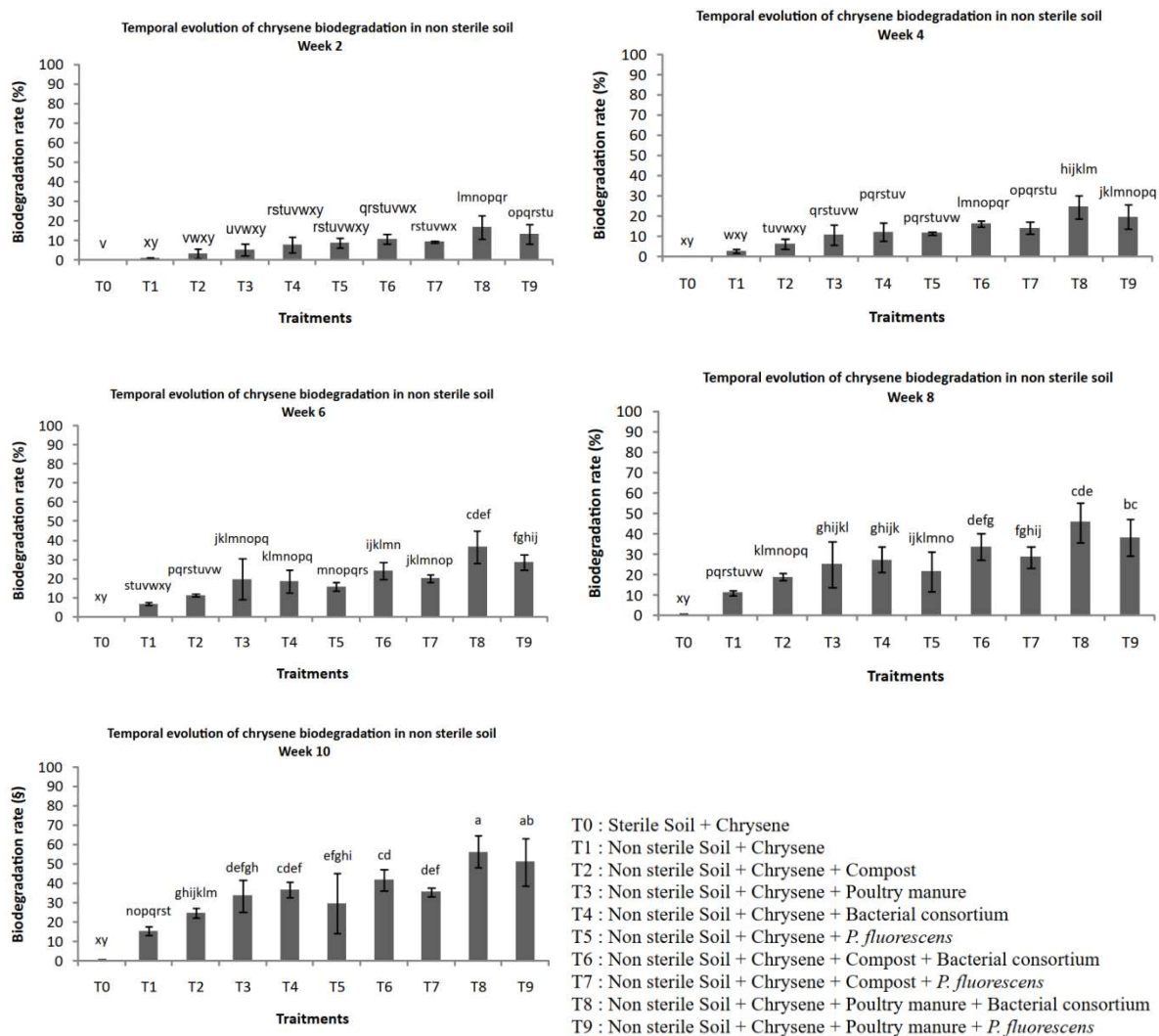


Figure 8. Chrysene degradation in non-sterile soils.

