RESEARCH ARTICLE

Performance study of biosurfactants in promoting microbial remediation of polycyclic aromatic hydrocarbon contaminated soil

Guang Hao[1], Na Meng[2, *], Xiaoxu Yu[3], Changliang Zhao[3]

[1]School of Life and Health Management, [2]School of Intelligence and Engineering, [3]Student Affairs Office, Shenyang City University, Shenyang, Liaoning, China.

Received: April 28, 2026; **accepted:** August 26, 2026.

Polycyclic aromatic hydrocarbons (PAHs) are typical persistent and toxic organic pollutants in soil, which seriously disrupt the ecological balance of the soil, endanger the safety of agricultural products and human health. Microbial remediation is a key technology for the green remediation of PAH-contaminated soil. However, the extremely low water solubility and poor biodegradability of PAHs have greatly limited the efficiency of natural microbial remediation. This study investigated the effects of rhamnolipids on the solubilization of PAHs, their uptake and accumulation by wheat, and the modulation of soil microbial communities. Furfural and pyrene were selected as representative PAH pollutants. Gradient concentration groups of raffinose lipids were set up. Through indoor solubilization experiments, wheat hydroponic experiments, and soil pot experiments, combined with high-performance liquid chromatography detection and microbial high-throughput sequencing technology, this study systematically analyzed the effects of raffinose lipids on the solubilization of PAHs. It further investigated the growth characteristics of wheat, the enrichment features of pollutants, the efficiency of soil remediation, and the structure of microbial communities. The results showed a significant positive correlation between the concentration of raffinose esters and both the apparent solubility and solubilization factor of PAHs. At high concentrations, the solubilization effect of pyrene was better than that of furfural. 200 mg/L of raffinose lipids could significantly enhance the enrichment ability of wheat for PAHs, and the pollutants were mainly concentrated in the roots of wheat. At a concentration of 500 mg/kg, the removal rate of pyrene in the soil could reach 84%, and it had no negative impact on the growth of wheat. Meanwhile, it could optimize the structure of soil microbial communities and enrich a large number of PAH-degrading functional bacterial genera. Raffinose lipids could enhance the bioavailability of PAHs through solubilization and synergistically enhance the remediation effect through plant enrichment and microbial degradation with good biological safety. The study clarified the remediation enhancement mechanism of biocatalytic surfactants and provided scientific basis and practical reference for the green engineering-based remediation of PAH-contaminated soil.

Keywords: biosurfactants; microorganisms; polycyclic aromatic hydrocarbons; pollution; soil.

[*]**Corresponding author:** Na Meng, School of Intelligence and Engineering, Shenyang City University, Shenyang, Liaoning 110112, China. Email: sycumengnana@126.com.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants composed of two or more benzene rings with strong hydrophobicity, difficult biodegradability, and bioaccumulation properties and tend to accumulate in the soil environment [1, 2]. Soil PAHs pollution not only damages the soil aggregate structure, deteriorates soil physical

and chemical properties, inhibits the metabolic activity of indigenous microorganisms, and causes soil ecological function degradation, but also can be progressively enriched through the soil-crop-food chain, posing risks of teratogenicity, carcinogenicity, and mutagenicity, seriously threatening the ecological security and human health of farmland [3, 4]. Therefore, developing green and efficient PAHs pollution soil remediation technologies is a key issue urgently needed to be addressed in the current environmental ecological restoration field. Microbial remediation technology, due to its environmental friendliness, low cost, and no secondary pollution advantages, is widely applied in the remediation of PAHs contaminated soil [5-7]. Rhamnolipid, as a common biological surfactant, has biodegradability, low toxicity, and good solubilization performance and shows broad application prospects in PAHs pollution remediation [8].

PAHs pollution has become an important issue in soil environment globally. Rabieimesbah *et al*. evaluated the concentration, sources, ecological and health risks of PAHs in agricultural soil and found that industrial activities and traffic emissions were the main pollution sources, posing potential risks to the local ecology and residents' health [9]. Ehtemae *et al*. studied the pollution status, sources, and risks of heavy metals and PAHs in street dust, confirming that PAHs were widely present in the urban environment and their cumulative effect could not be ignored [10]. Eze *et al*. conducted source analysis of soil PAHs in the crude oil pollution area, clarifying that oil extraction and leakage were the dominant factors of local PAHs pollution, providing a basis for regional pollution prevention and control [11]. At the microbial remediation level, Gao *et al*. revealed the geographical distribution patterns of soil microorganisms' degradation potential for PAHs through global-scale research and found significant differences in microbial community structure and function in different regions [12]. Although existing studies have achieved certain progress, there are still obvious shortcomings and technical bottlenecks. The strong hydrophobicity of PAHs makes them easily adsorbed by soil particles, resulting in extremely low biodegradability and a notable reduction in the degradation efficiency of indigenous microorganisms. On the other hand, most existing relevant studies focus either on the analysis of a single microbial community structure or the exploration of a single remediation path. The intrinsic correlation and regulatory mechanism among them remain unclear, which makes it difficult to achieve efficient and precise remediation of contaminated soil [13, 14].

In response to the above existing problems, this research took rhamnolipid, a typical green biological surfactant, as the research object to systematically explore the solubilization effect of different concentrations of rhamnolipid on soil PAHs, the absorption and enrichment patterns of PAHs by wheat, and the regulatory effect of soil microbial community structure. This study established treatment groups with varying concentrations of rhamnolipids and employed high-performance liquid chromatography (HPLC) and high-throughput sequencing of soil microorganisms to systematically investigate the effects of rhamnolipids on the apparent solubility and solubilization enhancement factor of PAHs, the physiological growth characteristics and PAH accumulation in wheat, the removal rate of PAHs from soil, and the structure, function, and functional genera of the microbial community. The research results could improve the theoretical system of biological surfactant-enhanced composite remediation, effectively solve the industry pain points of low bioavailability of PAHs and limited remediation efficiency, and provide new ideas for the research and application of similar organic pollution soil collaborative remediation technologies.

