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Environmental restoration of polyaromatic hydrocarbon-contaminated soil through sustainable rhizoremediation: insights into bioeconomy and high-throughput systematic analysis

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous hydrophobic environmental contaminants with carcinogenic properties. Due to their persistent nature, they can be present in diverse ecosystems, making their extraction and accurate assessment from contaminated environmental samples vital for quantification before implementing remediation strategies. Thus, this review explores the major sources of PAH pollution and their assessment techniques such as SPME, LPME, HF-LPME, and USAEME, which facilitate faster PAH extraction while minimizing the use of organic solvents. In recent years, there has been growing interest in nature-based, eco-friendly soil remediation approaches as compared to chemical and physical approaches. Rhizoremediation has emerged as a leading bioremediation method due to its effectiveness in field applications. However, understanding the interactions between the plant rhizosphere and its microbiome is essential, especially since current research predominantly focuses on *in situ* bioremediation and degradation of PAH compounds through plant-microbe partnerships. In natural environments, PAHs are present in intricate mixtures, and microorganisms operate within interconnected communities. Thus, this review explores the detailed mechanisms of plant-microbe interactions and the role of advanced omics approaches, including genomics, proteomics, and metagenomics, in enhancing the efficacy of rhizoremediation. Rhizoremediation not only aids in the removal of contaminants but also promotes biomass production, thereby enhancing soil fertility and productivity, leading to improved agronomic results. This article also reviews the ongoing advancements in PAH remediation techniques, evidenced by increased patent filings and innovative approaches, contributing to substantial growth in global bioeconomy revenue. Nevertheless, the widespread adoption of rhizoremediation faces hurdles related to marketing and commercialization. Furthermore, this review delves into strategies such as rhizosphere engineering and genetic modifications aimed at expediting rhizoremediation processes in PAH polluted soils.

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Environmental significance

The presence of polycyclic aromatic hydrocarbon (PAH) contamination results in degradation of soil and other ecosystems. PAHs are persistent and ubiquitous in nature and are considered highly carcinogenic, ranking among the most hazardous organic contaminants. Rhizoremediation is a highly effective nature-based technique, using plant-driven remediation mechanisms to restore contaminated air, soil, and water. However, toxic substances in contaminated environments can hinder plant growth and slow remediation, a challenge that can be addressed by introducing an efficient microbial consortium alongside plants. Plant roots provide essential nutrients to microbes in contaminated environments, while microbes, in turn, produce plant growth promoting metabolites and degrade PAHs, preventing their accumulation in plant tissues. This synergistic interaction enhances remediation efficiency.

1. Introduction

Polyaromatic hydrocarbons (PAHs) are a class of organic compounds primarily formed as byproducts of the incomplete combustion of fossil fuels such as coal, oil, and natural gas, as well as biomass sources like wood, waste materials, and

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tobacco.[1] Due to their semi-volatile nature, PAHs exist in both gaseous and particulate phases under ambient conditions. They are characterized by their non-polar and lipophilic nature, planar molecular structures, and lack of electrical charge, with many exhibiting no chromatic properties.[2] Naphthalene, consisting of two fused aromatic rings, is the simplest PAH. PAHs exhibit a distinctive ability to generate radicals and anions when treated with alkali metals. In environmental contexts, PAHs are typically classified based on the number of fused benzene rings, ranging from two (naphthalene) to seven (coronene) (Karadakov *et al.*, 2023), although PAHs with higher numbers of rings can also be found.[3] PAHs are further categorized based on their sources: pyrogenic PAHs result from fossil fuel combustion and are heavily alkylated and oxygenated, leading to the formation of PAH quinones;[4] petrogenic PAHs, on the other hand, are associated with crude oil and can enter aquatic environments following oil spills.[4]

A global study documented a broad spectrum of concentrations for 15 PAH homologues in soils, varying from less than 1 ng g^{-1} to 7840 ng per g dry weight (dw) of soil.[5] Further, in the past decade, PAH concentrations ranging from 10^{-6} to 10^{-3} g kg^{-1} have been observed on almost every continent.[6] The highest concentrations were found in Europe, followed by North America, Asia, Oceania, Africa, and South America. Vehicular emissions are recognized as a major anthropogenic source of PAHs in urban environments.[7] A study conducted in the United States reported that motor vehicle exhausts contribute approximately 36% of the total annual PAH emissions.[8]

Elevated levels of PAHs were associated with locations near long-term emission sources and significant atmospheric deposition inputs. Major contributors to PAH emissions include industries such as waste incineration, iron and steel production, coal-tar pitch manufacturing, dye and rubber production, asphalt industries, power generation, and diesel or gasoline-powered machinery.[9] Additionally, long-term emissions from exhaust produced by aircraft, ships, trains, and vehicles further contribute to PAH pollution.[10] Atmospheric deposition introduces PAH residues into terrestrial and aquatic ecosystems, primarily from fossil fuel combustion and industrial activities.[10] A study on the wet deposition of PAHs in Central South China (2014–2017) reported that coal combustion, petroleum sources, and vehicular emissions contributed 58%, 12%, and 30%, respectively.[11] Similarly, road vehicle emissions, accounting for 658 metric tons, along with atmospheric deposition, played a significant role in PAH accumulation in Haizhou Bay, China.[12] Furthermore, anthropogenic activities emitted nearly 191.5 tonnes of PAH compounds into the atmosphere in Germany.[13]

Additionally, a positive correlation was observed between microbial population density and PAH concentrations in soil, as well as between soil organic matter (SOM) and black carbon.[14] Anthropogenic activities account for approximately 85% of PAH emissions and 99% of related fatalities, with significant disparities in emissions and health effects across different regions.[15] Human exposure to PAHs poses substantial health risks, primarily cancer, including skin, lung, bladder, liver, and stomach malignancies, as evidenced by animal studies.[16] Additionally, PAH exposure may lead to cardiovascular disease and adversely affect foetal development.[17]

Biological systems are fundamental to PAH degradation, with plants and microorganisms serving as key contributors

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due to their diverse metabolic capabilities and pollutant detoxification mechanisms.[18] Microorganisms in both terrestrial and aquatic ecosystems exhibit adaptive potential, enabling them to degrade various PAHs over time.[19] Rhizoremediation, a bioremediation technique, relies on the mutualistic relationship between plants and microorganisms to sustainably clean environmental pollutants. This process is dependent on plant-microbe interactions with hydrocarbon-degrading capabilities.[20] Plant roots offer extensive surface areas for microbial growth and can penetrate soil to depths of 10–15 meters, aiding in contaminant degradation.[21,22]

In the field of environmental management, bioremediation initially represented only a small segment of the broader market for hazardous waste treatment until the late 1990s. In the United States, the bioremediation industry was valued at approximately $60 million in 1990, which grew to between $175 million and $300 million by 1995.[23] Today, bioremediation has significantly expanded, with revenues reaching $46 865.2 million and projected to grow to $333 470.0 million by 2027, according to Emergen Research (2020).[24] Bioremediation, along with bioeconomy, provides a common platform for researchers across various disciplines to develop sustainable solutions to environmental challenges.[25]

This review explores the application of rhizoremediation techniques for the degradation of PAH contaminants and their implications for the global market. It highlights the necessity of developing innovative rhizoremediation strategies to effectively remove PAH pollutants from soil, thereby protecting ecosystems and preserving biodiversity. The review emphasizes the role of plant-microbe interactions in enhancing the remediation of PAH-contaminated soils and discusses advanced molecular approaches aimed at improving rhizoremediation efficiency. Additionally, it provides insights into patent activities within the bioremediation sector, underscoring potential contributions from both governmental agencies and private enterprises in advancing this field.

2. Exposure, toxicity, and assessment of PAH contamination in soil

To date, more than 200 distinct PAHs have been identified in almost every ecosystem.[26] Their ubiquity facilitates their adsorption onto suspended particulates in the environment.[26] Studies have demonstrated that elevated PAH concentrations in soils and sediments from estuaries, lakes, and marine environments can exert toxic effects on living organisms.[27–29] Given their high toxicity, mutagenic potential, and carcinogenic properties, PAHs represent a significant environmental concern. Their toxicity is influenced by factors such as their molecular structure, the biological species exposed, and the

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specific pathways of exposure.[30] Therefore, assessing PAH contamination is crucial for implementing targeted remediation strategies, evaluating toxicity levels, and determining removal efficiency.

2.1 Exposure to PAH compounds

Over 90% of the PAHs present in soils and sediments are predominantly bound to the solid phase, particularly to organic materials. PAHs in soil are retained by soil particles, which reduces their mobility and availability for microbial breakdown.[31] PAHs exhibit persistent bioaccumulation and biotransformation characteristics, which contribute to the stability of organic matter components and enhance the resistance of soil-bound PAHs to degradation.[32] PAHs present in the air can be deposited into the soil and potentially infiltrate water systems *via* wet and dry deposition as well as precipitation. Furthermore, PAHs have a high affinity for soil particles and can disperse widely due to their hydrophobic nature.[33] They have been detected in remote regions, far from industrial activity, because of their persistence and atmospheric transport. For example, PAHs were found in tropical soils (0.5 to 49 mg per kg dw) and even in Arctic environments, despite the absence of local industrial emissions (186–11,600 mg per kg dw of soil).[34] Further, regional economic development, energy production, and population density significantly influence the existence and dispersion of soil PAHs in the environment.[35] Implementing a risk assessment strategy identifies potential contaminants and receptors at risk, determining the probability of adverse effects from exposure to specific substances or mixtures in a given area.[36] The detected range of 0.6 to 10 mg kg^{-1} of PAHs in soil signifies varying levels of contamination. PAH-contaminated soils are categorized based on concentration levels as follows: uncontaminated (<0.200 mg kg^{-1}), minimally contaminated (0.200–0.600 mg kg^{-1}), moderately contaminated (0.600–1.000 mg kg^{-1}), and highly contaminated (>1.000 mg kg^{-1}).[37] Sites exhibiting PAH concentrations exceeding 10 mg kg^{-1} are classified as severely contaminated, posing significant risks to agricultural systems by reducing crop productivity, inhibiting seed germination, and decreasing plant longevity.[38] Over the past three decades, there has been a substantial escalation in soil PAH concentrations, particularly in industrialized regions worldwide. It is anticipated that these concentrations will continue to rise over the next five years and beyond, primarily due to the ongoing expansion of anthropogenic emissions of PAHs into the environment.[39] Soil samples from such industrialised sites exhibit a wide spectrum of PAH contamination levels, with concentrations spanning from 0.001 to 300 000 mg kg^{-1} of total PAHs.[40] In non-industrial regions, PAH contamination is notably concentrated along roadsides, with PAH levels ranging from approximately 0.5 to 49 mg kg^{-1} of total PAHs.[41] Conversely, forested areas typically exhibit lower levels of PAH contamination, with concentrations ranging from approximately 0.2 to 1 mg kg^{-1} of total PAHs.[34] Residential areas tend to exhibit even lower PAH levels, typically falling within the range of approximately 0.1 to 4 mg kg^{-1} of total PAHs.[42]

Potential exposure to PAHs may arise *via* several routes, such as inhalation, ingestion, and direct skin contact. Research conducted on occupational exposure has provided evidence of various adverse health impacts resulting from elevated concentrations of PAHs, such as the onset of carcinogenesis.[43] The ingestion of PAHs has the potential to disrupt the normal functioning of cellular membranes and several enzyme systems. The primary focus of PAHs is the potential interaction between the epoxides and dihydrodiols with cellular proteins and DNA, resulting in physiological disturbances, cellular damage, genetic variations, and developmental abnormalities.[44] The extended use of industrial effluent combined with the utilization of municipal wastewater for irrigation has resulted in the excessive buildup of PAHs in agricultural soil. Crops cultivated in soil affected by wastewater contamination have the capacity to take up substantial quantities of these pollutants.[45] In fact, vegetables in PAH-contaminated soils have been reported to accumulate PAHs, with their concentrations ranging from 508.9 mg kg^{-1} to 197.3 mg kg^{-1} in home gardens and 589.9 mg kg^{-1} to 171.3 mg kg^{-1} in agricultural fields.[46]

2.2 Toxicity of PAH compounds

Various PAHs exhibit toxicity, mutagenicity, carcinogenicity, and teratogenicity. Due to their high lipophilicity, PAHs are efficiently absorbed into the gastrointestinal tract of animals and living beings.[47] The processing methods, such as smoking, drying, and heating, are the primary cause of contamination by PAHs. The quantity of PAHs in charcoal-grilled meals can reach up to 320 μg kg^{-1}. Seven PAHs have been identified by the Environmental Protection Agency (EPA) as potentially carcinogenic to humans: indeno[1,2,3-*cd*]pyrene, benzo[*b*]fluoranthene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, dibenz[*ah*]anthracene, and chrysene.[48] Experiments demonstrated the embryotoxic effects and early pregnancy associated with the several PAHs, including naphthalene, benzo[*a*]pyrene, and benzo[*a*]anthracene.[49] Benzo[*a*]pyrene is the first chemical carcinogen to be identified[50] and the predominant PAH responsible for inducing carcinogenesis in organisms. The effects of PAHs on aquatic species are determined by their metabolic and photo-oxidation processes, with increased toxicity observed in the presence of UV light. Furthermore, PAHs demonstrate a significant level of acute toxicity towards aquatic organisms and avian lifeforms.[51,52]

The effects of PAHs on plants include disruption of membrane-related physiological and biochemical processes such as changes in membrane permeability, enzyme malfunction, and interference with photosynthesis.[53] PAHs typically distribute into thylakoids, which may affect the chloroplasts and disrupt the electron transport system. The various effects of PAHs on treated plants show that thylakoid membranes accumulate hydrophobic PAHs.[54] According to reports, anthracene has been found to have inhibitory effects on the process of carbon fixation, leading to a reduction in net photosynthesis.[55] PAHs such as phenanthrene and pyrene, have been observed to have a negative impact on the overall process of net photosynthesis.[55] Exposure to phenanthrene and pyrene in higher plants

resulted in a reduction in growth, levels of photosynthetic pigments, stomatal conductance, the maximal quantum yield, the effective quantum yield of Photosystem II (PSII), and the photochemical quenching coefficient.[56]

The presence of PAHs in the top layers of agricultural soils may have an impact on the overall quality of the habitat, resulting in a reduction of its biological characteristics, such as enzyme activity and microbial populations.[56] PAHs can easily be absorbed into the soil, causing the aggregation of soil particles and reducing porosity.[57] Prolonged exposure to contaminants can have an impact on the geochemical characteristics of soils, including changes in Atterberg limits (a fundamental indicator of the crucial moisture levels in soils with fine particles), permeability, conductivity, and parameters related to strength, consolidation behaviour, compaction properties, infiltration capacity, and shear strength.[58] Furthermore, PAH contamination can alter the biological structure of soils, leading to changes in biomass levels and microbial activity. A scientific study reported a significant decline in the relative abundance of microbial phyla, including *Alphaproteobacteria*, *Actinobacteria*, *Chloroflexi*, *Crenarchaeota*, and *Deltaproteobacteria*, following exposure to PAH contamination.[59]

Additionally, the deposition of PAHs onto the soil surface is contingent upon their octanol–water partition coefficient (K_{ow}). A higher K_{ow} value correlates with reduced water solubility of PAH compounds, resulting in an augmented affinity for absorption onto soil particles.[60] This partitioning behaviour contributes to the retention and sequestration of PAHs in the soil environment. Soil productivity can decline due to PAH contamination, leading to reduced crop yields, with reports up to a 50% decrease in yields.[61] Several consequences of PAH contamination, including biodiversity loss, adverse effects on human health, carcinogenicity, alterations in soil structure and geochemical cycling, increased greenhouse gas emissions contributing to climate change, reduced crop productivity, and disruptions in microbial diversity, are illustrated in Fig. 1.