3.4 Overall assessment of the effect of nutrients on anthracene and chrysene biodegradation in sterile soil

Table 4. Biofertilizer contribution to Anthracene and Chrysene degradation in sterile soil

Experiments	Biofertilizers	PAHs	Biofertilizers contribution to degradation (%)				
			Week 2	Week 4	Week 6	Week 8	Week 10
Sterile soil + Consortium	Without compost	Anthracene	17.1	33.8	52	45.8	48.1
		Chrysene	66.6	66.4	36.1	41.4	39.1
	With compost	Anthracene	82.9 ± 0.5 ^{hi}	66.2 ± 2.5 ^{ghi}	48.0 ± 2.7 ^{fghi}	54.2 ± 4.9 ^{cdef}	51.9 ± 3 ^{bcd}
		Chrysene	33.4 ± 2.6 ⁱ	33.4 ± 5.7 ^{hi}	63.9 ± 7.7 ^{cdefg}	58.6 ± 6.2 ^{bcde}	60.9 ± 1.2 ^{abc}
Sterile soil + <i>P. fluorescens</i>	Without compost	Anthracene	25.4	56.2	72.5	65.3	71.4
		Chrysene	62.1	36.7	36.6	41.4	39.1
	With compost	Anthracene	74.6 ± 2.1 ⁱ	43.8 ± 0.8 ⁱ	27.5 ± 3 ⁱ	34.7 ± 5.4 ^{fghi}	28.6 ± 6.9 ^{fghi}
		Chrysene	37.9 ± 1.3 ⁱ	63.3 ± 1.4 ^{ghi}	63.4 ± 1.6 ^{efghi}	58.6 ± 4.9 ^{cdef}	60.9 ± 3.4 ^{abcd}
Sterile soil + Consortium	Without poultry manure	Anthracene	13.4	21.5	30.5	33.0	35.2
		Chrysene	34	37.8	30.2	51.2	35.6
	With poultry manure	Anthracene	86.6 ± 5 ^{fghi}	78.5 ± 4 ^{cdefg}	69.5 ± 5.2 ^{bcd}	67.0 ± 1.3 ^{ab}	64.8 ± 3.8 ^a
		Chrysene	66 ± 3.9 ^{fghi}	62.2 ± 6.8 ^{d^{efgh}}	69.8 ± 6.7 ^{bcde}	65.5 ± 5.9 ^{abc}	64.4 ± 6.3 ^a
Sterile soil + <i>P. fluorescens</i>	Without poultry manure	Anthracene	17.9	35.5	46.7	48.8	51.7
		Chrysene	27.4	21.7	29.9	34.1	33.9
	With poultry manure	Anthracene	82.1 ± 2.2 ^{fghi}	64.5 ± 3.1 ^{efghi}	53.3 ± 0.7 ^{defgh}	51.2 ± 4.7 ^{cde}	48.3 ± 6.9 ^{bc}
		Chrysene	72.6 ± 1.7 ^{fghi}	78.3 ± 0.5 ^{efghi}	70.1 ± 4.1 ^{cdefg}	65.9 ± 4.3 ^{bcd}	66.1 ± 3.5 ^{ab}

The effectiveness of biofertilizers in the biodegradation of anthracene and chrysene in sterile soil (Table 4) depended on the type of inoculum, organic amendment, and incubation period. Overall, treatments amended with compost or poultry manure exhibited higher degradation rates than non-amended treatments. In the absence of an amendment, anthracene biodegradation remained moderate, with *P. fluorescens* being more effective than the bacterial consortium, reaching a maximum degradation rate of 71.4% at week 10, whereas chrysene degradation remained low. The addition of compost significantly enhanced the degradation of both PAHs, with degradation rates reaching $82.9 \pm 0.5\%$ for anthracene (week 2) and $63.9 \pm 7.7\%$ for chrysene (week 6) with the bacterial consortium. Comparable performances were obtained with *P. fluorescens*, particularly for chrysene, for which degradation remained at approximately 60% until the end of the incubation period. Poultry manure was the most effective amendment. When combined with the bacterial consortium, it resulted in degradation rates of $86.6 \pm 5.0\%$ for anthracene and $69.8 \pm 6.7\%$ for chrysene, with degradation rates exceeding 64% after 10 weeks. The combination of *P. fluorescens* and poultry manure also resulted in substantial chrysene degradation, reaching $78.3 \pm 0.5\%$ as early as week 4.

Statistical analysis revealed significant differences among treatments ($p < 0.05$). Treatments amended with poultry manure were predominantly classified within the highest-performing statistical groups (a–ab), whereas non-amended treatments belonged mainly to the lowest-performing groups (hi–i). These findings confirm that organic amendments, particularly poultry manure, significantly enhance the biodegradation of anthracene and chrysene by both the bacterial consortium and *P. fluorescens* under sterile conditions.