Materials and methods

Resources of PAHs, biosurfactants, and test soil

Two typical PAHs, phenanthrene (CAS 85-01-8, molecular formula CH, logK = 4.57, water solubility 0.89 μg/L) and pyrene (CAS: 129-00-0, molecular formula CH, logK = 5.18, water solubility 0.14 μg/L) (Shanghai Maclin Biochemical Technology Co., Ltd., Shanghai, China), were selected as the target pollutants in this research. Chromatographic purity rhamnose lipids were used as the biological surfactants with critical micelle concentration of 33.4 mg/L (Shaanxi Ruijie Biotechnology Co., Ltd., Xi'an, China). The study used artificially contaminated soil as the test substrate. The clean soil was taken from the surface soil (0 – 20 cm) of the green plant area on the campus of Beijing University of Architecture and Technology (Beijing, China). After removing the impurities by passing through a 100-mesh sieve, the clean soil was thoroughly mixed with the actual multi-hydrocarbon contaminated soil taken from the pollution remediation demonstration site in Zibo, Shandong, China to obtain the basic mixed soil substrate. Phenanthrene and pyrene were dissolved in acetone to prepare a PAHs acetone solution, which was then sprayed evenly onto the mixed soil. The solution was stirred continuously until the acetone evaporated naturally. Water was added to keep the soil moist. After thorough mixing, the solution was placed in a dark place to age for one month to allow the PAHs to reach adsorption equilibrium in the soil [15].

Plant cultivation

Wheat seeds (Suqian Feili Xianghong Flowering Company, Suqian, Jiangsu, China) were selected as the test plants [16]. The semi-intensity Hoagland nutrient solution (Pu Shirui (Shanghai) Biomedical Company, Shanghai, China) was used for seedling cultivation. The wheat seeds were disinfected with 5% HO solution for 30 minutes followed by repeatedly rinsing with pure water to remove the residual disinfectant. The seeds were then soaked in pure water at room temperature for 12 hours to complete the seed swelling and activation. The activated seeds were evenly spread on the petri dishes with the semi-intensity Hoagland nutrient solution-soaked gauze and incubated at 28℃ with complete darkness for the germination. When the wheat seedlings grew to approximately 3 cm and had uniform and vigorous growth, the undamaged and consistent seedlings were selected and transplanted to hydroponic containers filled with semi-intensity Hoagland nutrient solution for further seedling cultivation. The cultivation continued for 2 weeks, and a stable cultivation environment was maintained throughout. The wheat plants with consistent plant height, biomass, and growth status were selected for subsequent hydroponic and potting experiments.

Biosurfactant effects on the apparent solubility and solubilization factor of PAHs

20 mg of each phenanthrene and pyrene solid powders were mixed with 20 mL of different concentration gradients of rhamnolipid solutions, respectively, and placed in a 200B constant temperature shaker (Shanghai Haixiang Instrument Equipment Factory, Shanghai, China) at 25℃, 150 rpm for 24 hours to ensure that the system fully reacted and reached a solid-liquid dissolution equilibrium. The samples were then centrifuged at 8,000 rpm for 15 minutes. 1 mL of the supernatant was accurately aspirated and 4 mL of chromatographically pure methanol was added for thorough mixing and dilution. After passing through the membrane, the samples were analyzed using the Ultimate 3000 high-performance liquid chromatography (Thermo Fisher Scientific, Waltham, MA, USA) to determine the concentrations of phenanthrene and pyrene in the samples [17]. The deionized water treatment group was used as the blank control. The apparent solubilities of phenanthrene and pyrene in different concentration rhamnolipid systems were determined and calculated. The corresponding PAHs solubilization factors were identified.

Effects of environmental factors on the apparent solubility of PAHs

Based on the results of the solubilization experiments, the concentration of rhamnolipid that had the best solubilization effect on phenanthrene and pyrene was selected as the fixed treatment concentration. The individual

influence laws of three typical environmental factors of temperature, solution pH, and ionic strength on the apparent solubility of PAHs were systematically explored with a single-factor variable design. The specific gradient settings of environmental parameters included the temperatures of 15℃, 25℃, 35℃, the solution pH of 5, 6, 7, 8, 9, the water body ionic strength of 0, 200, 400, 600, 800, 1,000, 2,000, and 5,000 mmol/L of NaCl.

Hydroponic experiment

100 mg of each phenanthrene and pyrene were dissolved in 100 mL of methanol to obtain a stock solution of 1,000 mg/L. 400 mL of Hoagland semi-strength nutrient solution was taken, and 1 mL of PAHs stock solution was added, bringing the volume to 500 mL to obtain a basal culture medium with a concentration of 2 mg/L for both phenanthrene and pyrene. Different concentrations of raffinose lipid solutions were added to the basal culture medium. Gradient treatment groups of 0, 20, 50, 100, 200, 300, 400, 500, 600, 800, and 1,000 mg/L were set up. A hydroponic working solution with phlorizin and pyropheophorbide concentrations of 1 mg/L and a raffinose lipid concentration gradient of 0 – 1,000 mg/L were prepared. The hydroponic solutions were transferred to laboratory-specific plant hydroponic cups (Ningbo ShuiGengfang Agricultural Machinery Co., Ltd., Yiwu, Zhejiang, China). Healthy wheat seedlings that had been pre-cultivated for 2 weeks with consistent plant height and growth status and without diseases or pests were selected for transplantation. Six (6) seedlings were planted in each hydroponic cup and uniformly placed in a constant temperature and lighted cultivation room with temperature of 25℃, light cycle of 12h/12h (light/dark), light intensity of 3,000 lx, indoor relative humidity of 60 - 70%. After 72 hours of continuous cultivation, the plant samples were uniformly harvested. The harvested wheat samples were divided into two parts for processing including the fresh plant samples for immediate determination of various physiological indicators and freeze-drying treatment for detecting the residual contents of phytiochrome and pyropheophorbide in the plant bodies.