2.3 Assessment of PAH compounds

Two advanced analytical techniques, such as microextraction and miniaturized extraction methods, have been employed for the extraction of PAHs from different samples.[62] Microextraction, including solid-phase microextraction (SPME), is a single-step, highly sensitive, and efficient method of sample preparation that eliminates the need for solvents, making it suitable for a diverse array of chemicals across multiple matrices.[63] Dispersive solid-phase extraction (d-SPE) is a variant of SPME, where the sorbent is introduced directly into the sample's aqueous solution, resulting in dispersion.[64] Ambade *et al.*[65] analysed PAH distribution in surface water and sediments from the Damodar River Basin using GC-FID and GC-MS,[66] reporting PAH concentrations between 0.036 mg kg^{-1} and 582 mg kg^{-1}, with acenaphthylene (ACY) and benzo(*a*)anthracene (BaA) as the most abundant. Xue *et al.*[66] developed a cost-effective method for PAH quantification in soils using ultrasonic-assisted extraction, solid-phase microextraction, and GC-MS. The 100-μm PDMS fiber exhibited superior performance with high repeatability, low detection limits, and a broad linear range, achieving recoveries above 79.3% in spiked samples and proving effective for field analysis of 16 PAHs.

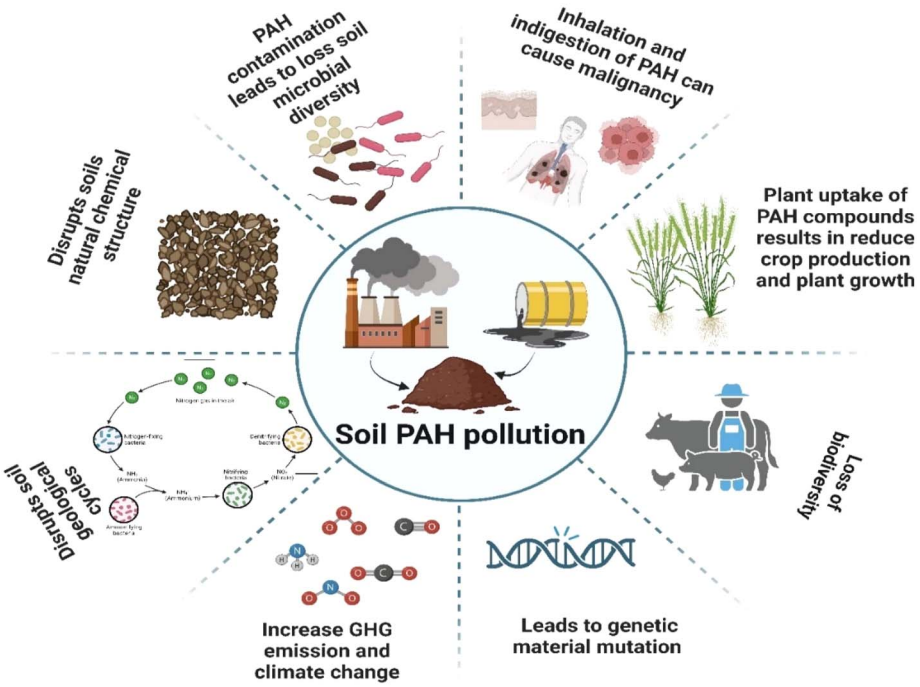

Fig. 1 Adverse effects of PAH contamination encompass biodiversity depletion, detrimental impacts on human health, carcinogenic properties, modifications in the soil structure and geochemical cycles, heightened greenhouse gas emissions exacerbating climate change, diminished agricultural yield, and reduction in microbial diversity.

Furthermore, among the various liquid-phase extraction (LPME) methods, such as single-drop microextraction (SDME), hollow fibre liquid-phase microextraction (HF-LPME), and ultrasound-assisted emulsification microextraction (USAEME), dispersive liquid–liquid microextraction (DLLME) is the most widely utilised technique.[67] The small volume (5 mm^3) within the microsyringe, along with the rapid equilibrium between gaseous analytes and the organic solvent film, SDME enabled the use of high vapor pressure solvents like cyclohexane without significant solvent loss during extraction.[68] HF-LPME employs disposable polypropylene porous hollow fibers filled with a minimal amount of extracting solvent, known as the acceptor phase.[69] The target analytes are extracted by immersing the fibers into the aqueous sample solution, termed the donor phase. Various DLLME techniques have been developed for PAH extraction.[70] Rezaee *et al.*[71] introduced a method using tetrachloroethylene and acetone for PAH quantification in surface water, though its reliance on high-density solvents limits efficiency. Guo *et al.*[72] developed a low-density solvent-based demulsification approach using *n*-hexane and acetone, eliminating the need for centrifugation and reducing extraction time to 2–3 minutes. Hosseini *et al.*[73] explored air flotation-assisted phase separation with toluene, further simplifying the process. The integration of ultrasound and vortex radiation has also improved mass transfer and extraction efficiency by reducing the diffusion distance and increasing the interfacial area. Fernandez *et al.*[74] developed a laboratory-based valve DLLME technique for PAH extraction, followed by analysis using HPLC-FLD, and the automated system improved efficiency, achieving enhancement factors of 86–95%. Further, fabric phase sorptive extraction (FPSE), magnetic solid-phase extraction (MSPE), flow injection solid-phase extraction (FI-SPE), and in-syringe solid-phase extraction of PAHs are novel miniaturized extraction methods of PAHs from different environmental samples.[75] FPSE utilizes a fabric substrate coated with a sol–gel organic–inorganic hybrid sorbent as the extraction medium. The coated FPSE medium is first cleaned with solvents and deionized water and then immersed in the sample solution with magnetic stirring to facilitate analyte adsorption.[76] After extraction, the FPSE medium is removed, and the analytes are eluted into a vial with an appropriate solvent for analysis following centrifugation or filtration. A trace-level analysis of specific PAHs in environmental water samples utilising FPSE before their quantification by HPLC-FLD has been documented.[77] FPSE-HPLC-FLD has been demonstrated to be direct, effective, rapid, sensitive, environmentally friendly, cost-effective, and dependable for the trace level analysis of significant PAHs.[78] Furthermore,[79] gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and ultra-high-pressure liquid chromatography (UHPLC) in conjunction with various detection techniques such as diode-array detectors (DAD), tandem mass spectrometry (MS/MS) detectors, flame ionization detectors (FIDs), fluorescence detectors (FDs), and ultraviolet (UV) detectors are the most widely applied PAH detection methods.[80] Wu *et al.*[81] proposed the use of flow injection solid-phase extraction (FI-SPE) for the extraction of PAHs from environmental samples, employing novel sorbents. Wu's research utilized a micro-column packed with multi-walled carbon nanotubes (MWCNTs) for PAH extraction, followed by gas chromatography-mass spectrometry (GC-MS) analysis. In contrast, Manousi and Zachariadis[82] synthesized a copper(II) isonicotinate coordination polymer as a pre-column sorbent, with subsequent high-performance liquid chromatography-diode array detection (HPLC-DAD) analysis. Both methods exhibited efficient extraction performance. For GC-MS detection, eluates from the FI-SPE process were manually injected, whereas for HPLC-DAD analysis, on-line elution in backflush mode allowed direct transfer of analytes into the chromatographic column, streamlining the analytical workflow. Mirzaee and Sartaj[83] demonstrated the effectiveness of magnetic granular activated carbon (MGAC) in removing PAHs from contaminated soil *via* an optimized soil washing process. XRD analysis confirmed the successful incorporation of Fe_3O_4 nanoparticles onto MGAC. Moreover, SEM-EDX and fluorescence microscopy used for the determination of PAH removal efficiency of *Medicago sativa* showed a dissipation rate of 96.2%, followed by *Helianthus annuus* and *Tagetes erecta*.[84] Ma *et al.*[85] employed Fourier transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and thermogravimetric-mass spectrometry (TG-MS) to investigate the composition and structure of soil aggregates (SAs) of varying particle sizes. The study revealed that partitioning played a dominant role in phenanthrene sorption in SAs larger than 0.002 mm, while adsorption was more pronounced in finer SAs (<0.002 mm), which exhibited the highest sorption coefficient (K_d). Lower aqueous equilibrium concentrations of phenanthrene further enhanced adsorption. Morphological and structural analyses indicated that micropores, soil organic matter (SOM), and minerals contributed to PAH sorption, while TG-MS demonstrated that SOM inhibited PAH release during heating. These findings improve the understanding of PAH-soil interactions and interface modelling.

3. Different remediation strategies used for remediation of PAHs from soil

The remediation of PAHs in soil possesses significant environmental challenges, necessitating the application of physical, chemical, and biological approaches. Among the physical remediation techniques, solvent-based soil washing and membrane filtration technologies, including ultrafiltration, microfiltration, nanofiltration, and reverse osmosis, have demonstrated effectiveness in the extraction of PAHs from contaminated soil matrices.[86] Thermal methods such as incineration and *in situ* thermal desorption (ISTD) are also employed to remediate PAH-contaminated soils.[87] Incineration is a highly effective strategy that utilizes temperatures between 900 and 1200 °C to eliminate PAHs. Despite its efficiency, incineration is an energy-intensive and costly remediation technique, largely due to the necessity for stringent control measures to manage off-gas emissions.[88] Similarly, *in situ* thermal desorption (ISTD), which involves heating the soil in place to volatilize and remove PAHs, encounters comparable challenges related to high energy

consumption and operational costs.[89,90] Physical remediation techniques, such as solvent extraction and electrokinetic remediation, are relatively simple to implement and often utilize non-toxic materials.[91] However, their major drawback lies in the necessity for repeated treatments to achieve effective contaminant removal. Additionally, these methods frequently require post-treatment measures to manage gaseous byproducts generated during the remediation process.

Chemical remediation of PAH-contaminated soil primarily relies on oxidation processes. Oxidizing agents such as ozone (O_3) and Fenton reagents (Fe^{2+}/H_2O_2) are widely utilized due to their high efficiency in breaking down PAH compounds.[92,93] *In situ* chemical oxidation (ISCO) represents another chemical remediation approach, particularly effective for both low-molecular-weight (LMW) and high-molecular-weight (HMW) PAHs in agricultural soils. ISCO involves injecting oxidants directly into the soil to break down PAHs. Despite its potential, ISCO can also generate toxic secondary intermediates, posing additional environmental risks.[94] Moreover, solvent extraction (SE) and soil washing (SW) are widely employed for the remediation of soils contaminated with HMW PAHs. These methods utilize solvents to dissolve and extract PAHs from the soil matrix, facilitating their removal and subsequent treatment. However, due to the hydrophobic nature of HMW PAHs and their strong binding to the soil matrix, SE/SW is not fully effective in removing all PAHs.[95] Furthermore, physical and chemical remediation methods for PAH-contaminated environments often fail to achieve complete removal of pollutants. These approaches may also lead to the formation of secondary intermediates, some of which can exhibit greater environmental toxicity than the original PAH compounds.[96] These PAH derivatives include oxygen (O-PAHs), nitrogen (N-PAHs and azarenes AZA), or sulphur (PASHs) inside the aromatic ring. The incorporation of oxygen, nitrogen, or sulphur into the aromatic rings of PAHs increases their toxicity.[97] Lundstedt *et al.*[98] illustrated that O-PAHs possess greater mobility compared to their parent PAHs, attributed to their polarity. Knecht *et al.*[99] investigated the toxicity of 38 oxygenated PAHs (O-PAHs) on zebrafish embryos (Danio rerio), revealing that structural variations significantly influence toxicity levels. O-PAHs with adjacent diones on 6-carbon moieties or terminal *para*-diones on multi-ring structures exhibited varying degrees of toxicity, whereas 5-carbon moieties with adjacent diones were the least toxic. The study further demonstrated that the toxicity of selected O-PAHs was differentially dependent on the aryl hydrocarbon receptor (AHR), emphasizing the role of oxidative stress in their toxicological mechanisms. Additionally, B[*a*]A or benzanthraquinone in zebrafish resulted in detrimental effects on protein biosynthesis, mitochondrial and cardiac function, and neural and vascular development.[100] Further, 3-nitrobenzanthrone (3-NBA), a derivative of benzanthrone, demonstrated superior toxicity relative to 1-nitropyrene (1-NP) or benzo[*a*]pyrene (B[*a*]P). This substance also caused an increase of cells in the S-phase, followed by the typical process of apoptotic cell death. The presence of 3-NBA triggers a substantial DNA response *via* the phosphorylation of the ataxia-telangiectasia mutant, checkpoint kinase (Chk) 2/Chk1, H2AX, and p53.[101] Similarly, Wang *et al.* demonstrated through *in vitro* and *in vivo* studies that 1-hydroxypyrene, 1-nitropyrene, and 1-methylpyrene, derivatives of pyrene, exhibit greater toxicity to lung health than the parent compound. Derivatives of benzo[*a*]anthracene, particularly 7-methylbenz[*a*]anthracene, exhibited the highest tumorigenic activity among the compounds analysed, leading to the development of subcutaneous sarcomas and multiple tumors in the lungs and liver.[102] Additionally, 7-bromomethyl-12-methylbenz[*a*]anthracene demonstrated comparable tumorigenic effects in the lung and liver. In contrast, 4-chloro-7-bromomethylbenz[*a*]anthracene exhibited minimal activity, with only a slight increase in liver tumor incidence in male mice.[103]

Bioremediation is considered a highly effective and environmentally friendly method for addressing PAH contamination in soil. It offers numerous benefits, including minimal energy usage, limited secondary pollution, and cost-effectiveness.[104] Bioremediation of PAH-contaminated soil utilizes microorganisms, including bacteria, fungi, and plants, to degrade and transform hydrocarbon compounds.[105] In recent years, various bioremediation strategies have demonstrated significant efficacy in the removal of PAHs from polluted environments[106–109] (Table 1). Guo *et al.*[129] isolated a bacterial consortium from PAH-contaminated soil capable of utilizing pyrene (PYR) as the sole carbon source, achieving 76% degradation in a liquid medium within 10 days. Xiong *et al.*[130] reported that *Mycobacterium gilvum*, isolated from PAH-contaminated soil, exhibited high efficiency in PYR removal, degrading 98% within just 5 days. Additionally, Zafra *et al.*[131] constructed a bacterial consortium that effectively degraded phenanthrene (PHE), pyrene (PYR), and benzo[*a*]pyrene (BaP) in soil, achieving 92% removal of PHE, 64% of PYR, and 65% of BaP within 14 days.