3.5 Overall assessment of the effect of nutrients on anthracene and chrysene biodegradation in non-sterile soil

Table 5. Biofertilizer contribution to Anthracene and Chrysene degradation in non-sterile soil

Techniques	Experiments	Biofertilizers	PAHs	Biofertilizers contribution to degradation (%)				
				Week 2	Week 4	Week 6	Week 8	Week 10
Biostimulation	Non sterile Soil	Without compost	Anthracene	45.8	32.6	65.4	76.3	86.7
			Chrysene	30.6	45.1	60.5	58.4	62.0
		With compost	Anthracene	54.2 ± 0.1 ^{fg}	67.4 ± 3.4 ^{efg}	34.6 ± 6.4 ^{efg}	23.7 ± 6.4 ^{efg}	13.3 ± 10.3 ^{fg}
			Chrysene	69.4 ± 2.3 ^h	54.9 ± 3.1 ^{gh}	39.5 ± 0.2 ^{fgh}	41.6 ± 0.7 ^{defgh}	38.0 ± 1.2 ^{bcdefgh}
		Without poultry manure	Anthracene	29.8	26.4	54.5	66.3	72.2
			Chrysene	18.7	26.6	34.4	54.1	55.4
		With poultry manure	Anthracene	70.2 ± 2.3 ^{efg}	73.6 ± 2.8 ^{defg}	45.5 ± 7.9 ^{defg}	33.7 ± 5.2 ^{defg}	27.8 ± 8.7 ^{defg}
			Chrysene	81.3 ± 2.7 ^{fgh}	73.4 ± 4.6 ^{defgh}	65.6 ± 10.1 ^{abcdef}	55.9 ± 10.7 ^{abcde}	54.6 ± 6.2 ^{ab}
		Without compost	Anthracene	74.0	70.3	83.5	77.7	79.7
			Chrysene	70.3	75.1	75.0	71.4	83.3
Combination Bioaugmentation and Biostimulation	Non sterile Soil + Bacterial consortium	With compost	Anthracene	26.0 ± 4.4 ^{fg}	29.7 ± 5.6 ^{efg}	16.5 ± 5.8 ^{efg}	22.3 ± 4.6 ^{efg}	20.3 ± 0.6 ^d ^{efg}
			Chrysene	29.7 ± 6.1 ^{gh}	24.9 ± 5.4 ^{fgh}	25.0 ± 2.9 ^{efgh}	28.6 ± 0.2 ^{efgh}	16.7 ± 6.5 ^{efgh}
		Without poultry manure	Anthracene	58.3	47.7	55.4	53	60.0
			Chrysene	43.1	49.8	51.4	60.3	64.9
		With poultry manure	Anthracene	41.7 ± 6.1 ^g	52.3 ± 8.7 ^{fg}	44.6 ± 2.1 ^{fg}	47.0 ± 5.6 ^{efg}	40.0 ± 10.9 ^{efg}
			Chrysene	54.9 ± 6.2 ^h	50.2 ± 4.8 ^h	48.6 ± 2.9 ^{fgh}	39.7 ± 6.7 ^{efgh}	35.1 ± 4.8 ^{efgh}
		Without compost	Anthracene	79.3	78.0	87.8	77.9	86.6
			Chrysene	94.1	80.9	77.8	75.7	83.6
		With compost	Anthracene	20.7 ± 1.0 ^{efg}	21.0 ± 4.5 ^{bcde}	12.2 ± 5.6 ^{abcd}	22.1 ± 10.9 ^{ab}	13.4 ± 16.6 ^a
			Chrysene	5.9 ± 2.2 ^{cd} ^{efgh}	19.1 ± 2.9 ^{abcdefg}	22.2 ± 3.7 ^{abc}	24.3 ± 4.8 ^{abc}	16.4 ± 15.4 ^a
	Non sterile Soil + <i>P. fluorescens</i>	Without poultry manure	Anthracene	56.3	50.1	64.1	53.0	58.2
			Chrysene	66.0	58.5	44.9	57.0	59.4
		With poultry manure	Anthracene	43.7 ± 3.9 ^{efg}	49.9 ± 5.6 ^{cdef}	35.9 ± 7.9 ^{cdefg}	47.0 ± 4.9 ^{abc}	42.8 ± 15.3 ^a
			Chrysene	34.0 ± 7.6 ^{fgh}	41.5 ± 5.7 ^{defgh}	45.1 ± 1.6 ^{abcdef}	43.0 ± 2.1 ^{abcd}	41.6 ± 3.8 ^a

The effect of biofertilizers on anthracene and chrysene biodegradation in non-sterile soil subjected to biostimulation alone or to a combination of bioaugmentation and biostimulation is presented in Table 5. The results show that biodegradation efficiency depended on the bioremediation strategy, type of amendment, and inoculated microorganism.