Pot experiment

The experimental period was from February 10, 2025 to April 10, 2025. Each pot contained 1.5 kg of artificially contaminated soil and applied different concentrations of rhamnolipin. Pretreated wheat seeds were sown with 60 seeds per pot. After germination, 40 seedlings of uniform growth were retained and cultivated in a natural light greenhouse with the daytime temperature ranged from 18 to 26℃, the nighttime temperature from 12 to 18℃, the relative humidity of the air from 55% to 75%. After 60 days of cultivation, the wheat was harvested, and the root and aboveground biomass were measured. Root, leaf, and rhizosphere soil samples were collected. Wheat samples were used to determine catalase (CAT) activity, peroxidase (POD) activity, malondialdehyde (MDA) content, and PAH content. Soil samples were used for PAH removal rate and microbial community analysis.

Soil microbial community and alpha diversity analysis

Two treatment groups were set up with one control group without biosurfactants and one treatment group with 500 mg/kg rhamnolipin. Three parallel samples were set up for each treatment and cultured under the same conditions as the pot experiment. After the culture was completed, 0.5 g rhizosphere soil samples were collected, freeze-dried, and used for microbial genomic DNA extraction and sequencing analysis [18]. E.Z.N.A™ Mag-Bind Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was employed for soil microorganisms' DNA extraction in the rhizosphere following manufacturer's instructions. The bacterial 16S rRNA gene was amplified with the forward primer of 5'-CAG CMG CCG CGG TAA TWC-3' and reverse primer of 5'-CCG TCA ATT CMT TTR AGT TT-3'. The polymerase chain reaction (PCR) was performed using the 2×Taq Plus Master Mix kit (Kangwei Century, Beijing, China) and Veriti gradient PCR instrument (Thermo Fisher

Scientific, Waltham, MA, USA). The reaction system composed of 25 μL with the reaction program of 94°C for 5 min followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and 10 minutes at 72°C. The amplified PCR products were purified using the Gel Recovery Kit (Kangwei Century, Beijing, China). The high-throughput sequencing was performed by Beijing Nojiao Zhiyuan Technology Co., Ltd., Beijing, China. Alpha diversity analysis was performed by evaluating the richness and evenness of soil microbial communities through the selection of the number of operational taxonomic units (OTUs), the Chao1 index, the Shannon index, and the Simpson index. The bacterial community relative abundance was analyzed at the major genera level using sequencing results, and the differences in community structure between the control group and the rhamnolipin treatment group were compared.

Soil and plant PAHs content determination

Soil PAHs were determined by mixing 2.0 g pre-treated soil samples with anhydrous sodium sulfate followed by adding deuterated trisphenyl (p-Terphenyl-d14) as a recovery rate substitute to monitor the loss and recovery efficiency of PAHs during the entire processing. The sample pretreatment adopted the Soxhlet extraction method based on the national environmental protection standard (HJ 805-2016) "Determination of Polycyclic Aromatic Hydrocarbons in Soil and Sediments by Gas Chromatography-Mass Spectrometry", using a mixture of acetone and n-hexane as the extraction solvent and extracting continuously for 16 to 18 hours. The extract was filtered and dehydrated. PAHs were determined using 1100 high-performance liquid chromatography (HPLC) (Agilent, Santa Clara, CA, USA) with qualitative and quantitative analysis using external standard method, corrected for moisture content on a dry basis, ensuring a relative deviation of ≤ 10% for parallel samples. Plant PAHs were determined by dehydrating crushed and sieved plant samples with anhydrous sodium sulfate and extracted three times with dichloromethane-n-hexane mixture using JP-080S ultrasonic cleaning machine (Jiemeng Technology Co., Ltd., Shenzhen, Guangdong, China). After combing the supernatants, the crude extract was saponified to remove chlorophyll, concentrated, and then purified in two steps using silica gel column and gel permeation chromatography. The final solution was obtained by membrane chromatography followed by determination of PAHs. Quantification was performed using either internal or external standard methods with the spiked recovery rate controlled within the range of 82.2% – 130% to ensure accurate and reliable results.

Physiological indicators of wheat

The CAT and POD activities of wheat root and leaf samples were measured using the CAT-1-W hydrogen peroxide enzyme activity detection kit and the POD-1-W peroxidase activity detection kit (Suzhou Koming Biotechnology Co., Ltd., Suzhou, Jiangsu, China) following manufacturer's instructions. The CAT activity was defined as nmol/min·g, while the POD activity was defined as U/g. MDA was determined by the thiobarbituric acid (TBA) method. Briefly, fresh plant sample was mixed with 5% trichloroacetic acid, grinded, centrifuged to collect supernatant. After mixing the supernatant with 0.67% TBA, the sample was boiled for 30 minutes, cooled down, centrifuged, and measured the absorbances at 450, 532, 600 nm using UV-1800 ultraviolet-visible spectrophotometer (Shimadzu, Kyoto, Japan) to calculate the MDA content.

Calculation of PAHs removal rate in soil

The removal rates of phenanthrene and pyrene in soil of different treatment groups were calculated as follows.

$$h = (C_0 - C_1)/C_0 \times 100\% \quad (1)$$

where h was the removal rate. C_0 was the initial concentration of PAHs in the soil. C_1 was the final concentration of PAHs in the soil.