During the degradation process, these bacteria utilize PAHs as a carbon source for energy production and growth. Specific bacteria, such as *Mycobacterium* sp., can oxidatively degrade PAHs *via* the cytochrome P450 monooxygenase enzyme, producing *trans*-dihydrodiols.[132] The phylum *Proteobacteria* is often predominant in hydrocarbon-contaminated environments.[133] Reports indicate that bacterial communities rapidly adapt to fuel contamination by shifting towards hydrocarbon-degrading species.[134–137] Utilizing indigenous microorganisms for ecological restoration is a widely adopted method for *in situ* bioremediation, where pollutants are converted into non-toxic compounds.[138] Conversely, *ex situ* bioremediation techniques, while effective, can be more costly, potentially less environmentally sustainable, and may increase the risk of secondary contamination compared to *in situ* methods.[139]

The primary metabolic pathways for the degradation of aromatic compounds begin with the *ortho*- and *meta*-cleavage of catechol molecules. Oxygen is essential for several steps in the aerobic degradation of PAHs by bacteria, including ring hydroxylation, ring cleavage, and the final electron uptake.[140,141] Pyrene mineralization can occur at the C-1 and C-2 positions or at the C-4 and C-5 positions of the aromatic ring due to the action of dioxygenase enzymes.[142,143] Numerous bacteria, including those from the genera *Pseudomonas* and *Rhodococcus*, can oxidize PAHs using dioxygenase enzymes.[144,145] Several

Table 1 Different PAH bioremediation techniques and their applications

SI. no	Bioremediation techniques	Application	References
1	Microbial bioremediation	Uses microorganisms (bacteria, fungi, archaea, and algae) for organic pollutant remediation	110
2	Phytoremediation	Involves plant-based *in situ* remediation, including methods like phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation, and phytodesalination	111–114
3	Microbe-assisted phytoremediation	The utilization of a bioremediation technique involves the establishment of a mutualistic relationship between plants and bacteria, such as rhizobacteria and endophytes. This association is employed to augment the effectiveness of remediation processes in contaminated environments	115
4	Electro-bioremediation	This approach employs a hybrid technology that combines bioremediation with electrokinetic mechanisms for the treatment of environmental pollutants. Electrokinetic phenomena play a crucial role in expediting and directing the transportation of environmental contaminants and microorganisms for the purpose of biological remediation	116
5	Electrokinetic-phytoremediation	It is a hybrid technology that combines phytoremediation with electrokinetic remediation. This method increases the metal mobility in polluted soil to facilitate their plant uptake	117 and 118
6	Enzymatic remediation	Utilizes catabolic enzymes to increase the degradation and detoxification of pollutants	119 and 120
7	Microbial fuel cells	The bio-electrochemical device harnesses the capabilities of aerobic microorganisms to efficiently convert organic substrates found in wastewater and other contaminants into electrical energy	121
8	Wetland's construction	Natural treatment involving wetland vegetation and microbes to improve soil quality	121
9	Nano-bioremediation	Integrated method using nanoparticles alongside bioremediation for sustainable remediation	122
10	Natural attenuation	Enhances indigenous microbiome's degradation capacity by improving soil conditions	123 and 124
11	Biostimulation	This technique involves enhancing the indigenous microbial activity in contaminated soil by introducing nutrients, fertilizers, humic acid, organic wastes, *etc.* These additions serve to stimulate the growth and activities of microorganisms, thereby promoting the remediation process	125
12	Bioaugmentation	The introduction of a highly efficient microbial consortium into soil contaminated with pollutants aims to enhance the degradation of these contaminants and enhance the catabolic capabilities of the existing native microbiome	110
13	Composting	Cost-effective method of increasing soil organic content and fertility, leading to enhanced degradation	126
14	Bioreactor	A controlled *ex situ* system is utilized, incorporating the use of surfactants, bioaugmentation, and biostimulation techniques for the purpose of bioremediating PAHs	127
15	Vermiremediation	Interactions between plants and microorganisms are employed for the purpose of PAH removal from fine soil with pore sizes smaller than 0.1 mm. PAHs that have accumulated within small pores exhibit limited accessibility for biodegradation. Through their burrowing, earthworms make soil pores bigger, which makes it easier for microbes and plant roots to get into the soil and break down PAHs	128

bacteria have been identified to have a bifunctional enzyme encoded by the *paaZ* gene. This enzyme features an N-terminal aldehyde dehydrogenase domain and a C-terminal enoyl-*CoA* hydratase domain.[146] The dioxygenase enzyme facilitates the breakdown of catechol through several pathways, ultimately leading to the production of succinyl-CoA, which then enters the TCA cycle.[147] Metagenomic function profiling indicates a high abundance of key enzymes involved in the central metabolism of petroleum contaminants, including catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, muconolactone D-isomerase, 3-oxoadipate enol-lactonase, and 4-oxalocrotonate tautomerase.[110,148] Various species such as *Bacillus subtilis, Rhodococcus erythropolis, Ochrobactrum, Hyphomonas* spp., and *Actinomycete* sp. have been identified for their ability to degrade *n*-alkanes and aromatic hydrocarbons.[111,112] The degradation pathways of PAHs in microbes are illustrated in Fig. 3. These pathways involve enzymatic transformations mediated by dioxygenases, monooxygenases, and oxidoreductases, leading

to the breakdown of complex PAH structures into less toxic intermediates and ultimately mineralization into CO_2 and H_2O.

Despite its advantages, on-site bioremediation is often constrained by several limiting factors, including high contamination levels, low nutrient availability, restricted microbial proliferation due to limited soil space, and competition among microbial populations. In such cases, rhizoremediation emerges as an effective strategy by leveraging plant-microbe interactions. Plants facilitate microbial proliferation by supplying nutrients and root exudates, while microbes enhance plant resilience in contaminated environments by degrading pollutants and improving soil conditions.

4. Rhizoremediation as a method for restoring natural ecosystems

According to the UN Environment Programme (2022), the estimated annual global loss of ecosystem services between 1997 and 2011 ranged from €3.5 to €18.5 trillion.[149] The primary focus of ecological restoration research has been on community and ecosystem ecology, with a significant emphasis on plant-based restoration efforts. Restoring degraded ecosystems plays a pivotal role in achieving the Sustainable Development Goals (SDGs),[149] particularly those related to climate change mitigation, poverty alleviation, and food security. Furthermore, a substantial £14 billion ($19.2 billion) has been allocated by public and private sectors to advance the SDGs, as reported by the United Nations in 2022.[150] Therefore, to tackle such environmental consequences, rhizoremediation is acknowledged as an effective approach for ecosystem restoration, resulting in substantial decreases in PAH concentrations, increased microbial degradation activity, and better soil health metrics in diverse polluted environments.[151] The synergistic interactions between microorganisms and plants in contaminant remediation represent a nature-based approach for producing environmentally safe end products. This method not only facilitates effective pollutant degradation but also contributes to the restoration of impaired ecosystems, promoting ecological balance and sustainability.[152] Bisht *et al.*[153] demonstrated that *Bacillus* sp. SBER3 degraded 83.4% of anthracene and 75.1% of naphthalene under laboratory conditions, while anthracene degradation in the rhizosphere of *Populus deltoides* was 45.6%. Liste[154] reported that pyrene degradation reached 74% in vegetated soil, compared to 40% in unplanted soil over an eight-week period. Rostami *et al.*[155] further investigated the role of cysteine in enhancing *Festuca* resilience to PAH-induced stress, demonstrating that pyrene and phenanthrene (200–400 mg kg^{-1}), in the presence of (100–200 mg kg^{-1}) cysteine, exhibited removal efficiencies of 47.78–93.31% and 55.95–98.16%, respectively.

Rhizoremediation, a specialized form of phytoremediation, leverages the symbiotic relationship between plants and microorganisms to environmentally remediate various waste materials.[156] The efficacy of this technique hinges on the synergistic interaction between plants and microorganisms capable of degrading pollutants, notably PAHs.[157] The extensive root system of plants offers a substantial surface area, promoting microbial proliferation and enabling the degradation of contaminants even at depths of 10–15 meters below the soil surface.[158] Plant growth-promoting rhizobacteria (PGPR) are pivotal in rhizoremediation, as they not only aid in pollutant degradation but also enhance plant growth through organic acid and hormone secretion.[159] PGPR strains provide resilience

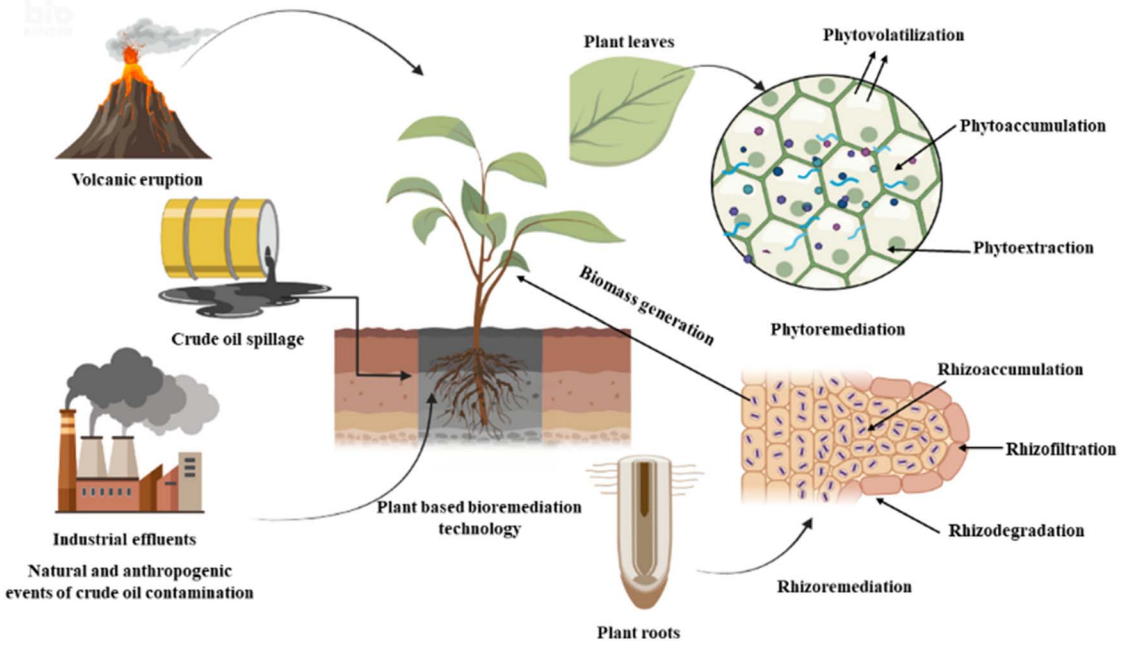

Fig. 2 Illustrations of various PAH-contaminated sources and diverse plant-based remediation mechanisms, including phytovolatilization, phytoaccumulation, phytoextraction, rhizodegradation, rhizoaccumulation, and rhizofiltration.

to environmental stresses and offer additional benefits such as metal detoxification, nitrogen fixation, and phosphate solubilization.[160] The use of PGPR in rhizoremediation enhances pollutant degradation, highlighting the multiple benefits of this practical, non-destructive, and cost-effective approach to environmental remediation.[161] Various PAH-contaminated sources and different plant-based remediation mechanisms, including phytovolatilization, phytoaccumulation, phytoextraction, rhizodegradation, rhizoaccumulation, and rhizofiltration, have been illustrated in Fig. 2. Further, Table 2 shows the comparison of different rhizoremediation methods to decontaminate PAH and petroleum hydrocarbons.

4.1. Plant-microbe interaction to degrade PAHs in soil

Sayyed *et al.*[161] highlighted the stimulation of microbial activity by root exudates, a phenomenon termed the "rhizospheric effect," which enhances the degradation of organic compounds by plants. The rhizosphere plays a pivotal role in facilitating crucial processes such as primary and secondary metabolism, as well as the establishment, survival, and ecological interactions with other organisms.[178] Plants in contaminated sites stimulate the accumulation of rhizospheric microorganisms near their roots through the release of nutrient-rich root exudates.[179] This microbial aggregation supports plant growth and promotes a healthy root system in contaminated environments.[180]

Hou *et al.*[181] conducted a study where they observed an increase in the biomass of *Festuca arundinacea* L. and the degradation of PAHs in oil-contaminated soil. This was achieved through bioaugmentation using bacteria that have plant growth-promoting properties and the ability to produce biosurfactants. Moreover, the concentration of bacteria specialized in the degradation of PAHs was observed to be significantly greater in the rhizosphere region of plant species in comparison to soil sites devoid of plant presence.[182] Sampaio *et al.*[183] showed that bacterial sp., *Bacillus* sp. and *Pseudomonas aeruginosa* effectively colonized the roots of*Rhizophora mangle* L. In addition to colonization, these bacterial strains played a crucial role in providing protection to the plant, enhancing propagule germination, and achieving the degradation of over 80% of PAHs present in sediment. In a field study conducted on a former coal mine site, researchers evaluated the ability of different legume tree species, including *Cassia siamea*, *Albizia lebbeck*, *Delonix regia*, and *Dalbergia sissoo*, to lower soil PAH levels. According to the findings, the degradation rates among the tested trees ranged from 51.5% to 81.6%.[184] The rhizosphere of *Zea mays* (maize) and *Sorghum sudanense* (Sudan grass), cultivated in the presence of benzo[*a*]pyrene (BaP) and pyrene (PYR), along with *Stenotrophomonas* sp. MAL1, *Arthrobacter* sp. MAL3, and *Microbacterium* sp. MAL2, showed complete PYR degradation and 38.7% BaP degradation over 10 to 14 days, when supplemented with low molecular weight organic acids (LMWOAs).[185] Further, Singha *et al.*[186] demonstrated the effectiveness of rhizoremediation in pyrene-contaminated soils through interactions between *Oryza sativa* (rice) plants and microbial consortia. A bacterial consortium of *Klebsiella pneumoniae* AWD5 and *Pseudomonas aeruginosa* PDB1 achieved 60% pyrene degradation. Similarly, Kotoky and Pandey[182] investigated the rhizodegradation of benzo[*a*]pyrene (BaP) using a bacterial consortium (*Bacillus subtilis* SR1, *Serratia marcescens* S2I7, and*Staphylococcus arlettae* S1I1) in the rhizosphere of *Melia azedarach*. BaP degradation reached 88% in the rhizosphere after 60 days, compared to 68.22% in bulk soil. In a separate study, *Bacillus flexus* S1I26 and *Paenibacillus* sp. S1I8 enhanced benzo[*a*]pyrene (BaP) solubilization by 24.41%, with pot trials, demonstrating even higher rhizosphere degradation efficiencies of 87.42% and 86.08%, respectively.[187] Mukhopadhyay and Masto[188] evaluated PAH degradation in coal mine sites, where *Cassia siamea* achieved the highest PAH reduction (81.6%), followed by *Albizia lebbeck* (55.6%), *Delonix regia* (51.9%), and *Dalbergia sissoo* (51.5%). Somtrakoon *et al.*[189] evaluated the impact of plant growth regulators on the phytoremediation potential of sweet grass (*Pennisetum purpureum* cv. Mahasarakham) in PAH-contaminated soil. The presence of sweet grass led to reductions in acenaphthylene (4.69 ± 0.50%), acenaphthene (10.69 ± 1.47%), and phenanthrene (3.61 ± 0.07%), whereas unplanted soil showed PAH reductions exceeding 30%, in field studies. Zhao *et al.*[190] further investigated the role of an indigenous BaP degrader, *Stenotrophomonas* BaP-1, in the ryegrass rhizosphere. Following bioaugmentation, the residual BaP mass in ryegrass and bioaugmented microcosms was 2.38 ± 0.10 mg kg^{-1} and 2.33 ± 0.07 mg kg^{-1}, respectively. Additionally, the degradation rates of Σ15PAHs after 45 days ranged from 32.80% to 74.35% with the application of the consortium to alfalfa plants. Similarly, Gawryluk *et al.*[191] observed an 8% increase in phenanthrene elimination in the rhizosphere after 14 days of ryegrass cultivation. Pyrene degradation in the rhizosphere of *Festuca arundinacea* (tall fescue) was 8.85–20.7% higher than that in non-plant soils. Dai *et al.*[107] reported a 21.8–28.0% increase in ∑PAH elimination (pyrene, chrysene, benzo(*b*)fluoranthene, and benzo(*k*)fluoranthene) in the rhizosphere of ryegrass as compared to unplanted soils.

Root-associated niches play a crucial role in pollutant dissipation, with phenanthrene and pyrene removal decreasing from the rhizoplane to the rhizosphere and near-rhizosphere soil (0–8 mm) after 40–50 days of ryegrass growth.[192] Moreover, rhizosphere activity significantly enhances the degradation of freshly spiked PAHs compared to weathered PAHs.[193] For instance, a phenanthrene dissipation rate of 86% was observed in the rhizosphere just one week after wheat planting.[194] Additionally, the elimination rates of phenanthrene in the rhizosphere of *Lolium perenne* exceeded those of *Elsholtzia splendens* under low copper (Cu) treatment. However, *Elsholtzia splendens* demonstrated higher phenanthrene degradation under elevated Cu treatment.[195] Examples of efficient plant-microbe pairs utilized for the degradation of PAH compounds are listed in Table 4.