Under biostimulation, non-amended soils showed a progressive increase in anthracene degradation, reaching 86.7% after 10 weeks. In contrast, compost and poultry manure promoted chrysene biodegradation more effectively during the early stages of incubation, with maximum degradation rates of $69.4 \pm 2.3\%$ and $81.3 \pm 2.7\%$, respectively, followed by a gradual decline.

The combination of bioaugmentation and biostimulation generally improved biodegradation performance. In the absence of organic amendments, treatments inoculated with the bacterial consortium or *P. fluorescens* achieved the highest degradation rates, reaching 79.7% and 86.6% for anthracene, and 83.3% and 94.1% for chrysene, respectively. In contrast, compost significantly reduced degradation rates, particularly for chrysene, for which values remained below 30% throughout the experiment. Poultry manure maintained intermediate performance, with degradation rates generally exceeding 40%. Statistical analysis revealed significant differences among treatments ($p < 0.05$). The highest degradation rates were obtained with the bioaugmentation–biostimulation strategy without organic amendment, whereas the lowest degradation rates were observed in compost-amended treatments, particularly for chrysene. These findings highlight the key role of indigenous microflora in PAH biodegradation in non-sterile soil and demonstrate that the effects of organic amendments depend on the bioremediation strategy employed.

3.6 Comparative analysis of anthracene and chrysene degradation rates in sterile soils

Table 6 shows that bioremediation strategies significantly affected (ANOVA, $p < 0.05$) the biodegradation of anthracene and chrysene in sterile agricultural soils. The control (T0) exhibited the lowest degradation rates ($1.5 \pm 0.9\%$ for anthracene and $0.4 \pm 0.3\%$ for chrysene), confirming the limited contribution of abiotic processes. Microbial inoculation significantly enhanced the biodegradation of both PAHs. The bacterial consortium (T1) resulted in degradation rates of $15.4 \pm 2.7\%$ for anthracene and $13.2 \pm 2.5\%$ for chrysene, whereas *P. fluorescens* (T2) achieved $19.8 \pm 2.4\%$ and $11.1 \pm 2.5\%$, respectively. The addition of compost (T3 and T4) further enhanced these performances, with no significant difference between the two treatments ($p > 0.05$).

The highest degradation rates were obtained with poultry manure, particularly when combined with the bacterial consortium (T5), which recorded the highest degradation rates ($43.6 \pm 4.9\%$ for anthracene and $37.1 \pm 3.8\%$ for chrysene), followed by treatment T6 (poultry manure + *P. fluorescens*), with $38.2 \pm 5.4\%$ and $32.45 \pm 0.9\%$, respectively.

Overall, anthracene was consistently more readily biodegraded than chrysene. The ranking of treatment efficacy was identical for both PAHs ($T5 > T6 > T3 \approx T4 > T2/T1 > T0$), confirming that the combination of poultry manure and the bacterial consortium was the most effective strategy for stimulating PAH biodegradation under sterile conditions.

Table 6 : Mass balance of PAHs amount remained (mg.kg⁻¹) and percentages in each fraction to total PAHs (Anthracene and Chrysene) added (input) in sterile soil after 10 weeks of degradation