Calculation of bioaccumulation coefficient

The bioconcentration factor (BCF) reflected the

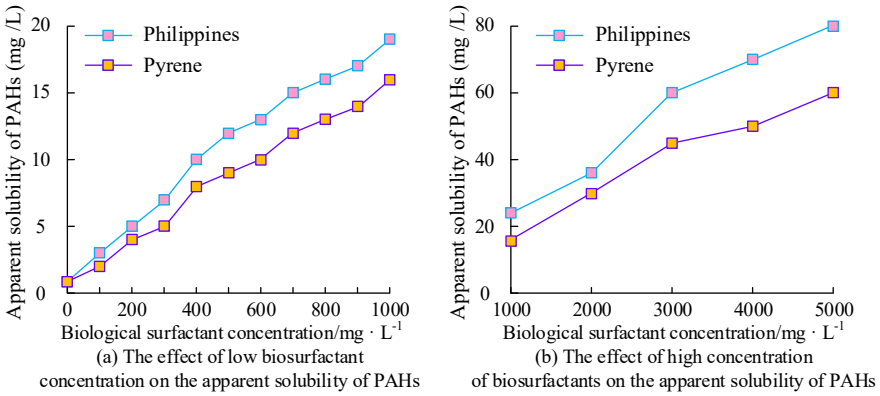


Figure 1. The effect of rhamnolipids concentration on the apparent solubility of PAHs.

ability of wheat to enrich PAHs and was calculated as follows.

$$BCF = C_a/C_b \quad (2)$$

where C_a and C_b were the PAH content in the plant and soil (mg/kg), respectively.

Data processing and statistical analysis

All experimental data was organized using Microsoft Excel. Data were expressed as mean ± standard deviation (SD) of three parallel experiments. SPSS 26.0 (IBM, Armonk, NY, USA) was employed for statistical analysis of this study. For the soil and plant physical and chemical indicators as well as pollutant content data of different treatment groups, one-way analysis of variance (ANOVA) combined with Duncan's multiple comparison was performed. *P* values less than 0.05 were defined as statistically significant differences, while *P* values less than 0.01 indicated extremely significant differences. The experimental data charts were drawn using Origin 2021 (https://www.originlab.com/).

Results

Analysis of apparent solubility and solubilization factor of PAHs

The correlation test results between biosurfactant concentration and apparent solubility of PAHs demonstrated that, when the concentration of the biosurfactant was 0 mg/L, the apparent solubility of PAHs for phenanthrene and pyrene was 1 mg/L. When the concentration of the biosurfactant increased to 1,000 mg/L, the apparent solubility of PAHs for phenanthrene and pyrene was 19 and 16 mg/L (Figure 1a). When the concentration of the biosurfactant was 5,000 mg/L, the apparent solubility of PAHs for phenanthrene and pyrene was the highest at 80 and 60 mg/L (Figure 1b). There was a positive correlation between the concentration of the biosurfactant and the apparent solubility of PAHs. In the high concentration range, the increase in apparent solubility of phenanthrene was significantly greater than that of pyrene, indicating that the biosurfactant had a better solubilizing effect on phenanthrene than on pyrene. The results of the effect of the biosurfactant on the solubilization factor indicated a notable and positive relationship between biosurfactant concentration and the solubility factors of phenanthrene and pyrene. The higher the concentration, the more pronounced the solubility effect. At low concentrations, the solubility factor increased slowly with both phenanthrene and pyrene solubility factors approaching 1, indicating that the surfactants did not exert a significant solubility effect at this level. When the concentration increased to 100 mg/L, the solubility factor began to rise rapidly, and the solubility rate of pyrene gradually surpassed that of phenanthrene. At high concentrations of 1,000

Table 1. The effect of rhamnolipids concentration on the solubilization factor of PAHs.

Surfactant concentration (mg/L)	Solubilization factor of phenanthrene	Solubilization factor of pyrene
20	1.03	0.98
50	1.09	0.97
100	2.50	2.81
200	5.66	5.62
300	7.47	7.59
400	10.61	12.52
500	12.61	17.55
600	14.00	20.98
700	15.79	24.73
800	17.04	28.63
900	19.44	31.59
1,000	21.91	36.48
2,000	40.92	90.52
3,000	65.40	132.88
4,000	77.31	199.25
5,000	84.15	252.87

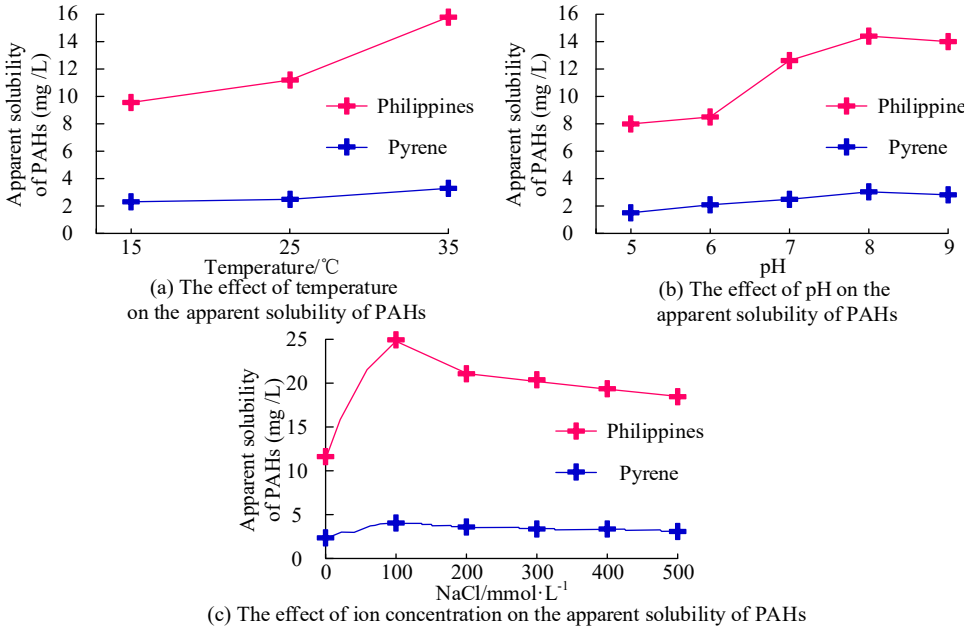


Figure 2. Effects of temperature, pH, and NaCl concentration on the apparent solubility of PAHs.