Plant growth-promoting microorganisms (PGPMOs) have gained recognition for their substantial influence on the rhizosphere environment. They augment the production of growth-promoting hormones, enzymes, siderophores, biosurfactants, and ACC deaminase.[196,197] Moreover, there are microorganisms referred to as "superbugs" capable of

Table 2 Comparison of different rhizoremediation techniques

Remediation type	Degraded compound	Microorganism used	PAH removal efficiency	Duration (days)	Advantages	Limitations	Field applicability	References
Biostimulation and bioaugmentation	PAHs	*Rhodococcus* sp., *Achromobacter* sp., *O. paurometabola, Pantoea* sp.,*Sejongia* sp. *Microbacterium* sp.	93%	56	The addition of nutrients to PAH-contaminated soils facilitates an increased rate of biodegradation during the biostimulation process	Microbial survival and activity depend on environmental factors; may require repeated inoculation	Applied in crude oil, PAH, and pesticide-contaminated soils	162–165
	PAHs	Rice straw-derived biochar pyrolyzed at 600 °C	40.00–58.84%	180				
	PAHs	—	73%	534				
	Alkane PAHs	Solid inoculants of *Bacillus* LZ-2	21.6%	30	Native microorganisms that are well-suited to the subsurface environment enhanced degradation	The effectiveness of microbial population density, soil pH, moisture content, temperature, nutrient concentration, soil texture, PAH volatility, chemical structure, concentration, and toxicity are all crucial factors	Biostimulation aids groundwater, soil, and wastewater remediation and may also facilitate quorum quenching by enhancing signal-degrading bacteria	
	Petroleum hydrocarbon	*Bacillus altitudinis* strain HRG-1	20%	60				
	PAHs	*Rhodococcus* sp. (NH2), *Achromobacter* sp. (NH13), *Oerskovia paurometabola* (NH11), *Pantoea* sp. (NH15), *Sejongia* sp. (NH20), *Microbacterium maritypicum* (NH30) and *Arthrobacter equi* (NH21)	99%	30				
Phytoremediation	Fluoranthene Pyrene Benzo(*a*)anthracene Chrysene Benzo(*b*)fluoranthene Benzo(k)fluoranthene Benz(*a*)pyrene Dibenz(a,h)anthracene (PAH)	*Echinacea purpurea* (L.)	38–60%	120	Effective for organic pollutants like PAHs and hydrocarbons; promotes microbial diversity	Limited to biodegradable contaminants; slow degradation rates in some cases	Widely used for petroleum-contaminated soils and pesticide degradation	166–169
	Phenanthrene, pyrene	Wheat	98%	90				
	PAH and crude oil	*T. arundinacea*	64%	90				
	PAHs	*Mimosa*, *Zinnia, gazania*, *Cypress vine*	45–49%					
Rhizofiltration	Cd and Pb	*Brassica juncea*	48–59% accumulation	—	Efficient for removing heavy metals; applicable for wastewater treatment	Requires proper plant disposal after pollutant accumulation; limited to waterborne contaminants	Used in wetlands, constructed treatment systems, and contaminated aquatic environments	170–172
	Al, Fe and Mn	*Pistia stratiotes* L.	90%	15				
	Cd and As	*Cynara cardunculus*	10%	30				
Phytovolatilization	TCE (trichloroethylene)	*Eucalyptus*	0.97 flux (μmol m^{-2})^{b}	45	Effective for volatile organic pollutants and	Risk of atmospheric recontamination;	Used for chlorinated solvents and heavy metal volatilization	173 and 174
	TCE (trichloroethylene)	Poplar	0.1 (μmol m^{-2})^{b}	5				

Table 2 (*Contd.*)

Remediation type	Degraded compound	Microorganism used	PAH removal efficiency	Duration (days)	Advantages	Limitations	Field applicability	References
Rhizoremediation	Naphthalene	Poplar	2.8 (summer) (μmol m^{-2})[b]	—	heavy metals like mercury	limited to specific pollutants	Applied in crude oil, PAH, and pesticide-contaminated soils	175–177
	Trichloroethylene (TCE)	Wheat	63%	36	Increases	Microbial survival		
	PAHs	*Brachiaria serrata* and *Eleusine coracana*	63–96%	70	biodegradation efficiency; can target	and activity depend on environmental		
	PAHs	*Phragmites australis and alfalfa* (*Medicago sativa*)	68.7–74.5%	2 years	specific contaminants	factors; may require repeated inoculation		
	Phenanthrene and pyrene	Wheat	87–97% and 65–70%	—				

biodegrading a diverse array of contaminants. Consortia of microorganisms, wherein individual strains fulfil complementary functions, also exhibit advantageous characteristics. The rhizosphere effect, driven by the secretion of PAH compounds, biosurfactants, and organic molecules, positively influences microbial diversity, activity, and the rhizoremediation process.[198] Biosurfactants, amphiphilic molecules produced by microorganisms, form micelles in the presence of hydrophobic PAHs, increasing their bioavailability and promoting biodegradation.[199] Microbes generate organic acids, which lower soil pH and enhance PAH solubility. Additionally, enzymatic synthesis of degradative agents, like oxidoreductases, significantly boosts the degradation process.[200] The application of *Verbascum sinuatum* L. and a microbial consortium effectively remediated polluted soils, reducing PAHs and 6-ring compounds by up to 68%.[201] Mehmannavaz *et al.*[202] found that the introduction of *Sinorhizobium meliloti* strain A-025, a rhizobacterium that forms a symbiotic relationship with *alfalfa* and fixes nitrogen, increased the conversion of several polychlorinated biphenyls (PCBs). A wide range of bacterial genera, including *Pseudoxanthomonas*, *Burkholderia*, *Mycobacterium*, *Prevotella*, *Cellulomonas*, *Actinobacillus*, *Anaeromyxobacter*, *Paraburkholderia*, *Sphingomonas*, *Novosphingobium*, *Acetivibrio*, *Acetobacter*, *Cycloclasticus*, *Microbulbifer*, *Gordonia*, and *Micrococcus*, have been identified as involved in the degradation of PAHs in the rhizosphere.[203–206]

4.2. Role of plant-root exudates in shaping microbial diversity in PAH contaminated soil

The presence of root exudates plays a crucial role in shaping the composition and population of microorganisms in the rhizosphere, while also playing an essential role in the growth and development of the rhizosphere.[207] The chemical composition of root exudates is influenced by the specific type of plant and various environmental factors.[208] Root exudates play a crucial role in regulating the soil rhizosphere microbiome, promoting beneficial symbiotic interactions, suppressing the growth of competitive organisms, and enhancing the chemical and physical conditions of the soil.[209] Root exudates can be categorised into different groups depending on their chemical composition, such as passive root exudates, root tissue lysates, mucilage chemicals, and secondary metabolites.[210]

Plants develop various interactions, both beneficial and harmful, through the release of root exudates. These interactions influence relationships among different plants as well as interactions with microorganisms, shaping the rhizosphere environment and affecting processes like nutrient cycling, microbial colonization, and contaminant degradation.[211] Soil amended with root exudates containing high concentrations of organic acids has been found to have a reduced capacity for absorbing organic pollutants.[182,212] Rajkumari *et al.*[213] found that the application of certain substances, such as organic acids, glucose, and serine, can significantly improve the degradation of PAHs. The presence of glucose in the root exudates triggers the production of dehydrogenase enzyme, which aids in the breakdown of pyrene and promotes the

growth of *Mycobacterium* sp. A study conducted by Jin *et al.*[214] found that *Arabidopsis* plants with enhanced phenolic exudate secretion experienced significant changes in the microorganisms present in the rhizosphere. The biodegradation of phenanthrene was observed to be most effective within a distance of 3 mm from the roots, with a degradation rate of 86%. However, the degradation rate decreased to 48% at a distance of 3–6 mm and further declined to 36% at a distance of 6–9 mm. There is a positive relationship between the proximity to the roots and the abundance of heterotrophs and PAH-degrading bacteria. In the rhizosphere of perennial ryegrass (*Lolium perenne* L.) grown in soil contaminated with petroleum hydrocarbons, a majority of the hydrocarbon degraders were found within a 3 mm distance.[215] Muungo[216] showed that *Pseudomonas* and *Arthrobacter* exhibited the highest levels of activity in degrading phenanthrene. Interestingly, this was observed both in the presence and absence of artificial root exudates. The area surrounding the roots of perennial ryegrass, known as the rhizosphere, exhibited the highest levels of microbial activity and contamination.[217] The microbial community responsible for phenanthrene degradation shifted when ryegrass exudates were applied. Initially, *Pseudoxanthomonas* spp. and *Microbacterium* spp. were identified as the main phenanthrene degraders. However, after the application of ryegrass exudates, the dominant species changed to *Arthrobacter* spp. and *Pseudomonas stutzeri*.[218] A variety of microbial species have the ability to utilise both root exudates and hydrocarbons as their source of carbon.[218] Table 2 shows the different plant-microbe pairs to degrade PAH in soil. Yergeau *et al.*[219] found that key bacterial groups, including *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Acidobacteria*, exhibited increased activity in the willow rhizosphere, while most showed reduced activity in bulk soil. Additionally, fungi such as *Basidiomycota*, *Ascomycota*, and *Glomeromycota* were notably more active in the rhizosphere than in bulk soil. Microbial taxa in the rhizosphere exposed to PAHs became dominant, with *Eurotiomycetes* increasing from 20.6% to 52.2% and *Eurotiales* from 20.4% to 51.8%, suggesting their role as primary PAH degraders.[107] Rhizosphere stimulation varied significantly depending on soil pollution levels,[219] and it enhances PAH-degrading bacterial populations in the rhizosphere compared to bulk soil.[219] Bacterial taxa such as *Sphingobacteriia* and *Actinobacteria*, along with genera including *Pseudomonas*, *Rhizobium*, *Sphingomonas*, *Ilumatobacter*, *Singulisphaera*, and *Ensifer meliloti*, exhibit increased relative abundances in the rhizosphere of ryegrass and lucerne.[220] DNA-SIP studies confirm their role in PAH degradation, as demonstrated by ^{13C}-PAH-labeled components.[218,221] Additionally, certain indigenous herb species from coking facility soils promote PAH-degrading bacterial communities like *Sphingomonas*, *Pedomicrobium*, and *Pseudomonas* in the rhizosphere.[222] Research indicates that over 90% of phenanthrene and more than 60% of pyrene were eliminated from planted soils after 40 to 80 days of development, surpassing the removal levels in non-planted soils.[223] For instance, phenanthrene removal efficiency in the rhizosphere increased by 8% following 14 days of ryegrass cultivation,[221] while pyrene removal efficiency in the rhizosphere of tall fescue (*Festuca arundinacea*) was elevated by 8.85% to 20.7% compared to non-planted soils.[223]

Huang *et al.*[224] found that different subspecies of *Arabidopsis* release unique exudates that specifically target the microbiome in the rhizosphere. These exudates play a significant role in altering the microbial community in the root zone. Kimani *et al.*[225] showed that the phenolic substance has the ability to alter the microbial composition, distinguishing it from other compounds like sugar and carbohydrates. In addition to phenolic compounds, the exudates from cucumber roots, specifically *p*-coumaric acid and vanillic acid, have been found to impact the microbiome in the rhizosphere, leading to alterations and increased abundance.[226] Certain compounds found in root exudates have the ability to imitate the signalling molecules known as bacterial quorum-sensing *N*-acyl homoserine lactones (AHLs).[227] These compounds play a role in regulating the interaction between plants and microbes. Additionally, the bacterial functions in the host plant can be regulated by inducing the gene for host infection, promoting the production of biofilms and biosurfactants, enhancing nitrogen fixation, and increasing the production of degrading enzymes.[228] Some plant species, like *Coronilla varia*, *Pisum sativum*, and *Oryza sativa*, release compounds similar to AHLs found in certain microbes. These compounds help these plants regulate the population of microbes in the rhizosphere, while also repelling others.[229] Enzymes like peroxidase, laccases, and phenol oxidases produced by plants play a crucial role in the oxidation of hydrocarbon products into intermediate derivatives.[230] Singha *et al.*[231] showed *Pseudomonas fragi* DBC and *Jatropha curcas* interacted for pyrene biodegradation, with *yfc* upregulation under pyrene stress in the presence of artificial root exudates, enhancing plant growth and stress response in *Jatropha* roots mediated by *P. fragi* DBC. The release of flavonoid compounds from plant roots can trigger the co-metabolism of polyaromatic hydrocarbons. This is because flavonoids have a similar structure to aromatic compounds, which enhances the degradation and mineralization process carried out by microorganisms.[232] The degradation of non-aromatic plant compounds, specifically linoleic acid-induced pyrene and benzo [*a*]pyrene, by Gram-positive microorganisms has also been investigated. Various studies have highlighted the enhanced bioavailability of xenobiotic compounds when plant roots secrete low molecular weight organic acids like malic acid, citric acid, succinic acid, tartaric acid, and oxalic acid.[233]

Despite these beneficial approaches, very few large-scale and long-term studies have been conducted on the rhizoremediation of PAH-contaminated soils. Most existing studies are limited to greenhouse conditions and focus primarily on bioremediation strategies involving microbial populations. Therefore, there is an urgent need to promote awareness and popularize rhizoremediation techniques for global applications. Table 3 provides examples of large-scale field studies on bioremediation, specifically addressing TPH and PAH remediation.

The rhizoremediation of PAHs using plants and microbes in the soil is illustrated in Fig. 3. Root exudates, such as carbohydrates, flavonoids, and amino acids, facilitate interactions with rhizospheric microbes, enhancing the microbial degradation of

Table 3 Details of field case studies on bioremediation conducted worldwide

SI. no	Sites	Type of remediation	Degradation efficiency	References
1	University of Calabar (spiked with crude oil)	Bioremediation	*Bacillus*, *Pseudomonas*, *Vibrio*, *Micrococcus*, and *Alcaligenes* reduced crude oil from 26.7% to 43.3% after 16 days	234
2	University of Port Harcourt spiked with crude oil	Biostimulation and phytoremediation	Biostimulation of soil with NPK was more effective than phytoremediation using *Vigna* sp.	235
3	Former oil refinery site in Montreal, Canada	Rhizoremediation	Willow plantations showed a 60–80% reduction in organic contaminants and heavy metals	236
4	Kuwait	Native microbial species bioremediation	Over a 12-month period, reduction of TPH up to 82.5% and 90.5% of alkanes	237
5	Crude oil polluted farmland in Bodo	Phytoremediation	In 90 days, *M. alternifolius* and *F. ferruginea* showed 99% TPH removal and 78% PAH removal	238
6	Northern France	Phytoremediation	Miscanthus × giganteus. 3.19–53.85% removal of PAHs	239
7	China	Phytoremediation	7 month rhizoremediation using alfalfa and tall fescue. 7.5–17.2% 5(+6)-ring PAH removal by alfalfa and 25.1−30.1% in tall fescue	240
8	Shengli Oil Field in Dongying City, China	Rhizoremediation	In 150 days, the removal rate of $\sum$ 8 PAHs was up to 99.40% using Fire Phoenix	241
9	India	Rhizoremediation	*Populus deltoides* with *Kurthia* sp. SBA4, *Micrococcus varians* SBA8, *Deinococcus radiodurans* SBA6 and *Bacillus circulans* SBA12 degraded 43.6% of PAHs in 120 days	242
10	Jianghan Oil Field, China	Bioremediation	*Pseudomonas* sp., with rice husk and plowing, showed 95% TPH degradation in 150 days	243

PAH compounds. Additionally, the secretion of various compounds such as indole acetic acid (IAA), siderophores, ACC deaminase, and phytohormones promotes plant growth and provides protection in contaminated environments. Microbial cells contribute to PAH degradation by producing enzymes such as dioxygenases, monooxygenases, and oxidoreductases, which break down complex PAH structures into less toxic intermediates and ultimately lead to their mineralization into CO_2 and H_2O. Similarly, plants produce enzymes like peroxidases, laccases, and phenol oxidases, which play a crucial role in the oxidation of hydrocarbon compounds into intermediate derivatives, further facilitating PAH degradation and accumulation.