Different biodegradation strategies	Polluted agricultural sterile soils				
	Fate of PAHs	Anthracene		Chrysene	
		Amounts (mg.kg ⁻¹)	% Input	Amounts (mg.kg ⁻¹)	% Input
Sterile soil (T0)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	3,318.33 ± 110.7	98.5	3,348.66 ± 132.4	99.6
	Losses	49 ± 31.2	1.5 ± 0.9 ^{rs}	13.66 ± 10.5	0.4 ± 0.3 ^p
Sterile soil + Bacterial Consortium (T1)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	2,850.33 ± 50.5	84.6	2,917.33 ± 153.6	86.8
	Losses	517 ± 112.4	15.4 ± 2.7 ^{hij}	445 ± 84.1	13.2 ± 2.5 ^{ij}
Sterile soil + <i>P. fluorescens</i> (T2)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	2,701.66 ± 100	80.2	2,662.33 ± 187.7	88.9
	Losses	665.66 ± 100.9	19.8 ± 2.4 ^{gh}	370 ± 74.6	11.1 ± 2.5 ^{jk}
Sterile soil + Compost + Bacterial Consortium (T3)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	1,075.66 ± 81.13	68.1	2,301.33 ± 98	68.4
	Losses	2,291.66 ± 128.3	31.9 ± 2.2 ^{cd}	1,061 ± 86.2	31.6 ± 2 ^{bc}
Sterile soil + Compost + <i>P. fluorescens</i> (T4)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	932.66 ± 126.2	72.3	2417 ± 104.5	71.8
	Losses	2,484.66 ± 249.8	27.7 ± 4.6 ^{de}	945.33 ± 57.3	28.2 ± 1.4 ^{cd}
Sterile soil + Poultry manure + Bacterial Consortium (T5)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	1,467.33 ± 219.5	56.4	2,113 ± 102.7	62.9
	Losses	1900 ± 100	43.6 ± 4.9 ^a	1,248.66 ± 159.3	37.1 ± 3.8 ^a
Sterile soil + Poultry manure + + <i>P. fluorescens</i> (T6)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
	In soil phase	1287 ± 214	61.8	2,271.33 ± 57.6	67.55
	Losses	2,080.33 ± 158.9	38.2 ± 5.4 ^b	1,091 ± 70	32.45 ± 0.9 ^b

3.7 Comparative assessment of the mass balance of anthracene and chrysene in non-sterile soils

The mass balance of anthracene and chrysene after 10 weeks of biodegradation in non-sterile agricultural soils is presented in Table 7. Statistical analysis (ANOVA, $p < 0.05$) showed that bioremediation strategies significantly affected the degradation of both PAHs.

The control (T0) exhibited the lowest losses ($1.46 \pm 0.9\%$ for anthracene and $0.5 \pm 0.2\%$ for chrysene), confirming the limited contribution of abiotic processes. Indigenous microflora (T1) significantly enhanced biodegradation, resulting in degradation rates of $30.2 \pm 7.4\%$ and $15.2 \pm 2.2\%$ for anthracene and chrysene, respectively. Under biostimulation, poultry manure (T3) was more effective than compost (T2), with degradation rates of $41.8 \pm 3.5\%$ versus $34.8 \pm 3.3\%$ for anthracene, and $33.6 \pm 8.2\%$ versus $24.6 \pm 2.5\%$ for chrysene. Bioaugmentation also enhanced degradation, with the bacterial consortium (T4) generally outperforming *Pseudomonas fluorescens* (T5).

The highest degradation rates were obtained with the combined bioaugmentation–biostimulation strategy. Treatment T8 (poultry manure + bacterial consortium) achieved the highest degradation rates ($74.8 \pm 4.6\%$ for anthracene and $56.4 \pm 8.4\%$ for chrysene), followed by T9 (poultry manure + *Pseudomonas fluorescens*), with $69.1 \pm 3.8\%$ and $50.9 \pm 12.1\%$, respectively.

Overall, anthracene was consistently more readily biodegraded than chrysene. The treatment efficacy ranking was identical for both PAHs ($T8 > T9 > T6 > T7 \approx T4 > T3 > T5 > T2 > T1 > T0$). The substantially higher degradation rates observed compared with sterile soils highlight the synergistic effects of indigenous microflora, inoculated microorganisms, and organic amendments, particularly poultry manure.

Table 7. Mass balance of PAHs amount remained (mg.kg⁻¹) and percentages in each fraction to total PAHs (Anthracene and Chrysene) added (input) in non sterile soil after 10 weeks of degradation under different techniques