– 5,000 mg/L, the increase in solubility factor was significantly greater. At 5,000 mg/L, the solubility factor of phenanthrene reached 84.15, and that of pyrene reached 252.87, approximately three times that of phenanthrene (Table 1). Overall, the solubility-promoting effect of biosurfactants on pyrene was significantly better than that of phenanthrene, especially in the high concentration range. The effects of temperature, pH, and ion concentration on the apparent solubility of PAHs showed that, at temperatures of 15℃, 25℃, and 35℃, the apparent solubility of phenanthrene PAHs was 9.5, 11.2, and 15.8 mg/L, while the apparent solubility of pyrene PAHs was 2.3, 2.5, and 3.3 mg/L (Figure 2a). At pH 8, the apparent solubility of phenanthrene and

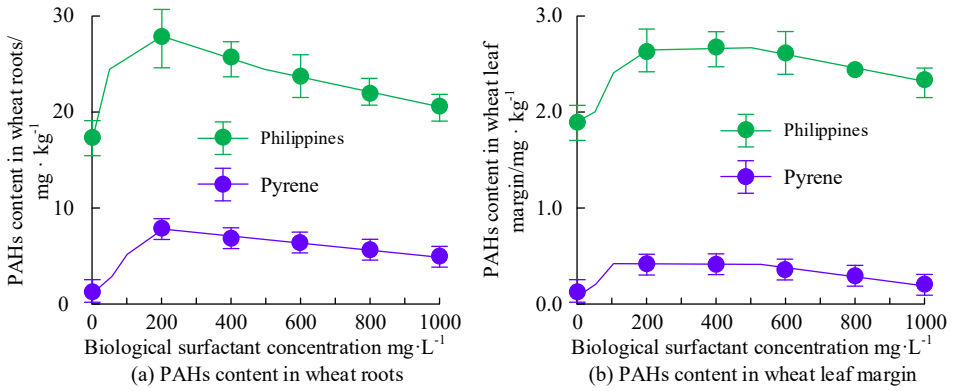


Figure 3. The effect of rhamnolipids on the content of PAHs in wheat in a hydroponic system.

pyrene PAHs was the highest at 14.4 and 3.0 mg/L (Figure 2b). When the NaCl concentration was 100 mmol/L, the apparent solubility of phenanthrene and pyrene PAHs was the highest at 25 and 4 mg/L (Figure 2c). The results indicated that temperature, pH, and ion concentration all made a big difference to the apparent solubility of PAHs. Neutral to weakly alkaline conditions, a suitable temperature around 35℃, and a NaCl concentration of 100 mmol/L were more conducive to improving the apparent solubility of PAHs.

Effects of rhamnolipids on the physiological characteristics of wheat and the repair effect on PAHs

To clarify the distribution characteristics of phenanthrene and pyrene in wheat in a hydroponic system, the PAH contents in wheat roots and leaves were measured under different concentrations of rhamnolipin treatment. When the biosurfactant concentration was 200 mg/L, the PAH contents of phenanthrene and pyrene in wheat roots were the highest at 27 and 8 mg/kg (Figure 3a). When the biosurfactant concentration was 200 mg/L, the PAH contents of phenanthrene and pyrene in wheat foliage were the highest at 2.6 and 0.4 mg/kg (Figure 3b). Appropriate concentrations of biosurfactants could significantly promote the absorption and accumulation of PAHs in wheat. Under the same treatment conditions, the phenanthrene content in wheat was much higher than pyrene, and the PAH content in roots was significantly higher than that in foliage, demonstrating that wheat had a stronger ability to accumulate low-cyclic PAHs, and that PAHs were mainly accumulated in root tissues. The effects of rhamnolipids on the removal rate of soil PAHs and wheat biomass showed that the removal rates of phenanthrene and pyrene both increased with increasing rhamnolipin concentration. Overall, the removal effect of phenanthrene was better than that of pyrene in all treatment groups, which was closely related to the number of benzene rings in PAHs. Low-ring PAHs were more easily degraded and utilized by microorganisms (Figure 4a). There was no significant difference between the root biomass and shoot biomass of wheat after adding different concentrations of rhamnolipin, indicating that within the experimental concentration range, rhamnolipin had no significant inhibitory or promoting effect on wheat growth and had good biosafety (Figure 4b). The effects of rhamnolipin on wheat CAT activity, POD activity, and MDA content demonstrated that CAT activity reached its peak when the rhamnolipin concentration was 500 mg/kg with root CAT activity at 470 nmol/min·g and leaf CAT activity at 370 nmol/min·g (Figure 5a). POD activity was the highest in both roots and leaves when the rhamnolipin concentration was 200 mg/kg at 15,400 U/g and 15,000 U/g, respectively (Figure 5(b). Rhamnolipin treatment significantly affected wheat MDA content. At low concentration of 50 mg/kg, MDA content fluctuated significantly. However, when the rhamnolipin concentration was 200 mg/kg, the

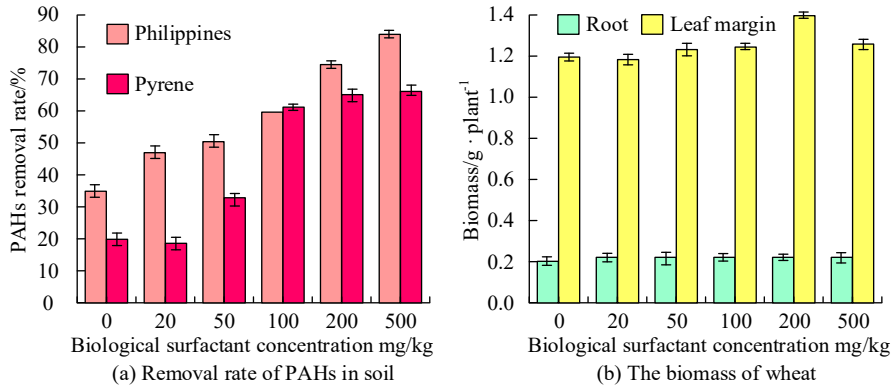


Figure 4. The effect of rhamnolipids on soil PAHs removal rate and wheat biomass.