4.3. Genetic attributes for rhizoremediation of PAHs

The rhizosphere enriched both PAH-degrading microorganisms and functional genes associated with PAH degradation.[263] The alteration in the chemical properties of PAHs, such as their hydrophobic or hydrophilic character, affects their absorption by microbial communities and plant cells. The use of *Trichoderma virens*-derived glutathione transferase (GST) enhanced the effectiveness of phytoremediation for recalcitrant PAHs, such as anthracene, by genetically incorporating the gene into *Nicotiana tabacum* (tobacco) plants.[264] Ibáñez *et al.*[265] found that the use of *TPX1* (tobacco transgenic hairy roots) in conjunction with arbuscular mycorrhizal fungus (AMF) enhanced the efficiency of phenol phytoremediation as compared to only relying on transgenic hairy root technology. AMF-enhanced transgenic tobacco hairy roots have a notable capacity to withstand elevated concentrations of phenol. This may be attributed to the existence of strong anti-oxidative enzyme systems that protect against oxidative damage caused by phenol. Horizontal gene transfer is a common occurrence in the endophytic niche, where microbial communities adapt to environmental stress.[266–268] For instance, the plasmid *pTOM-Bu61*, which carries genes for enzymes that break down toluene, may spontaneously transfer to many types of plant endophytes. This transfer has played a significant role in facilitating the effective breakdown of toluene in poplar plants. *Pseudomonas* endophytes carrying plasmids *pWWO* and *pNAH7* exhibited significant levels of horizontal transfer to other endophytes.[269] In a separate study, Barac *et al.*[270] documented the process of conjugative transformation of natural endophytes to improve the degradation of toluene. The expression of the *bphA* gene, which encodes biphenyl dioxygenase in *Pseudomonas* sp., is apparently induced by the presence of salicylate in root exudates during the degradation of PCBs.[271] Enhanced variants of *bphA*, which demonstrate improved PCB degradation capabilities, were generated from *Burkholderia cepacia* strain LB400, *Comamonas testosteroni* B-365, and *Rhodococcus globerulus* P6 using a family of shuffling technique.[272] In the context of

Table 4 Plant-microbe association in rhizoremediation of pollutants

SI. no	Plant species	Microbial species	Type of contaminant	References
1	*Hordeum vulgare*	*Burkholderia cepacia*	2,4-Dichlorophenoxyacetic acid	244
2	*Populus deltoides*	*Actinomycete Amycolata* sp. CB1190	1,4-Dioxane	245
3	*Populus deltoides*	*Sphingomonas yanoikuyae*	Benzo[*a*]pyrene	246
4	*Spartina alterniflora*	Gram-negative bacteria and endophytes	Phenanthrene, pyrene	247
5	*Glycine max*	*Glomus caledonium* GM24, *Glomus intraradices* GG31, *Glomus coronatum* GU53, *Pseudomonas fluorescens* PA28, *Pseudomonas borealis* PA29, *Bacillus subtilis* BA41	Pyrene and others	248
6	*Cucumis sativus*, *Daucus carota*, *Allium cepa*, *Cucurbita*, *Petroselinum sativum*	Mixed culture	Total 16 PAH	249
7	*Lotus corniculatus* L., *Oenothera biennis* L.	*Rhizobium, Pseudomonas*, *Stenotrophomonas Rhodococcus*	Hydrocarbon	250
8	*Trifolium repens*, *Lolium perenne*	AMF Glomus mosseae	PAH	251
9	*Festuca arundinacea*, *Sorghum x drummondii Lolium perenne*, *Lolium multiflorum*	*Fulvivirga kasyanovii*,*Massilia niabensis*,*Novosphingobium indicum*	Phenanthrene, fluoranthene and pyrene	252
10	*Jatropha curcas*	*Pseudomonas aeruginosa* PDB1	Pyrene	253
11	*Vallisneria spiralis*	Mixed consortium	Phenanthrene and pyrene	254
12	*Melia azedarach*	*B. subtilis* SR1, *B. subtilis* S1I26, *Paenibacillus* sp. S1I8 and *S. arlettae* S1I1	Benzo(*a*)pyrene (BaP)	182
13	*Populus deltoides*	*Kurthia* sp., *Micrococcus varians*, *Deinococcus radiodurans* and *Bacillus circulans*	Chrysene, benzene, toluene, xylene, anthracene and naphthalene	255
14	*Vigna unguiculata, Helianthus annus, Austrodanthonia caespitosa, Zea mays, Sorghum sudanense, Vetiveria zizanoides*	*Pseudomonas sphingomonas*	Mixed PAH	256
15	*Italian ryegrass*	*Pseudomonas poae*, *Actinobacter bouvetii*, *Stenotrophomonas rhizophila*, *Pseudomonas rhizosphaerae*	Hydrocarbon contamination	257
16	*Lolium perenne*, *Medicago sativa*	Mixed culture	Pyrene	258
17	*Populus deltoides*	*Burkholderia fungorum* DBT1	Dibenzothiophene, naphthalene, fluorene and phenanthrene	259
18	*T. patula*, *M. jalapa*	Mixed culture	Benzo[*a*]pyrene	260
19	*Brassica napus* L.	*Rhodoccocus equi*, β-proteobacterium, *Enterobacter* sp., *Acinetobacter calcoaceticus*, *Comamonas* sp., *Pseudomonas alcaligenes*	Petroleum hydrocarbon	261
20	*Medicago sativa* L.	*Sinorhizobium meliloti* strain 1021	Dioxin-like PCB	262

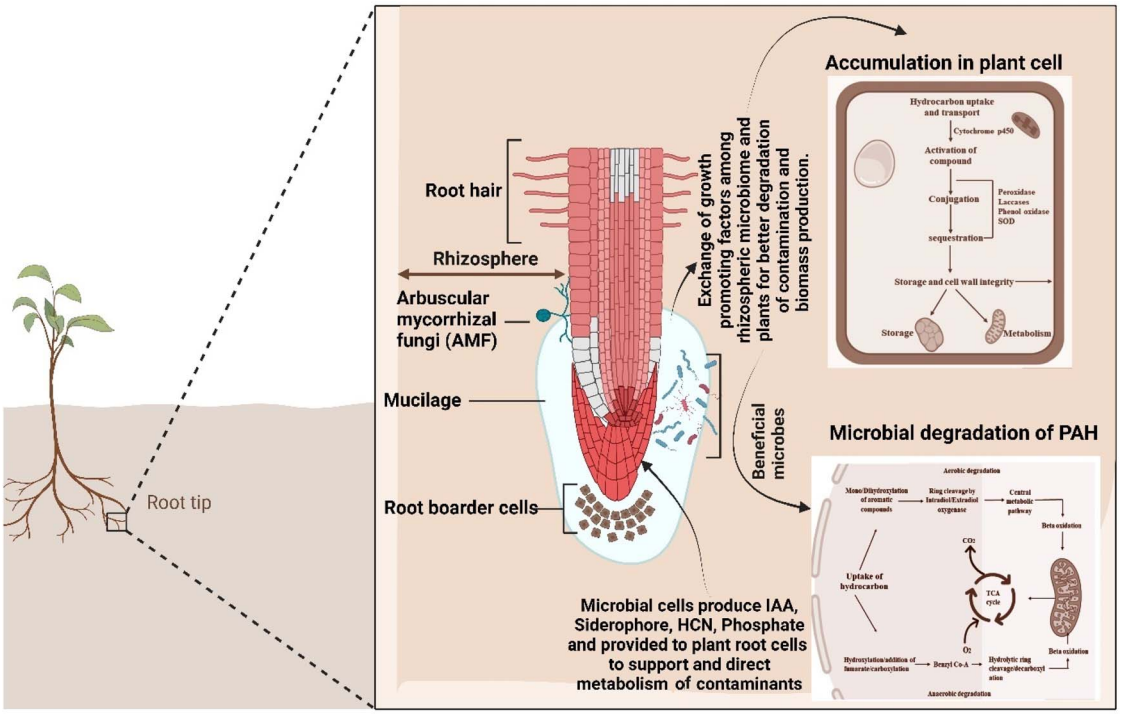

Fig. 3 The microbe-associated rhizoremediation of PAHs is a multistep process involving complex plant-microbe interactions. Root exudates secreted by plant roots enhance both plant growth and microbial degradation of contaminants. In the rhizospheric space, plants and microbes engage in chemical signalling and quorum sensing to facilitate cross-talk. In PAH-contaminated soil, microbial populations increase the bioavailability of hydrocarbon contaminants and metabolize them as a carbon source. Additionally, plants can directly absorb and store simpler forms of these contaminants in their biomass, contributing to the overall remediation process.

hydrocarbon remediation, several genes, including *alkB*, have been identified as contributing to stress tolerance.[272] In *Pseudomonas* sp., additional genes such as *nah, pah*, and *phn* enhanced microbe-assisted phytoremediation.[273,274] Nie *et al.*[275] studied the *Dietzia* genome, focusing on the *alkB* and *CYP153* genes, which encode alkane monooxygenase and P450 alkane hydroxylase potential for PAH phytoremediation. The expression of *NahAa, NahAb, NahAc*, and *NahAd* genes in *Pseudomonas putida* G7 (flavoprotein reductase, ferredoxin, and terminal dioxygenase subunits, respectively) was shown to enhance phenanthrene rhizoremediation in *Arabidopsis thaliana* and *Oryza sativa*. Enzymes such as *nahAc* and C23O (catechol 2,3-dioxygenase) play a vital role in starting the conversion of BaP, as they have evolved over time due to prolonged contact with petroleum.[276] Research has demonstrated that certain microorganisms, including *Pseudomonas* sp., *Burkholderia* sp., *Mycobacterium* sp., and *Sphingomonas* sp., harbour highly conserved catabolic gene clusters (*nah*, *phd*, *nid*, and *phn*) that encode enzymes responsible for PAH degradation.[277] Studies have shown the enrichment of key PAH-degrading genes in the plant rhizosphere, including PAH-RHD (PAH-ring hydroxylating dioxygenase), *phtA* (phthalate dioxygenase), *P34O* (protocatechuate 3,4-dioxygenase), and *C12O*/*C23O* (catechol dioxygenases).[263] These genes were elevated by 6.93–8.33-fold in ^{13C}-DNA metagenomes of the rhizosphere compared to bulk soil.[263] The PAH-*RHD* gene showed a significantly higher abundance in ryegrass rhizospheres exposed to benzo[*a*]pyrene,[190] while tall fescue enhanced PAH-*RHDα* Gram-negative gene expression.[278] Predominant PAH-degrading genes, including those encoding PAH dioxygenase and ring-cleavage dioxygenase, were more abundant in rhizospheres of *Betula pendula* in PAH-contaminated soil than in bulk soil.[190] Additionally, *C12O* and *C23O* gene expression in *tall fescue* rhizospheres increased 1.2–1.9 times relative to bulk soil.[273] The relative abundances of PAH-*RHDα* genes showed a strong correlation with the degradation rates of ^{13C}-phenanthrene, highlighting their role in PAH biodegradation. The current understanding of the genetic basis of PAH degradation in the rhizosphere remains limited, particularly in comparison to non-rhizosphere environments.[279] There is a need for further investigation into additional functional genes associated with PAH biodegradation to enhance rhizoremediation efforts. Multi-omics approaches, such as metagenomics, metatranscriptomics, and metabolomics, as explained in the following sections, can provide deeper insights into the functional diversity, metabolic pathways, and regulatory mechanisms involved in microbial PAH degradation. Different peripheral and central metabolic pathways related to PAH metabolism in bacterial systems are illustrated in Fig. 4. Aerobic degradation pathways involve dioxygenase-catalyzed oxidation of aromatic rings, leading to the formation of dihydrodiol intermediates. These intermediates undergo further cleavage *via* the ortho or meta pathways, generating key intermediates such as protocatechuates and catechols, which are subsequently integrated into the tricarboxylic acid (TCA) cycle for complete mineralization.

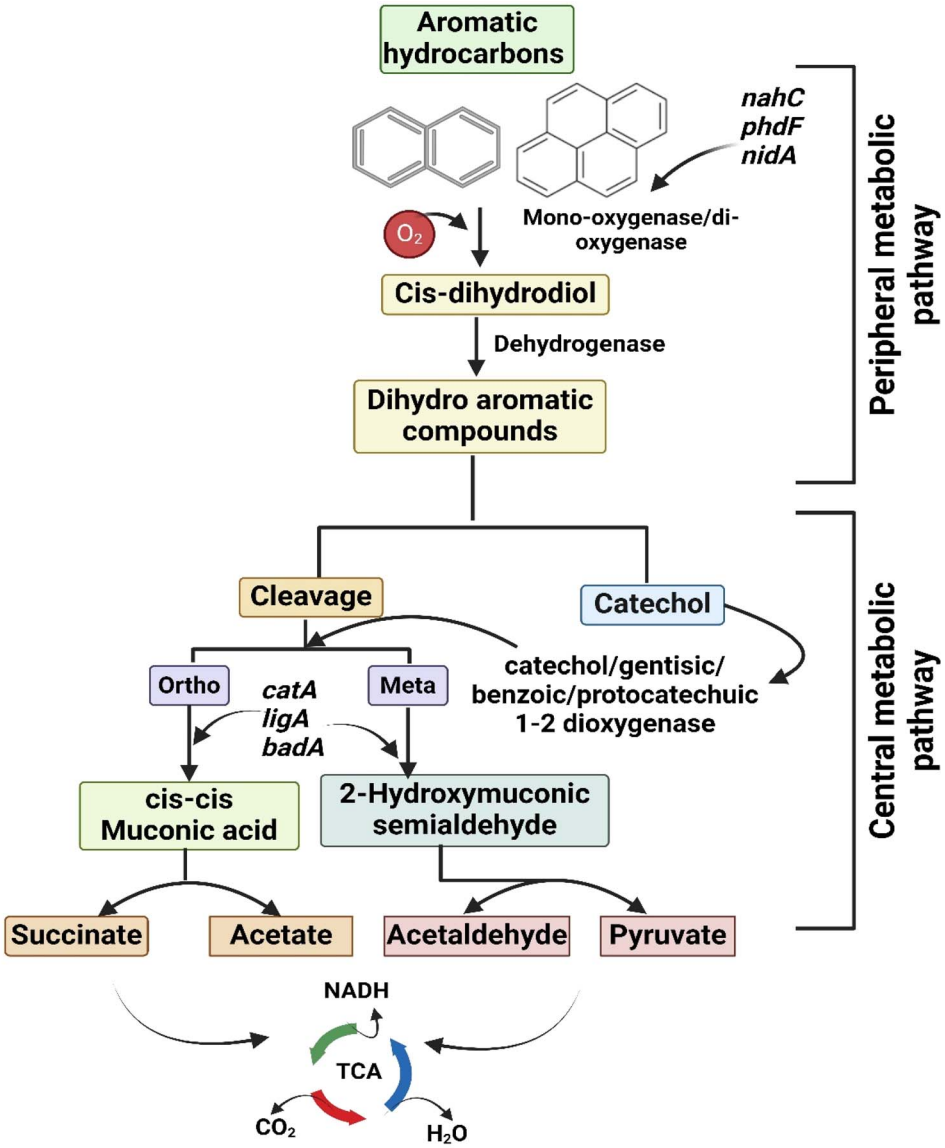

Fig. 4 Illustration of an overview of the peripheral and central metabolic pathways, including the genes responsible for the enzymes involved in PAH metabolism.[178,179]

5. Microbial diversity and functional analysis of PAH contaminated soil

A significant and persistent challenge in the study of the microbial role in PAH rhizoremediation lies in the restricted knowledge to discern the majority of microbial taxa within the community.[280] To address this limitation, emerging methodologies and advanced omics technologies have been developed to explore the physiological, metabolic, and structural aspects of the microbiome linked to the degradation of pollutants.