Biodegradation Techniques	Differents biodegradation strategies	Fate of PAHs	Polluted agricultural sterile soils			
			Anthracene		Chrysene	
			Amounts (mg.kg ⁻¹)	% Input	Amounts (mg.kg ⁻¹)	% Input
Sterile control	Sterile soil (T0)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	3,318.33 ± 110.7	98.54	3,347 ± 130.4	99.5
		Losses	49 ± 31.2	1.46 ± 0.9 ^w	15.33 ± 8.1	0.5 ± 0.2 ^{xy}
Natural attenuation	Non sterile soil (T1)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	2,351.33 ± 227.4	69.8	2850 ± 132.3	84.8
		Losses	1,016 ± 280.3	30.2 ± 7.4 ^{hijkl}	512.33 ± 78	15.2 ± 2.2 ^{nopqrst}
Biostimulation	Non sterile soil + Compost (T2)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	2,196 ± 133.6	65.2	2,535 ± 155.5	75.4
		Losses	1,171.33 ± 130.5	34.8 ± 3.3 ^{fghi}	827.33 ± 73.5	24.6 ± 2.5 ^{ghijklm}
	Non sterile soil + Poultry manure (T3)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	1,960.66 ± 72.6	58.2	2,233.33 ± 321.4	66.4
		Losses	1,406.66 ± 166.6	41.8 ± 3.5 ^{def}	1,129 ± 271	33.6 ± 8.2 ^{defgh}
Bioaugmentation	Non sterile soil + Bacterial consortium (T4)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	1859 ± 250.5	55.2	2,133.33 ± 152.7	63.4
		Losses	1,508.33 ± 254.7	44.8 ± 7.1 ^{de}	1,229 ± 150.8	36.6 ± 4.1 ^{cdef}
	Non sterile soil + <i>P. fluorescens</i> (T5)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	2,038.33 ± 330	60.5	2,363.33 ± 539.3	70.3
		Losses	1,329 ± 459	39.5 ± 12.4 ^{efg}	999 ± 534.2	29.7 ± 15.6 ^{efghi}
Bioaugmentation and Biostimulation	Non sterile soil + Compost + Bacterial consortium (T6)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	1,473.66 ± 233.8	43.8	1,966.66 ± 251.6	58.5
		Losses	1,893.66 ± 239.5	56.2 ± 6.6 ^b	1,395.66 ± 132.6	41.5 ± 5.4 ^{cd}
	Non sterile soil + Compost + <i>P. fluorescens</i> (T7)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	1,833.33 ± 208.2	54.4	2,166.66 ± 152.7	64.4
		Losses	1,534 ± 115.9	45.6 ± 4.5 ^{de}	1,195.66 ± 32.9	35.6 ± 2.2 ^{def}
	Non sterile soil + Poultry manure + Bacterial consortium (T8)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	850 ± 180.3	25.2	1,466.66 ± 305.5	43.6
		Losses	2,517.33 ± 101.4	74.8 ± 4.6 ^a	1,895.66 ± 277.8	56.4 ± 8.4 ^a
	Non sterile soil + Poultry manure + <i>P. fluorescens</i> (T8)	Input	3,367.33 ± 139.5		3,362.33 ± 122.4	
		In soil phase	1,040.66 ± 150.9	30.9	1,650 ± 409.3	49.1
		Losses	2,326.66 ± 132.4	69.1 ± 3.8 ^a	1,712.33 ± 424.1	50.9 ± 12.1 ^{ab}

4. Discussion

Overall, the results obtained demonstrate that the efficiency of polycyclic aromatic hydrocarbon (PAH) biodegradation results from the interaction among soil physicochemical properties, pollutant bioavailability, microbial community activity, and nutrient availability. The characteristics of the studied soil, particularly its loamy texture, moderate organic matter content, C/N ratio of 11, and slightly acidic pH (5.7), generally provide favorable conditions for the development of hydrocarbon-degrading microorganisms. However, the presence of clay particles and organic matter also promotes PAH adsorption, particularly for high-molecular-weight compounds, thereby reducing their bioavailability and slowing their biodegradation (Titaley et al., 2020; Ukalska-Jaruga & Smreczak, 2020). This limitation is particularly pronounced for chrysene, whose high hydrophobicity promotes strong retention within the soil matrix, unlike anthracene, which is more soluble and therefore more readily available for microbial uptake (Rajput et al., 2023).

The comparison between sterile and non-sterile soils clearly highlights the central role of indigenous microflora in bioremediation processes. The significantly higher performance observed in non-sterile soils indicates that indigenous microorganisms possess an intrinsic capacity to utilize PAHs as carbon and energy sources. Their presence promotes synergistic interactions with the inoculated bacteria, enhancing enzymatic diversity and the complementarity of metabolic pathways involved in the degradation of aromatic compounds. This microbial cooperation explains the higher degradation efficiencies observed under non-sterile conditions and is consistent with observations reported in several recent studies on the bioaugmentation of contaminated soils (Wu et al., 2020).