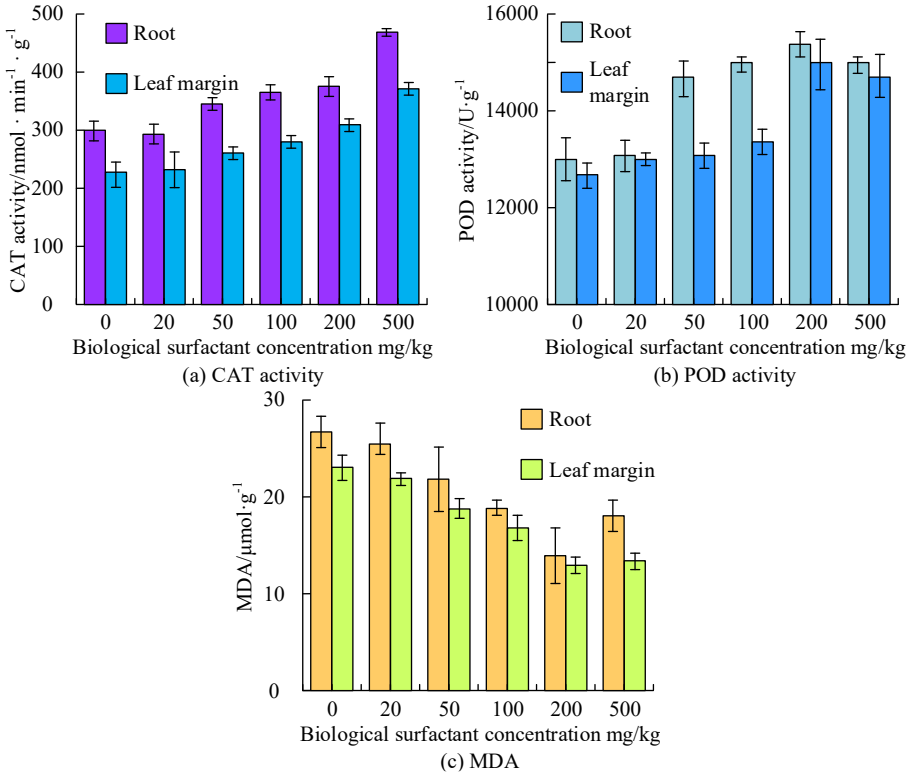


Figure 5. Effects of rhamnolipids on wheat CAT activity, POD activity and MDA content.

MDA content in the rhizosphere and leaves of wheat decreased to its lowest levels at 14 μmol/g and 13 μmol/g, respectively (Figure 5c). Rhamnolipids could enhance the antioxidant capacity and alleviate oxidative stress of wheat by regulating CAT activity and MDA content and showed better regulatory effect in the concentration range of 200 – 500 mg/kg, offering a physiological foundation for its utilization in the rehabilitation of soil contaminated with PAHs.

Effects of rhamnolipin on the enrichment capacity of wheat PAHs and soil microbial community

To investigate the regulatory effect of rhamnolipin on the absorption and enrichment of PAHs in wheat, the contents of phenanthrene, pyrene, and BCF in the plant were measured. The

Table 2. Effects of rhamnolipids treatment on phenanthrene, pyrene content and BCF in wheat.

Concentration (mg/kg)	Root (mg/kg)		Leaf margin (mg/kg)		BCF	
	Philippines	Pyrene	Philippines	Pyrene	Philippines	Pyrene
0	0.428 ± 0.024^{b}	0.682 ± 0.030^{d}	0.110 ± 0.008^{c}	0.039 ± 0.005^{b}	0.025	0.029
20	0.371 ± 0.050^{b}	0.625 ± 0.073^{d}	0.130 ± 0.007^{c}	0.028 ± 0.005^{b}	0.024	0.027
50	0.402 ± 0.026^{b}	0.799 ± 0.027^{c}	0.145 ± 0.029^{c}	0.024 ± 0.008^{b}	0.027	0.033
100	0.430 ± 0.029^{b}	0.967 ± 0.056^{b}	0.145 ± 0.009^{c}	0.035 ± 0.015^{b}	0.027	0.040
200	0.628 ± 0.004^{a}	1.350 ± 0.018^{a}	0.141 ± 0.009^{c}	0.061 ± 0.009^{b}	0.035	0.055
500	0.613 ± 0.027^{a}	1.282 ± 0.089^{a}	0.185 ± 0.019^{c}	0.052 ± 0.003^{b}	0.036	0.054

Note: The data represented the average values of three parallel experiments ± standard deviation. Different lowercase letters in the same column indicated significant differences among treatments ($P < 0.05$).

Table 3. Number of OTUs, Chao1, Shannon and Simpson before and after rhamnolipid treatment.

	No biological surfactants	500 mg/kg rhamnolipids
OUT	946	938
Chao1 Index	943	937
Shannon index	8.031	7.934
Simpson's Index	0.989	0.985

results showed that different concentrations of rhamnogalactoside had differential effects on the enrichment levels of phenanthrene and pyrene in wheat roots and leaves. As the concentration of rhamnogalactoside increased, the content of phenanthrene and pyrene in wheat roots generally showed a gradual increase and then stabilized. The peak value was reached in the 200 mg/kg treatment group, which was 0.628 ± 0.004 mg/kg and 1.350 ± 0.018 mg/kg, respectively. This group had the same results as the 500 mg/kg treatment group for the PAHs content in the roots, and there was no significant difference between the two groups, but both were significantly higher than the low to medium concentration (0 - 100 mg/kg) treatment groups ($P < 0.05$). The contents of phenanthrene and pyrene in wheat leaves in each treatment group were consistent, indicating that different concentrations of rhamnogalactoside had no significant effect on the PAHs enrichment levels in wheat leaves. The fluctuation of PAHs content in the leaves was small, and the increase was much lower than that in the roots. The BCF analysis results showed that the biological enrichment coefficients of phenanthrene and pyrene in wheat were overall increased with the increase of rhamnogalactoside concentration and maintained a relatively high level in the 200 mg/kg and 500 mg/kg treatment groups (Table 2). The results suggested that an appropriate concentration of rhamnogalactoside could significantly promote the enrichment and absorption of PAHs by wheat roots and effectively enhance the ability of wheat to accumulate PAHs from contaminated soil. Compared with phenanthrene, rhamnogalactoside had a more prominent enrichment enhancement effect on pyrene. After treatment with 500 mg/kg rhamnolipin, the number of OTUs, Chao1, Shannon, and Simpson of soil microorganisms only decreased slightly. Specifically, the number of OTUs decreased from 946 to 938, while the Chao1 from 943 to 937, the Shannon from 8.031 to 7.934, and the Simpson from 0.989 to 0.985 (Table 3). Overall, rhamnolipin treatment did not significantly reduce the richness and diversity of the soil microbial community, and the community structure maintained high stability and evenness, indicating that this concentration of rhamnolipin did not significantly inhibit the alpha diversity of the soil microbial community while enhancing pollution remediation. The effect of horizontal