5.1. Metagenomics

The study of metagenomes in the rhizosphere offers valuable insights into the organization and content of the microbiome, particularly focusing on bacterial 16SrDNA.[281] Advancements in next-generation sequencing and bioinformatics methodologies have enabled scientists to develop techniques and pipelines that enhance understanding of metagenomes.[282] Metagenomics enables the comprehensive understanding of microbial diversity and their ecological roles within a specific habitat.[283] The phylum Proteobacteria continues to be prominently featured in such habitats, highlighting its significant role in the natural attenuation of PAH-contaminated soils.[284] In the context of soil samples collected from crude oil wells, the predominant microbial diversity was characterized by the presence of various taxonomic groups, including members of the Acetobacteraceae, Hyphomicrobiaceae, Rhodobacteraceae, and Sphingomonadaceae families.[285] In contaminated soil, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* exhibited a robust association with other biological processes.[286] A greater prevalence of Rhodocyclaceae and *Polaromonas* in agricultural soils

polluted with PAHs was positively correlated with the degradation of LMW PAHs.[287–289]

In a recent study, Wang *et al.*[290] observed a higher abundance of *Alphaproteobacteria* and *Actinobacteria* in contaminated soil. Similarly, the metagenomics analysis of a soil sample amended with anthracene has indicated that the relative abundance of *Gammaproteobacteria* was greater than that of *Alphaproteobacteria*.[291] Further, the prevalence of *Actinobacteria* was significantly higher in soil that has been spiked with anthracene, while *Acidobacteria* was predominantly found in soil that has not been spiked.[292] Redfern *et al.*,[293] in metagenomic studies, showed that the *Geobacter* species was a predominant biostimulation candidate in PAH-contaminated soil due to its significant prevalence and high abundance of degradative genes. Further, *Mycobacterium* and *Sphingomonas* were found to interact with different PAHs and have notable abundance in contaminated soil, making them suitable candidates for bioaugmentation studies.[294] Metagenomic analyses revealed the presence of key metabolic intermediates such as phthalate, protocatechuate, naphthalene, and salicylate in the rhizosphere, reconstructing PAH degradation pathways based on functional gene annotations.[190]

5.2. Genomics

Genomic methodologies that include the comprehensive analysis of an organism's whole genome or partial draft sequencing provide distinct benefits for investigations related to a group of genes participating in the complete breakdown of hydrocarbon compounds. These high-throughput molecular techniques play a crucial role in the identification of microbial traits and operons that exhibit collective activity involving numerous genes.[295] Additionally, these techniques aid in the identification of genes with similar functions, which may be linked to various additional genes in different microbes. The whole genome of the newly identified sp. of *Stenotrophomonas* had a total of 145 genes that were associated with the breakdown of PAHs such as anthraquinone, biphenyl, naphthalene, phenanthrene, and phenanthridine.[296]

The whole genome sequencing of *Sphingobium yanoikuyae* B1 revealed the presence of around 5140 putative open reading frames, composed of 35 dioxygenase genes including catechol 1, 2-dioxygenase, biphenyl 2,3-dioxygenase, and biphenyl-2, 3-diol 1, 2-dioxygenase.[297] Similarly, whole genome sequencing of the *Bacillus marisflavi* Bac 144 strain revealed the existence of genes associated with hydrocarbon degradation along with significant plant growth-promoting (PGP) attributes. Several species, including *Gordonia bronchialis*, *Gordonia sputi*, *Williamsia muralis*, and *Corynebacterium efficiens*, were detected to express the catechol 1,2-dioxygenase gene under different PAH stress conditions demonstrating its role in degradation pathways.[298–300] The genomic analysis of *Bacillus subtilis* SR1 reveals 12 genes involved in the metabolic processes of aromatic compounds, including peripheral pathways like biphenyl 2,3-dioxygenase, gentisate 1,2-dioxygenase, fumarylacetoacetase, and catechol 2,3-dioxygenase, which catalyze the degradation of hydrocarbons.[301] *Pseudomonas aeruginosa* DN1 was found to be proficient in breaking down HMW PAHs and crude oil from soil samples contaminated with petroleum at Changqing Oilfield. The strain's genome contains numerous genes and gene clusters that contribute to the degradation of aromatic compounds, including *catA* (catechol 1,2-dioxygenase), *pcaG*, which encodes the beta subunit of protocatechuate 3,4-dioxygenase, *hmgA* (homogentisate 1,2-dioxygenase), *dad* (2,4′-dihydroxyacetophenone dioxygenase), benzoate/toluate 1,2-dioxygenase and gentisate 1,2-dioxygenase. Several genes, including *nah*, *phn*, and *nid*, which encode enzymes such as naphthalene dioxygenase, salicylate hydroxylase, and phenanthrene dioxygenase, have been identified as being responsible for PAH degradation.[302] Ivanova *et al.*[303] documented that *Paraburkholderia aromaticivorans* strain BN5 possesses a total of 29 monooxygenase and 54 dioxygenase genes associated with the biodegradation of various hydrocarbons.

5.3. Metatranscriptomics

Metatranscriptomic analysis is employed to investigate the mRNA expression patterns of genes within a specific microorganism or a community of microorganisms present in an ecosystem.[304] Mukhtar *et al.*[305] studied the metatranscriptome of functional gene expression in the Willow plant soil microbiome grown in contaminated and uncontaminated soil. The contaminated rhizosphere showed increased expression of genes related to competitive traits like antibiotic resistance and biofilm formation due to selective pressure from pollutants and the rhizosphere environment.[306] Additionally, soils contaminated with pollutants had higher expression levels of genes associated with PHC degradation. A study conducted by Peng *et al.*[307] employed a transcriptomics-based approach to elucidate the specific microbial species involved in the degradation of PHCs within the context of willow-microbe systems. The study observed an increased expression of four key genes associated with PHC degradation. This enhanced gene expression was notably detected within several bacterial orders, including *Actinomycetales*, *Rhodospirillales*, *Burkholderiales*, *Alteromonadales*, *Solirubrobacterales*, *Caulobacterales*, and *Rhizobiales*. In a study, de Menezes *et al.*[308] investigated the effects of phenanthrene on the soil microbial community. It was demonstrated that the addition of phenanthrene resulted in a significant increase in the abundance of transcripts related to dioxygenase, stress response, and detoxification. The relative quantities of heavy metal P-type adenosine triphosphatases (ATPases) and thioredoxin proteins in microorganisms, specifically in relation to their response to PAH stress, have also been identified.

5.4. Metaproteomics

The field of proteomics, or metaproteomics analysis, involves the comprehensive examination of the whole protein composition within a certain ecological environment.[309] Metaproteomics can also be used for ongoing monitoring of the soil microbial community as remediation efforts progress, providing feedback on the effectiveness of bioremediation strategies. Guazzaroni *et al.*[310] used shotgun metagenomics and

metaproteomics to study microbial diversity in soil contaminated with PAHs in northern Spain. Their primary objective was to gain insights into how biostimulation influenced the microbial community after exposure to naphthalene. The researchers successfully reconstructed the metabolic pathway responsible for the degradation of naphthalene, focusing on the gentisate pathway, activated by specific bacterial groups within the soil's complex microbial communities. Bastida *et al.*[311] investigated the compost-assisted bioremediation process in semiarid soil contaminated with petroleum. Surprisingly, they found that only 0.55% of the proteins identified in the compost-treated soils were associated with biodegradation, despite the successful removal of 88% of alkanes and PAHs within 50 days of compost treatment. The primary influencers in the compost-assisted bioremediation process were the *Sphingomonadales*. These microorganisms exhibited a higher abundance of catabolic enzymes, including dioxygenases and *cis*-dihydrodiol dehydrogenases. Furthermore, in the presence of benzoate, *p*-hydroxybenzoate, and vanillin, *Pseudomonas putida* KT2440 induced around 80 unique proteins, including various dioxygenases, hydrolases, and thiolases.[312] Through proteomic methods, researchers found that *nidA* is closely linked to the metabolism of pyrene, while *nidA3* is associated with fluoranthene. This suggests that the bacterium employs different initial RHO enzymes in response to HMW-PAHs when serving as a carbon source.[313]

Rabus[314] reported metaproteomics analysis and found *Burkholderiales* as the active community member responsible for the degradation of PAHs in the presence of dioxygenase enzymes within this microbial group. Further, the work by Guazzaroni *et al.*[310] unveiled the presence of the naphthalene degradation pathway within certain bacterial species inhabiting complex microbial communities. A metaproteomic approach identified 847 proteins from microorganisms involved in naphthalene and fluorene degradation. About 70% of these proteins came from taxonomic groups like *Burkholderiales, Actinomycetales*, and *Rhizobiales*.[315]

5.5. Metabolomics

A metabolomic platform has the potential to be used for the purpose of quantitatively and extensively investigating the metabolic reactions of living organisms in response to external influences.[316] The application of metabolomic technology has the potential to enhance the detection of biological reactions resulting from soil changes, thereby elucidating distinct phenotypic variations such as alterations in the composition and quantity of soil metabolites.[317] Bao *et al.*[318] carried out an extensive investigation to study the microbial community of petroleum-contaminated soil. The results of their research revealed a remarkable level of diversity within the microbial population, as well as the presence of numerous metabolites. Experimental investigations have shown a substantial increase in the expression of enzymes during the breakdown of various external aromatic compounds. Similarly, Li *et al.*[319] investigated the profound impact of crude oil pollution on the composition of soil microorganisms and their metabolites. The levels of metabolites derived from PAH degradation pathways included 9-fluorenone and gentisic acid. Wang *et al.*[320] determined the metabolites producing during phenanthrene degradation using *Rhodococcus qingshengii* strain FF. The primary metabolite identified was pyrogallol, and notably, 59% of the metabolites were oxygen-containing PAHs with a single benzene ring.

PAHs reduce hydroxypyruvate levels and alter amino acid metabolism, thereby influencing gluconeogenesis. Furthermore, phenanthrene exposure in wheat root cells leads to decreased cellular pyruvate levels and downregulation of key metabolic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (involved in NADH production) and adenosine kinase (related to ATP generation), which significantly impact the dynamics of the TCA cycle.[321] The existence of PAHs triggers the activation of metabolic pathways related to galactose, sucrose, inositol galactoside, and melibiose in plants. This activation leads to elevated levels of D-mannose, D-galactose, raffinose, galactinol, melibiose, sucrose, and D-glucose metabolites in plant tissues. The findings indicate a suppression of energy-producing processes, specifically the synthesis of ATP and NADPH, coupled with an induction of fermentative metabolism within plant cells in the presence of different kinds of PAHs.[321] Fig. 5 illustrates various omics approaches, including metagenomics, genomics, metatranscriptomics, metabolomics, and metaproteomics, for the identification of PAH-degrading genes, metabolic pathways, enzyme expression, and associated microbial abundance in soil.

6. Factors affecting the rhizoremediation process

Rhizoremediation, being a mutually beneficial biological process, is influenced by a multitude of factors, where the interplay between plants and microorganisms is pivotal for effective degradation.[6] Numerous abiotic and biotic factors affect the biodegradation of polyaromatic hydrocarbons in contaminated sites.[322] These include soil properties, pollutant concentrations, pH, soil composition, organic matter content, temperature, nutrient availability, soil moisture, oxygen levels, contaminant solubility, microbial community diversity, metabolic capabilities, substrate specificity, carbon sources, and biofilm/biosurfactant production. Also, the intensity of the plant-microbe interactions has a significant impact on different stages of rhizoremediation.[323] Furthermore, the rhizoremediation process is influenced by several interconnecting factors. These factors include the physical and chemical complexity of PAH compounds, the history of pollution, the composition, porosity, and permeability of the soil, the density of the soil, the distribution of contaminants, the metabolic functioning of microbes, and the diversity of the microbial population involved in PAH mineralization.[324]

Contamination by PAHs modifies the organic matter and composition of soil, specifically influencing parameters such as carbon-to-nitrogen ratio, carbon-to-phosphorus ratio, salinity, pH, and electrical conductivity.[325] Maintaining a neutral soil pH is crucial for effective PAH biodegradation. Wu *et al.*[326] observed

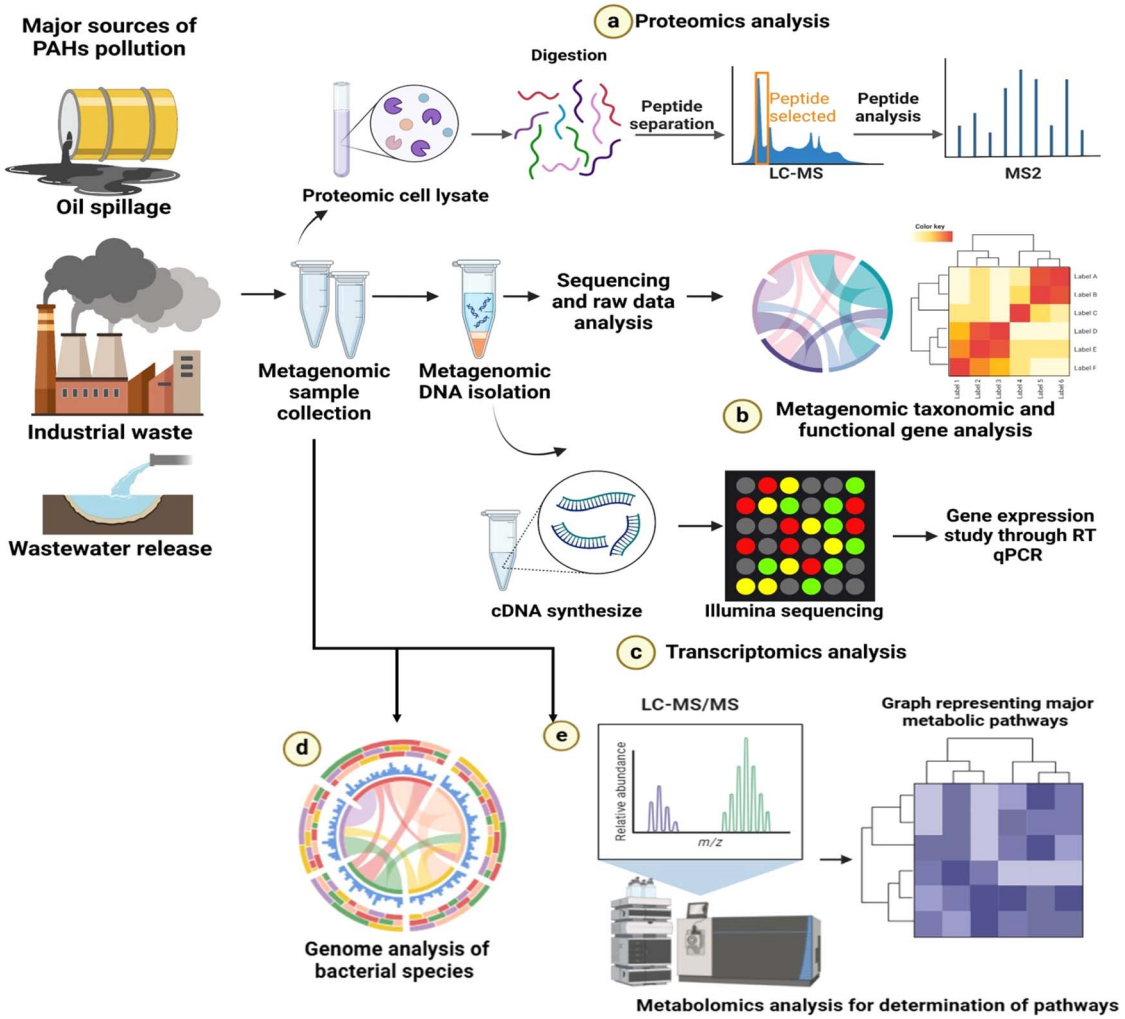

Fig. 5 Various omics approaches employed to analyze contaminated soil samples from PAH-contaminated environments.

that in soils contaminated with PAHs (0.18–20.68 mg kg^{-1}), the pH varied widely (4.26–8.43), significantly influencing bacterial diversity. Moreover, the benzo[*a*]pyrene degradation rate was noted to be pH-dependent, with the highest efficiency observed at pH 8.0 and the lowest at pH 5.0. Pawar reported that 50% of PAHs degraded within three days at pH 7.5, while the same degradation took 21 days at pH 5.0–6.5.[327] Vipotnik *et al.*[328] observed that approximately 85–90% of fluorene, pyrene, and benzo[*a*]pyrene degraded more efficiently in soil at pH 5, while chrysene exhibited greater degradability at pH 7 through fungal remediation processes. Qi *et al.*[329] also demonstrated that PAHs significantly increased soil organic carbon while reducing total phosphorus levels. The abundance of *Nitrospinota*, *Dadabacteria*, *Planctomycetota*, and *Acidobacteria* showed a positive correlation with soil PAH and total phosphorus levels but a negative correlation with total salt and organic carbon content.