The evolution of microbial populations throughout the experiment further confirms the progressive adaptation of microorganisms to PAHs. The continuous increase in competent microflora, for both anthracene and chrysene, reflects a process of natural selection favoring populations capable of metabolizing these compounds. Treatments combining the bacterial consortium with organic amendments consistently exhibited the highest microbial densities, indicating greater nutrient availability and enhanced biological activity in the soil. Bacterial consortia possess greater metabolic diversity than individual strains such as *P. fluorescens*, which may facilitate the sequential degradation of different hydrocarbon fractions and limit the accumulation of potentially inhibitory intermediate metabolites (Brzeszcz & Kaszycki, 2018; Xu et al., 2018). This metabolic complementarity may explain their superior performance throughout the study.

The results also demonstrate that bioaugmentation alone improves PAH biodegradation, but its effectiveness is markedly enhanced when combined with biostimulation. Organic amendments, particularly poultry manure, provide substantial amounts of nitrogen, phosphorus, potassium, and readily assimilable carbon, thereby promoting microbial growth and the synthesis of enzymes involved in PAH oxidation. The greater effectiveness of poultry manure compared with compost may be attributed to its lower C/N ratio and the more rapid availability of nutrients. These findings support the view that combining bioaugmentation and biostimulation represents an effective approach for accelerating the bioremediation of contaminated soils (Semboung et al., 2016; Xue et al., 2023).

The changes in pH observed during the experiment also provide important information regarding the progression of biological processes. The progressive decrease in pH in inoculated treatments likely reflects the production of organic acids and intermediate metabolites resulting from the microbial oxidation of PAHs. This acidification was more pronounced in treatments combining bioaugmentation and biostimulation, indicating greater metabolic activity promoted by the organic amendments. In contrast, the relatively stable pH observed in the sterile controls confirms that the recorded variations were primarily of biological rather than abiotic origin (Liu et al., 2023; Beljin et al., 2024). The greater acidification observed in anthracene-contaminated soils also reflects its more rapid biodegradation compared with

chrysene, consistent with differences in the bioavailability of low- and high-molecular-weight PAHs (Semboung & Ngo Nkot, 2026).

Across all treatments, anthracene exhibited significantly higher biodegradation rates than chrysene. This difference is directly related to the physicochemical properties of the two compounds. Anthracene, which consists of three fused aromatic rings, has a lower molecular weight, greater solubility, and higher bioavailability, thereby facilitating its uptake by microorganisms. In contrast, chrysene, which consists of four fused aromatic rings, is more hydrophobic and more strongly adsorbed onto soil organic matter, limiting its transfer to microbial cells and explaining its greater persistence (Rajput et al., 2023). Although nutrient supplementation and microbial inoculation enhanced its degradation, they did not completely overcome the constraints imposed by its molecular structure.

The mass balance results further confirm that the highest degradation efficiencies were achieved when bioaugmentation was combined with biostimulation, particularly with the poultry manure–bacterial consortium combination. This strategy simultaneously benefits from increased nutrient availability, high microbial functional diversity, and the contribution of indigenous microflora in non-sterile soils. It therefore optimizes metabolic interactions, enhances the production of degradative enzymes, and promotes more complete PAH mineralization. These observations are consistent with recent recommendations favoring integrated approaches that combine bioaugmentation, biostimulation, and the valorization of organic amendments for the remediation of contaminated soils.

From an environmental perspective, this study demonstrates that the valorization of organic residues such as poultry manure represents a sustainable alternative to conventional physicochemical treatments. By simultaneously improving soil biological fertility and biodegradation efficiency, this strategy contributes to a circular-economy approach and sustainable organic waste management. It appears particularly suitable for tropical regions, where such amendments are readily available and can be incorporated into low-cost programs for the restoration of hydrocarbon-contaminated soils.

5. Conclusion

In conclusion, this study demonstrates that successful bioremediation of PAH-contaminated soils relies on an integrated approach combining favorable soil properties, active indigenous microflora, appropriate nutrient supplementation, and efficient microbial consortia. The combination of bioaugmentation and biostimulation, particularly with poultry manure, was the most effective strategy for accelerating the degradation of anthracene and chrysene. These findings provide a solid scientific basis for the development of sustainable bioremediation technologies applicable to agricultural soils contaminated with polycyclic aromatic hydrocarbons.

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Data availability : No datasets were generated or analysed during the current study.

Conflict of Interest : The authors declare no conflict of interest.

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