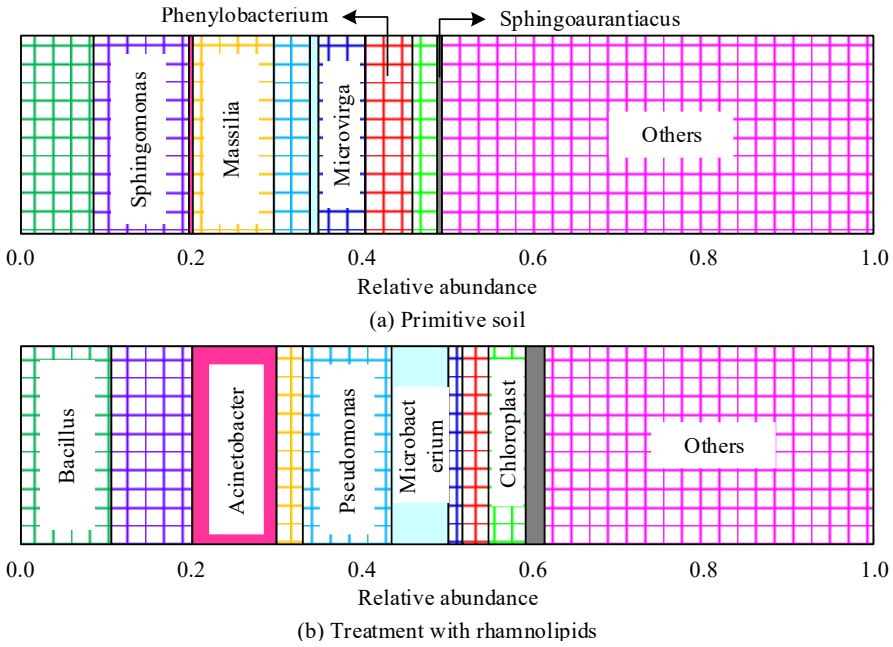


Figure 6. Relative abundance of bacterial community before and after treatment.

relative abundance showed that the bacterial community in the original soil was dominated by *Sphingomonas*, *Massilia*, *Microvirga*, and *Phenylobacterium* along with a large number of unclassified "Others" taxa, which indicated that the original soil had a relatively stable and diverse bacterial community structure with the presence of genera *Sphingomonas* possibly related to the metabolic potential of natural organic pollutants in the soil (Figure 6a). After treatment with rhamnolipin, the soil bacterial community structure underwent significant reconstruction. The relative abundance of genera of *Bacillus*, *Acinetobacter*, and *Pseudomonas* significantly increased, becoming new dominant taxa, while the abundance of dominant genera in the original soil decreased significantly (Figure 6b). This change indicated that rhamnolipin as a biosurfactant could selectively enrich functional bacterial genera with PAH degradation potential.

Discussion

The study explored the enhancing effect of rhamnolipin on microbial remediation of PAHs-contaminated soil. The results showed that rhamnolipin could enhance the remediation effect through multiple pathways. Its mechanism of action was closely related to PAHs solubilization, plant enrichment, and microbial community regulation. It also reflected the unique advantages of biosurfactants in pollution remediation. The solubilization effect of rhamnolipin on PAHs was the basis for its enhanced remediation. The study found that the concentration of rhamnolipin was significantly positively correlated with the apparent solubility and solubilization factor of PAHs, which was consistent with the solubilization mechanism of most biosurfactants. As a typical amphiphilic molecule, rhamnolipin could effectively reduce the water-soil interfacial tension, improve the bioavailability of PAHs through micellar solubilization, and reduce the adsorption and locking of hydrophobic organic matter by soil colloids, thus providing the preconditions for subsequent microbial degradation and plant absorption. The result was consistent with the previous research conclusion that improving the solubility and migration capacity of hydrophobic pollutants was the key technical path to break through the bottleneck of bioremediation efficiency [19]. Regarding plant responses,

appropriate concentrations of rhamnolipin could promote the absorption and accumulation of PAHs in wheat while ensuring plant growth safety. The PAH content in wheat roots reached its peak under the 200 mg/L treatment, and the accumulation in roots was significantly higher than that in the leaf margins, indicating that PAHs mainly accumulated in the roots and had a weak ability to be translocated to the aboveground parts, which was related to the hydrophobicity of PAHs and the absorption characteristics of plant roots. Simultaneously, rhamnolipin could alleviate PAH stress by increasing CAT activity and reducing MDA content, further demonstrating its biosafety and providing a guarantee for plant-microbe co-remediation. The targeted regulation of soil microbial communities by rhamnolipin was the key to enhancing microbial degradation efficiency. Studies showed that 500 mg/kg rhamnolipin did not significantly reduce soil microbial alpha diversity, but it significantly increased the relative abundance of PAH-degrading genera such as *Bacillus* and *Pseudomonas*, promoting the evolution of community structure towards a direction favorable to pollutant degradation. Su *et al*. also confirmed in their study on the remediation of PAHs-contaminated soil in cold regions that exogenous additives could improve the *in situ* remediation effect by enriching functional microorganisms and optimizing community structure, which was highly consistent with the results of this research [20]. Rhamnolipid achieved efficient remediation of PAHs-contaminated soil *via* the synergistic effect of chemical solubilization, plant accumulation, and microbial degradation. Nevertheless, this study only explored the single remediation effect of rhamnolipid. The synergistic remediation potential of rhamnolipid combined with physical, chemical, or other biological remediation technologies remained unclear. Future studies should focus on combined remediation systems to optimize technical parameters and further improve the remediation efficiency and practical applicability of PAHs-contaminated soil.