Soil contamination with PAHs typically involves a complex mixture of hydrocarbon molecules. The presence of multiple pollutants in varying combinations increases toxicity compared to individual contaminants. PAH concentrations in contaminated soils are often accompanied by co-contaminants such as heavy metals (Cu, Ni, V, Zn, Pb, and Cr) and elevated salt levels. Co-contamination of PAHs and heavy metals can have both synergistic and antagonistic effects on pollutant removal in the rhizosphere. For instance, Cd-PAH co-contamination enhanced Cd removal in the rhizosphere of *Fire Phoenix*.[107] Similarly, low concentrations of pyrene-Cu/Cd co-contamination promoted metal removal and pyrene degradation in maize.[330] However, high Cu levels (1000–1500 mg kg^{-1}) reduced PAH removal efficiency in maize, *Elsholtzia splendens*, and *Lolium perenne*.[330] This inhibition may result from heavy metal-induced suppression of root growth and exudation, altering enzyme activity and microbial communities in the rhizosphere.[330] Su *et al.*[194] reported that microbial diversity in the rhizosphere was higher than in the endosphere under phenanthrene stress. Additionally, organic pollutants like phenanthrene had a substantial effect on microbial communities in the endosphere and rhizosphere compared to bulk soil.[330]

Studies have elucidated the mechanisms driving differences in the microbial community structure and activity among plant species and genotypes, which can be attributed to variations in the root structure and plant-specific exudates.[331] Under pollution stress, root exudation patterns differ across plant species in

terms of composition and concentration,[332] leading to changes in microbial biomass, activity, and structural composition.[331] The release of root exudates by plants contributes to the availability of carbon and nitrogen sources, which in turn creates a conducive environment for microorganisms to degrade organic contaminants. Moreover, certain plants, such as maize, can synthesize secondary metabolites like benzoxazinoids, which influence the selective depletion and enrichment of microbial species in the rhizosphere.[333] Such plant-specific microbial recruitment can lead to cultivar-specific rhizosphere microbiota. However, the primary factors shaping rhizosphere microbial diversity remain inconsistent across studies. While Robertson *et al.*[334] identified plant species and soil factors as the main determinants of microbial community structure, Pritchina *et al.*[335] suggested that PAH pollution levels had a more pronounced impact than plant species. Therefore, further research is needed to determine the dominant factors controlling rhizosphere microbiome composition under organic pollution, which could aid in optimizing plant-microbe interactions for improved bioremediation efficiency. Additionally, in soils co-contaminated with Cu and phenanthrene, the rhizosphere microbiome of *Elsholtzia splendens* and *Lolium perenne* exhibited substantial shifts in the enrichment and depletion of microbial phyla such as *Actinobacteria* and *Bacteroidetes*.[336] These findings highlight the complexity of plant-microbe interactions in contaminated environments and underscore the need for targeted microbiome engineering to enhance rhizoremediation.

Organic pollution exerts significant toxicity on microbial populations, leading to reduced microbial biomass and diversity in the rhizosphere.[337] Phenanthrene exhibits greater toxicity to microorganisms than pyrene, due to its higher bioavailability.[338] As PAH concentrations increase, microbial biomass declines markedly in both rhizospheres.[338] Gram-positive bacteria demonstrate heightened susceptibility to PAHs compared to Gram-negative bacteria. Bacteria exhibit greater sensitivity to organic pollutants such as lindane, PCBs, and co-contaminants like cadmium and DDT compared to fungal communities.[337,339]

Further, Eriksson *et al.*[340] investigated PAH biodegradation under low temperatures and anaerobic conditions, demonstrating that aerobic conditions at 20 °C facilitated the removal of 52% to 88% of PAHs over 90 days, whereas the highest PAH degradation at 7 °C reached 53%. Similarly, Amponsah *et al.*[341] found that increasing soil temperatures from 10 °C to 20 °C and subsequently to 25 °C reduced pyrene, fluorene, chrysene, and anthracene concentrations at three Canadian well sites. These findings align with previous studies,[342–344] which reported enhanced PAH degradation with increasing soil moisture. Similar studies have highlighted the critical role of soil moisture in organic pollutant degradation by regulating microbial oxygen availability, with optimal moisture levels varying by climate and soil type, ranging from 30–90%[345] to 12–32%.[346]

Moreover, electrical conductivity quantifies the soil's capacity to conduct electricity, directly correlating with the quantity of dissolved salts and nutrients. Elevated electrical conductivity may signify heightened salinity, potentially detrimental to plant development and microbial activity. Maintaining suitable electrical conductivity in the soil is essential for facilitating effective rhizoremediation processes. Cation exchange capacity refers to the soil's capability to retain and exchange positively charged ions (cations) such as potassium, calcium, and magnesium. A high cation exchange capacity signifies that the soil has an enhanced ability to store and provide necessary nutrients to plants and microbes. This directly impacts the efficacy of rhizoremediation by guaranteeing that plants and microorganisms get sufficient nutrients to sustain their development and metabolic functions.[347]

7. Methods for improving the effectiveness of rhizoremediation

To enhance the effectiveness of rhizoremediation in addressing PAH-contaminated soil, a combination of carefully chosen strategies can be employed to optimize plant-microbe interactions and the degradation processes within the rhizosphere. These strategies include plant selection, bioaugmentation, nutrient management, and several others, with a focus on long-term sustainable solutions.[348]

7.1. Microbiome engineering

The process of microbiome engineering involves the *in situ* application of a "bacterial consortium" as an artificial community. This community is used to manipulate and control the existing microbial community in order to achieve remediation objectives.[349] The primary objective of bioaugmentation is to enhance the removal of contaminants by leveraging the metabolic activities of these externally introduced microorganisms.[350–352] Derz *et al.*[353] demonstrated the effectiveness of bioaugmentation by introducing a mixed bacterial culture into soil contaminated with PAHs, specifically pyrene and benzo[*a*]pyrene. They observed a pyrene mineralization rate of approximately 36% after 150 days and a benzo[*a*]pyrene removal rate of 5% after 70 days. The application of bioaugmentation in landfarming led to an 86% reduction in total petroleum hydrocarbons within a 90-day timeframe. The phytochelating activity of *Mesorhizobium haukuii* was enhanced by introducing a chelating gene (phytochelatin synthase; *PCSAt*) from *A. thaliana.*[354] The enhancement of siderophore production in polluted soil can be achieved through the regulation of the transcriptional unit *pvdS* regulator on the *pvdD* and *pvdA* genes of *Pseudomonas fluorescens*.[355] The evaluation of bioaugmentation involving the introduction of efficient hydrocarbon-degrading Pseudomonas bacteria into the rhizosphere of teak (*T. grandis*), gmelina (*G. arborea*), neem (*Azadirachta indica*), and champak (*Michelia champaca*) plants has been conducted. The objective of this evaluation is to enhance the biodegradation of crude oil.[356] From a practical standpoint, employing a microbial consortium instead of a pure culture for bioremediation offers distinct advantages, primarily due to its capacity to deliver the necessary metabolic diversity and resilience essential for field applications.[357] However, the effectiveness of this procedure significantly relies on the chosen microbial consortia's adaptability to the specific site conditions

and their capability to outcompete the indigenous microorganisms.[358] The bacterial culture is introduced into the polluted area using methods like spraying, injection, or soil or water blending, depending on the specific environment. After the bacteria become established, they initiate the process of metabolizing the PAH compounds found in the polluted surroundings.[359] This process at play is referred to as mineralization, resulting in the complete removal of PAH contaminants.[360] Throughout the course of the bioaugmentation procedure, continuous monitoring of the site is conducted to assess the progress in PAH degradation and the efficacy of the introduced bacterial strains.[361] Further, *Lolium perenne* and *Medicago sativa* can improve pyrene degradation in soil up to 46% when biocompost is applied.[362]

7.2. Soil engineering

Biostimulation is a procedure used to increase the growth of indigenous organisms in habitats polluted by contaminants. This is achieved by providing nutrients that promote co-metabolism.[363] Biostimulation is the introduction of organic nutrients into the polluted area to enhance the development and functioning of native microorganisms.[364] As the microbial population experiences growth, it initiates the synthesis of various enzymes, such as dioxygenases and ring-hydroxylating enzymes. The enzymes are essential in the process of breaking down PAHs, converting them into compounds that are simpler and pose less risk.[365] The PAHs undergo subsequent enzymatic reactions, ultimately resulting in their complete conversion into CO_2, H_2O, and biomass. This process ensures the complete removal of PAHs from the environment.[366,367] The utilisation of diverse materials, including crop residues, sugarcane bagasse pith, sewage sludge compost, vermicompost, food waste compost, corn stalks, corn fermentation byproduct, peat and sawdust,[368] wastewater sludge, ground rice hulls, and dried blood,[369] has been observed to lead to a noticeable increase in PAH degradation. When introduced into a PAH-polluted environment, the addition of 5% manure resulted in an increase in available phosphorus, potassium, and hydrolysable nitrogen. This, in turn, supported the growth of bacteria capable of degrading PAHs. The utilisation of PGPR and biochar in the process of bioremediation for soil contaminated with hydrocarbons has gained significant importance in recent years.[370] Furthermore, a thorough examination was conducted to analyse the impacts of nutrients (NPK), aeration, and the bioinduction of native soil microorganisms, as well as the stimulation of external microbial communities. This investigation revealed that these factors have positive effects on the remediation of oil-contaminated soil.[371]

7.3. Phyto-engineering

The initial step in the phyto-engineering approach for crude oil degradation is the selection of plants that possess high contaminant degrading efficiency. The presence of a plant species with a significant amount of aboveground biomass is crucial in the phytoextraction process.[372] The successful implementation of rhizoremediation requires the inclusion of a plant species that has an extensive root system or significant belowground biomass. However, the presence of contaminants frequently hinders the growth of plants in polluted regions.[373] The incorporation of *ACC* deaminase genes into genetically modified plants has been demonstrated to successfully decrease ethylene levels, leading to a stronger and more extensive root system.[374] The process of plant breeding and genetic modification can lead to several beneficial outcomes, including enhanced nutrient intake, increased production of root exudates, improved survival rates, and more efficient mineralization of pollutants.[375] The researchers Uchida *et al.*[376] conducted genetic engineering on Arabidopsis plants to introduce a root-specific laccase (LAC1) obtained from cotton plants.[377] The researchers conducted an observation and found that the modified plants demonstrated enhanced tolerance to phenolic compounds and 2, 4, 6-trichlorophenol when these substances were secreted into the rhizosphere. Moreover, the successful enhancement of the degradation process of polychlorinated biphenyls (PCBs) was observed through the insertion of the *bphC* gene derived from *Pandoraea pnomenusa* B-356 into tobacco plants.[277] In addition, Uchida *et al.*[376] reported that the introduction of estradiol dioxygenase genes responsible for aromatic cleavage (*DBfB*) into Arabidopsis plants resulted in an increased degradation rate of 2,3-dihydroxybiphenyl (2,3-DHB).

8. The global bioremediation markets

According to Biospace Reports (2022), the bioremediation market had a value of USD 105.68 billion in 2019 and is expected to see a compound annual growth rate (CAGR) of 15.5%. It is forecast to reach USD 334.70 billion by 2027.[378] The environmental remediation market in the United States is projected to experience significant growth over the forecast period. It is anticipated to increase from USD 19.96 billion in 2021 to USD 22.86 billion in 2022 and further reach USD 37.26 billion by 2027, reflecting a CAGR of 10.96%. In recent years, there has been a significant increase in global awareness and acknowledgment of natural-based bioremediation methods. The biotreatment industry in the United States is currently composed of around 130 businesses, which can be categorised into three primary sectors: product vendors, transdisciplinary environmental services, and bioremediation services.[379] Due to its cost-effectiveness and accessibility, widespread promotion of rhizoremediation, along with bioaugmentation and biostimulation, is recommended.[380]

In 2018, the *in situ* bioaugmentation segment held the largest market share at 23.9%, followed by the biostimulation segment at 16.91%. Soil remediation accounted for the highest revenue, with a market share of 46.64%, driven by the increasing prevalence of soil pollutants. In terms of application, the industrial sector led the market with a 27.09% share, followed by agriculture and aquaculture at 21.18%.[381] In 2022, the phytoremediation segment accounted for the largest portion of global revenue, exceeding 32.02%. This growth in the industrial segment is attributed to the significant number of contaminants and pollution originating from this sector (Global Bioremediation Market, 2020).[382]

The phytoremediation market was valued at USD 1.07 billion in 2019. The fungal bioremediation method, which is employed

for the purpose of eliminating radioactive contaminants, is currently experiencing a CAGR of around 15.2%. The market is projected to be dominated by the soil-based bioremediation category, with a CAGR of 15.8% from 2019 onwards.[383] However, *in situ* bioremediation is anticipated to generate the most revenue in the coming years due to its cost-effectiveness and minimal risk of cross-contamination. *In situ* bioremediation methods include bio-slurping, bio-vending, and rhizoremediation.[384] The bio-stimulation sector generated revenues of $12,094 million in 2021 and is projected to grow at an annual rate of 7.1%, reaching $24,500.2 million by 2030 (Share and Trends Report, 2030).[385]

The North American region has recently dominated the market for bioremediation, accounting for a market share of approximately 41.8%. This can be attributed to the presence of numerous major industrial firms in the region. Asia-Pacific is projected to experience the highest CAGR of 16.5% during the forecast period (Biospace Reports, 2022).[386] The expansion of the bioremediation market in these areas is mainly propelled by the rising environmental concerns and regulatory objectives for environmental protection established by diverse government sectors (Environmental Remediation Market, 2022).[387]

The Netherlands has achieved successful remediation of over 6000 sites since 1982, establishing itself as the European country with the most significant advancements in this field (Europe Sustainable Development Report, 2021).[388] Companies like Gist-Brocades are marketing improved anaerobic wastewater clean-up techniques. The Dutch government endorses the utilisation of compact fermenters for the conversion of agricultural waste into commercially viable fertilisers, with a specific focus on providing support to underdeveloped nations. Additionally, they are actively engaged in conducting research on soil bioremediation.[389] The Ministry of Construction in Japan launched a project in the 1980s with a budget of five billion yen. The project aimed to develop and implement biotechnological methods for treating wastewater (Sanitation and Sustainable Development in Japan, 2016).[390] The Swedish National Environment Protection Board has recently contracted a biotreatment company from the UK to carry out *in situ* bioremediation of soil contaminated with creosote. The bioremediation process will involve the use of *Pseudomonas* bacteria. The total value of the contract is estimated at US $1.6 million.[390] The progress in bioremediation underscores its critical role in addressing environmental challenges across various sectors, including industry, agriculture, and remediation, highlighting its potential for continuous innovation and global application.