Acknowledgements

The research was supported by Natural Science Foundation of Liaoning Province, China (Grant No. 2024-MS-254).

References

1. Emre M, Tongco JV, Permana R. 2024. Environmental impact and decomposition of polycyclic aromatic hydrocarbons (PAHs) in urban soils: Challenges and future directions. Trop Aquat Soil Pollut. 4(2):111-126.
2. Rouhani A, Hejcman M. 2025. A review of soil pollution around municipal solid waste landfills in Iran and comparable instances from other parts of the world. Int J Environ Sci Technol. 22(10):9711-9728.
3. Ihenetu SC. 2024. Ecological and human health risks associated with soil pollution by PAHs and heavy metals in the Niger Delta. Carpathian J Earth Environ Sci. 19(1):187-200.
4. Kosheleva NE, Zhaxylykov NB, Zavgorodnyaya YA, Kasimov NS. 2024. First assessment of soil cover pollution with polycyclic aromatic hydrocarbons in the city of Ulan-Ude. Dokl Earth Sci. 518(2):1770-1775.
5. Anju VT, Busi S, Mohan MS, Salim SA, Ar S, Imchen M, *et al*. 2024. Surveillance and mitigation of soil pollution through metagenomic approaches. Biotechnol Genet Eng Rev. 40(1):589-622.
6. Dudnikova T, Minkina T, Sushkova S, Barbashev A, Antonenko E, Shuvaev E, *et al*. 2024. Profile distribution of polycyclic aromatic hydrocarbons in coastal soils of the Lower Don and Taganrog Bay, Russia. Eurasian Soil Sci. 13(2):111-124.
7. Liu Y, Wang Z, Liu M, Zhang T, Liu S, Lu K. 2025. Characterization, sources and risk assessment of organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs) and polycyclic aromatic hydrocarbons (PAHs) in agricultural soils from Jilin Province in Northeast China. Stoch Environ Res Risk Assess. 39(4):1741-1755.
8. Li YM, Wang YC, Zhang HS, Han L, Liu Z, Liu Y, *et al*. 2024. Investigation of thermal desorption characteristics and pilot-scale study of polycyclic aromatic hydrocarbons (PAHs)-contaminated soil during cement manufacturing. J Mater Cycles Waste Manag. 26(3):1633-1648.
9. Rabieimesbah A, Sobhanardakani S, Cheraghi M, Lorestani B. 2024. Concentrations, source identification and potential ecological and human health risks assessment of polycyclic aromatic hydrocarbons (PAHs) in agricultural soils of hamedan county, west of Iran. Soil Sediment Contam. 33(4):482-506.
10. Ehtemae N, Ghanavati N, Nazarpour A, Babaeinejad T, James Watts M. 2024. Status, source, and risk assessment of heavy metal (loid) s and polycyclic aromatic hydrocarbons (PAHs) in the street dust of ilam, Iran. Polycycl Aromat Compd. 44(10):6475-6500.
11. Eze V, Ononogbo C, Anyanwu GC, Egbucha JN, Nlemchukwu BN, Emeagubor AO, *et al*. 2025. Source identification of polycyclic

aromatic hydrocarbons (PAHs) in soils around crude oil contaminated area in Ohaji/Egbema, Imo State. Int J Environ Anal Chem. 105(16):3862-3871.

12. Gao M, Zhang Q, Chen B, Lei C, Xia Q, Sun L, *et al*. 2025. Global geographic patterns of soil microbial degradation potential for polycyclic aromatic hydrocarbons. Environ Sci Technol. 59(15):7550-7560.
13. Razek A. 2025. Risk appraisal and protection against the adverse effects of electromagnetic fields on living tissues and near-tissues health devices. Smart Wearable Technol. 1:A1.
14. Janeiro-Tato I, Rodríguez E, Lopez-Anton MA, Baragaño D, Arrojo L, Parra-Benito P, *et al*. 2024. Bio-based carbon foams assembled with Fe nanoparticles for simultaneous remediation of As, Hg and PAHs in co-contaminated industrial soils. Environ Sci Nano. 11(6):2683-2692.
15. Dhara A, Dutta R. 2025. A review on sources and distribution of polycyclic aromatic hydrocarbons (PAHs) in wetland ecosystem: Focusing on plant-biomonitoring and phytoremediation. Environ Sci Pollut Res. 32(14):8743-8765.
16. Wang X, Wang C, Zhang Y, Yan Y. 2025. Pathway to identify and assess regional potential soil pollution risk from industrial activities: Case of Pearl River Delta region, China. Int J Sustain Dev World Ecol. 32(5):511-524.
17. Dhanapal AR, Thiruvengadam M, Vairavanathan J, Venkidasamy B, Easwaran M, Ghorbanpour M. 2024. Nanotechnology approaches for the remediation of agricultural polluted soils. ACS Omega. 9(12):13522-13533.
18. Mungai TM, Yang Y, Ngumbau VM, Mureithi M. 2024. Evaluation of heavy metals and polycyclic aromatic hydrocarbons pollution in residential, agricultural and commercial soils in Ngong, Kenya. Int J Environ Sci Technol. 21(3):2863-2880.
19. Muhsin ZAA. 2024. A systematic review of bioremediation of soil pollution using different types of bacteria 2015-2023. Wasit J Pure Sci. 3(4):136-150.
20. Su D, Dong Y, Liu Y, Yang C, Wang X. 2024. Strengthening effect of mixed biochar on microbial remediation of PAHs-contaminated soil in cold areas. J Soils Sediments. 24(3):1092-1110.