9. The global landscape of patents related to bioremediation

Patents play a crucial role in measuring economic development as they enable the efficient exchange and spread of technology between different countries.[391] The United States is the leading country in terms of bioremediation technology patents, accounting for 61.85% of the total. China follows with 79% and Japan with 67%.[392] The contributions of South Korea and India are approximately 4.51% and 2.93%, respectively.[393] Bioremediation research is less prevalent in other nations such as Australia, Belgium, France, Spain, Canada, Great Britain, and Russia. The distribution of patent applications among different regions is as follows: out of a total of 443 applications, North American countries accounted for 67%, Asian countries accounted for 23%, and European countries accounted for 10%. The data suggest that Asian nations, specifically Japan and Korea, are making significant investments in research and development (R&D) to improve their technological capabilities.[394]

Developed countries possess robust research infrastructures to address oil contamination issues due to their financial resources, availability of trained scientific personnel, and stable economies (National Innovation Systems, OECD).[395] PAH contaminants are prevalent in developing and economically disadvantaged countries as well. The capacity of biological systems to break down aromatic hydrocarbons and the utilisation of bioremediation techniques have been well-documented since the early 1970s.[396] The earliest patent applications, dating back to 1971, discuss the use of emulsifiers or fertilizers to promote oil-degrading bacteria. The Exxon Valdez oil spill in 1989 marked a significant increase in hydrocarbon degradation patent applications in the early 1990s.[397] Initially, chemical and physical methods were employed to mitigate the damage, but bioremediation soon emerged as a viable cleanup technique.[398]

An analysis was conducted on the global database maintained by the European Patent Office to examine the growth of bioremediation technologies for water, soil, and sludge. The results indicate a steady increase in these technologies, with water accounting for 53% of the patents, soil accounting for 36%, and sludge accounting for 11%.[399] In India, government agencies are less involved in cutting-edge bioremediation R&D compared to private entities. Organizations such as the Council of Scientific and Industrial Research (CSIR), the Indian Council of Agricultural Research (IARI), Bharat Petroleum Corporation Ltd, Indian Oil Corporation Ltd, and M/S Avestha Gengraine Technologies Pvt. Ltd have secured numerous patents, yet private entities have obtained more patents than government institutions.[400] Fig. 6 highlights the leading continents in the bioremediation market based on market revenue and patent filings. Continents with a significant bioremediation market, along with market size and CAGR values, are presented in Table 5, which also lists several key bioremediation companies involved in the sector.[386]

10. Limitations and challenges

Rhizoremediation, as a plant-microbe-driven strategy for PAH degradation, offers a promising approach for restoring contaminated soils. However, its long-term sustainability is influenced by several critical factors. Some limitations of rhizoremediation include the need for a large field area for *in situ* remediation, the age of the plant being used, hindered plant and microbial growth due to severe pollution, dependence on environmental factors, uncertainty regarding the disposal of plant parts containing PAHs, unknown by-products of

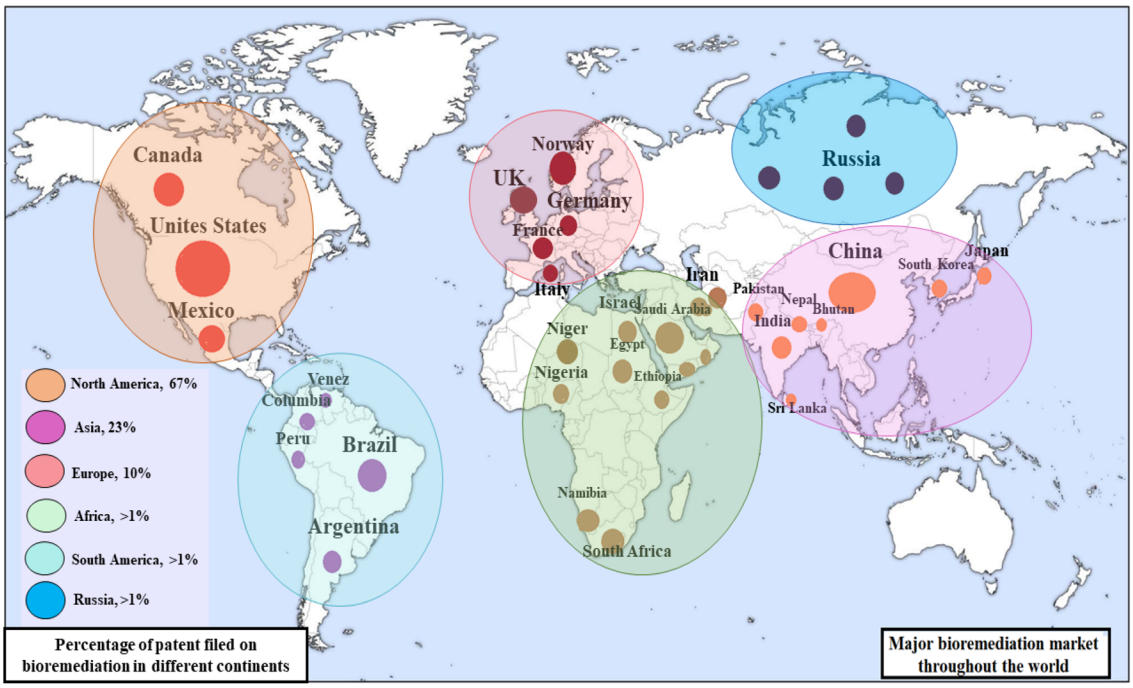

Fig. 6 A map illustrating countries with major bioremediation markets and the percentage of patents filed from different geographic regions. Transparent bubbles represent continents with significant patent activity, while coloured dots within the bubbles indicate countries with high bioremediation market revenue. The size of each dot corresponds to the market share percentage of the respective country.

Table 5 Overview of the bioremediation market across different continents, including market size, CAGR, and key companies actively involved in bioremediation[386]

SI no.	Continents	CAGR	Market size	Key companies
1	North America	8.7%	US$ 5040.96 million	• Altogen Labs • InSitu Remediation Services Ltd • Probiosphere • Xylem • REGENESIS
2	Europe	7.70%	US$ 3487.61 million	• Altogen Labs • InSitu Remediation Services Ltd • Probiosphere • Xylem • REGENESIS
3	Asia-Pacific	8.43%	US$ 2249.68 million	• Aquatech International Carus Group Inc • Drylet Inc • InSitu Remediation Services Ltd • Remediation Solutions • RT Environmental Services Inc. • Soilutions Ltd, Verde Environmental Group • Xylem Inc.
4	Latin America	8.26%	US$ 3898.20 million	• Aquatech International Carus Group Inc. • Drylet Inc. • InSitu Remediation Services Ltd • Remediation Solutions • RT Environmental Services Inc. • Soilutions Ltd • Verde Environmental Group • Xylem Inc.
5	Middle East & Africa (MEA)	6.4%	US$ 695.57 million	• Aquatech International LLC • InSitu Remediation Services Limited • Ivey International Inc. • Xylem Inc.

biodegradation, and the possibility of PAHs entering the food chain. The use of seasonal plants in rhizoremediation may be limited in certain situations.[401–403] To facilitate efficient rhizoremediation, it is crucial to monitor and regulate soil pH, electrical conductivity, and cation exchange capacity. The soil pH affects the activity of microorganisms and influences the solubility and transportation of contaminants in the soil. A further crucial factor for rhizoremediation is the possibility of unforeseen ecological repercussions. The introduction of non-indigenous plant species or microbes might provide unforeseen consequences for the surrounding environment. Hyperaccumulator plants, capable of absorbing and withstanding elevated concentrations of certain pollutants, are exemplary candidates for bioremediation. Moreover, the efficacy of bioremediation may be enhanced by using plants with broad root systems that foster interactions between plant roots and the rhizosphere microbial population, enhancing biotransformation activities.[404] Prioritizing climate-adaptive plant-microbe systems is crucial for sustainable remediation. Plant health and growth metrics act as indirect markers of rhizoremediation effectiveness. Monitoring root exudation patterns, stress responses, and biomass buildup is essential for evaluating plant-microbe interactions and their influence on PAH breakdown.

The effectiveness of rhizoremediation is contingent upon the persistence of PAH-degrading microbial populations within the rhizosphere. Soil that is contaminated can offer the combined advantages of increased fertility and bioremediation through microbial processes, leading to soil that is both healthy and productive.[405] Microorganisms in the soil play a crucial role in increasing the availability of nutrients and aiding in the production of regulators that promote plant growth. Concurrently, they participate in numerous transformations of organic substances within the soil and play a role in the degradation of xenobiotics. Another function involves participating in the processes of adsorption and desorption of various substances, as well as the detoxification of both organic and inorganic contaminants.[405] Specific strains exhibit the ability to break down particular pollutants, notably those that include functional genes like PAH-*RHDα* and dioxygenase genes, which are essential for maintaining degradation efficiency.[405] Conventional bioremediation techniques encompass continuous biostimulation, which involves the supplementation of nutrients to promote microbial activity, and bioaugmentation, which entails the introduction of external microflora, aimed at achieving long-term sustainability.[406] For instance, the inoculation of plant growth-promoting rhizobacteria in soil cultivated with tall fescue and rice plants augmented the extraction of total petroleum hydrocarbons (particularly the C21–C34 fraction) and phthalate esters during phytoremediation.[407] However, more advanced strategies are required to optimize rhizoremediation efficiency for diverse organic contaminants.

The particular genes associated with the biodegradation of various organic contaminants and their degradation pathways within the rhizosphere are not yet fully understood. Furthermore, numerous recent investigations concerning rhizosphere microorganisms were predominantly conducted in controlled greenhouse environments utilising artificially contaminated soil, which markedly differs from natural field conditions. Li *et al.*[407] explored the impact of ryegrass root exudates on phenanthrene biodegradation using both ^{12}C- and ^{13}C-labeled phenanthrene. Understanding microbial community shifts within the rhizosphere, including the role of pollutant-degrading microbes and their functional genes, is essential for optimizing *in situ* remediation strategies.

To ensure the long-term effectiveness of rhizoremediation, continuous monitoring of PAH concentrations and microbial activity is crucial. Advances in nanocomposite technologies have enhanced PAH analysis across various matrices. Pretreatment, extraction, and clean-up techniques, primarily using gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography-ultraviolet (HPLC-UV), enable precise detection. GC-MS and GC-MS/MS offer high sensitivity and selectivity, with GCxGC-FID achieving recovery rates of 95–120% for 24 PAHs in soil. Eventually, to quantify the PAHs adsorbed, techniques such as Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) play a crucial role in the initial identification of PAHs.[408] Ma *et al.*[409] developed a sensor integrating pre-concentration and *in situ* electrochemical analysis using electropolymerized poly(3-methylthiophene) (P3MT) for 1-hydroxypyrene detection, a key PAH exposure marker.

Despite these advancements, large-scale PAH degradation in field studies remains underexplored. Post-rhizoremediation monitoring is essential to evaluate residual PAH levels and ensure successful long-term remediation outcomes. Following rhizoremediation, soil rehabilitation occurs alongside microbial community shifts that enhance nutrient cycling, predominantly involving native microbes. Incorporating amendments such as biochar, compost, or surfactants can further stimulate microbial activity and improve PAH bioavailability, thereby increasing remediation efficiency. Integrating these strategies can optimize rhizoremediation as a sustainable, long-term solution for PAH-contaminated soils.

In addition, several significant factors are anticipated to restrict the revenue growth of the global bioremediation market. These include the high cost of excavation equipment and the slow adoption of environmental protection regulations. The costs related to the use of heavy machinery in treatment operations, such as bulldozers, loading trucks, and excavators, are considerably higher. Environmental protection regulations and policies pertaining to environmental flow are being implemented globally; however, several interconnected challenges are evident. Expanding bioscience research, whether in the academic sector or the commercial sphere, depends on having advanced laboratory infrastructure and cutting-edge technology. Modern research facilities must be established, upgraded, and expanded. Strong business relationships must be forged.[410] Collaboration initiatives with other countries' premier reference centres must be encouraged.[411] The exploration of better alternatives to the use of technology and natural resources and to organise economic growth without endangering the ecosystem's sustainability must be implemented in the future.[412] To achieve the bioeconomy and sustainability

goals, land decontamination should be viewed as critical. The remedial technology of bioremediation seems to be sustainable.[413] While lacking statutory authority, sustainability criteria are nonetheless infrequently used to evaluate remedial technology.[414]

11. Conclusion and future prospects

The pollution of PAHs is a long-lasting global issue. Nevertheless, higher levels of production are also associated with a greater number of instances of contamination in various ecosystems. The soil is a primary ecosystem that is consistently contaminated by numerous PAH molecules as a result of human interference. Inadequate management practices and accidents result in the pollution of PAHs, leading to the depletion of soil fertility and negative effects on plants and indigenous soil bacteria. Consequently, there exists a significant disparity in the commercialization of rhizoremediation for the purpose of treating soil contaminated with PAHs. This method has the potential to offer a sustainable solution for restoring ecosystems. Rhizoremediation not only removes contaminants from damaged soil, but also improves soil fertility by restoring its natural structure. The bioremediation technique often involves the use of microorganisms to clean up contaminated places or to enhance the activity of naturally occurring microbes in polluted areas. Therefore, by employing suitable combinations of plants and microbes in polluted areas, this issue could be effectively resolved. The use of modern sequencing techniques to manipulate the rhizosphere also improves our understanding of the intricate plant–microbe relationship. Nevertheless, it is imperative for both the public and private sectors involved in bioremediation to prioritise the commercialization of rhizoremediation techniques and the global dissemination of knowledge. Additionally, the process must be improved to achieve both environmental and commercial advantages. Effective strategic planning and the formulation of enduring policies on rhizoremediation will support nations in attaining their objectives for a sustainable future.

The process of rhizoremediation is regarded as an efficient approach; however, a significant research gap remains that must be addressed for future advancements. As highlighted in previous sections, large-scale field studies on rhizoremediation are still very limited. There is a need to raise awareness for the beneficial attributes of rhizoremediation and effectively commercialize such plant-microbe pairs for field application. Rhizoremediation not only degrades contaminants but also enhances soil health and microbial communities, making it a promising strategy for restoring farmlands and agricultural sites. Conducting more data-driven studies will provide a comprehensive analysis and reinforce confidence in the efficacy of rhizoremediation. Additionally, process optimization is crucial, as rhizoremediation depends on several biotic and abiotic factors. The selection of efficient plant-microbe pairs and the assessment of proper remediation strategies are essential for ensuring successful implementation. Furthermore, the effectiveness and duration of the rhizoremediation process must be considered for long-term field applications. Research is also needed to optimize the benefit-to-cost ratio, particularly regarding the cost of implementing rhizoremediation relative to the biomass generated. The processing of rhizoremediated biomass requires additional precautions and refinement. However, there is significant potential to utilize this biomass for other applications like biochar production, contributing to the circular economy, as reviewed in previous studies.[21] To overcome current limitations in evaluating nature-based remediation systems, it is essential to refine life cycle assessment (LCA) and cost-benefit analysis (CBA) approaches.[415] Addressing these challenges will facilitate the broader adoption of rhizoremediation as a sustainable and cost-effective strategy for environmental restoration.

Data availability

No data were used for the research described in the article.

Author contributions

Writing – original draft: ND; conceptualization: PP; data curation: ND; formal analysis: ND, PP; funding acquisition: ND, PP; investigation: ND, VK, KC; validation: PP; visualization: PP; writing – review & editing: PP.

Conflicts of interest

There are no conflicts to declare.

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