Current Pollution Reports
https://doi.org/10.1007/s40726-024-00324-8

REVIEW

Bioremediation of Polyaromatic Hydrocarbons in Soils: A Review of Recent Progress

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Accepted: 17 July 2024

Abstract

Purpose of Review Recent progress in bioremediation of soils contaminated with polyaromatic hydrocarbons (PAHs) is reviewed. Innovative techniques, traditional approaches, and combinations of technologies are examined.

Recent Findings Bioremediation was heavily researched in past decades and continues to be studied with excellent advances. Phytoremediation, bioaugmentation, biostimulation, and natural attenuation remain important but are now studied in conjunction with genetic analyses, community dynamics, and extracellular enzymes and/or surfactants. Field soils contaminated with heavy matrices have lower rates of degradation (often < 25%), even for the most aggressive techniques.

Summary Significant strides have been taken in improving the efficacy of bioremediation of PAH-contaminated soils and understanding the fundamental processes. Key genes, important enzymes, and optimal conditions have been identified. Research continues in the challenging and important area of degradation of PAHs in anaerobic environments. Bioremediation endures as a viable approach to decontamination of soils and a fertile area for future research.

Keywords Phytoremediation · Bioaugmentation · Natural attenuation · Surfactants · Bioaugmentation

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a broad group of semivolatile organic compounds that are naturally occurring, potentially toxic, frequently carcinogenic, and persistent in soils. The most well-known and studied PAHs are the 16 priority pollutants from the U.S. Environmental Protection Agency [1]. Alkyl-substituted PAHs are suspected to have health impacts similar to the 16 priority pollutants but are studied far less frequently [2]. Nearly all environments are contaminated with low levels of PAHs because of the large quantities of semi-volatile PAHs entering the atmosphere from the burning of fossil fuels and natural processes, such as forest fires and volcanoes.

PAHs persist in soil because they are somewhat recalcitrant and strongly sorbed to organic and inorganic surfaces. However, the similarity in chemical structure between the PAHs and certain chemical components of plants and soil organic matter allows PAHs to be degraded by many of the same microorganisms that attack lignin, fulvic acid, and humates.

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The degradation of PAHs by organisms has been long recognized for its potential to efficiently remove PAHs from contaminated soils. Over the decades, bioremediation of PAHs has taken many forms: landfarming, adding nutrients and water with frequent tilling; bioaugmentation, adding microorganisms to the soil that target PAHs; phytoremediation, using higher plants to stimulate rhizosphere degradation; and natural attenuation, simply allowing indigenous microbes to slowly take their course. Bioremediation can be a passive, in situ approach (e.g., natural attenuation), an active in situ approach (bioaugmentation, phytoremediation), or aggressively active ex situ (stirred reactors with high inputs of nutrients and microorganisms). Microbes of various types have been selected or even genetically modified to enhance bioremediation. Several reviews from the past decade provide an overview of previous research [2–6]. Some of the reviews went into great depth on specific aspects of bioremediation, and they will be referenced in the appropriate sections.

The objective of this review is to provide an update of progress in key areas of bioremediation of PAHs in soils from the past 5 years. Bioremediation of PAHs continues to be a vibrant area of study, and the science has evolved to combine multiple facets of bioremediation to enhance efficiency.

Published online: 02 August 2024

Progress in Bioremediation of PAHS in Soils

Many areas of bioremediation of PAHs in soils have witnessed steady growth since 2018, others have languished, and new approaches are emerging. In preparing this review, Clarivate's Web of Science (accessed through Texas A&M Libraries) was used to search peer-reviewed journals. Phytoremediation and the genetics of bioremediation have been major growth areas, while the number of published articles regarding bioslurries, composting, anaerobic environments, and soil-dwelling fauna remains small. With over 1200 peer-reviewed publications on the general topic of "bioremediation of PAHs in soils" and over 1000 articles on the specific subjects covered in this review (Table 1), only a fraction of the recent work can be cited here.

The rows in Table 1 correspond to the subtopics in this review:

- Natural attenuation: the use of unmanaged, naturally occurring processes to remove pollutants.
- Biostimulation: alteration of the environment to enhance the remediation capability of existing microorganisms.
- Composting: aerobic decomposition of organic solids through careful management of nutrient concentrations, moisture content, and aeration.
- Bioslurry: another name for slurry bioreactors, soil is excavated and placed in a stirred tank with enough water to form a flowable mixture. Nutrients and other materials may be added.
- Phytoremediation: the use of higher plants to accelerate the degradation of PAHs in the rhizosphere.
- Bioaugmentation: microorganisms are added to the soil to create a more effective bioremediating population.
- Wood decay fungi (includes white rot fungi): adding or enhancing existing populations of fungi that decay wood to degrade PAHs. Lignin degraders appear to possess an enhanced ability to degrade PAHs.
- Surfactants: generally limited to naturally produced compounds such as rhamnolipids, the addition of surface-active compounds to desorb PAHS and make the compounds susceptible to degradation.
- Laccase enzymes: this class of extracellular enzyme is capable of cleaving the rings of aromatic compounds.
- Extracellular exudates and enzymes: the study of compounds that may be responsible for enhanced PAH degradation by microorganisms and/or in the rhizosphere of higher plants. This area of research is more fundamental and not often considered as a remediation method; however, this research led to the study of laccase enzymes.
- Fauna: the use of soil-dwelling animal species to enhance PAH degradation, usually limited to earthworms.

For this review, the mandatory criteria for the consideration of published research included the following: (1) research published in peer-reviewed journals in 2018 or after; (2) studies addressing the remediation of PAHs in contaminated soils; (3) no review articles. Other criteria were deemed as highly important but could not always be met: (4) the contaminant PAHs were introduced in the field and aged at least 1 year; (5) individual PAHs must be analyzed before and after treatment. The requirement of field-contamination eliminated "spiking" soils in the laboratory,

Table 1 Summary of bioremediation publications from 2018 to 2023

Technology	Publications	Reviews	Significant advances
Natural attenuation	82	11	Combining multiple technologies, benchmark for comparisons, long-term studies
Biostimulation	95	8	Expanding range of amendments; combining with bioaugmentation
Composting	7	1	Application to complex or heavy waste matrices
Bioslurry	34	1	Combining with bioaugmentation, biostimulation; branching into genetic analyses; mechanisms
Phytoremediation	314	21	Expanding species database; combining with bioaugmentation, enzymes, biostimulation
Bioaugmentation	97	6	Wide-ranging variety of microorganisms added to soil; increased focus on indigenous species
Woody decay fungi	34	4	Examining more species of fungi; adding surfactants
Surfactants	68	6	Application to all forms of bioremediation; broadening the number of chemicals; microbial exuders
Laccase enzymes	39	1	Immobilization of laccase to enhance activity; exploring a wide range of mediators
Extracellular enzymes	145	15	Rapidly increasing field of study; isolation/characterization of enzymes
Biological communities	195	11	Tracing communities through remediation; molecular/genetic approaches; dynamics
Genes/genetics	379	34	Rapidly growing field; combining with other technologies; expanding applications
Fauna	15	0	Increasing the number of species; expanding beyond earthworms
Anaerobic/anoxic	8	0	Anaerobic degradation is slow, but this research is needed for PAHs in anoxic environments; wetlands

and this criterion was met for all areas except wood decay fungi, extracellular enzymes, and remediation using fauna. In the case of extracellular enzymes, all the articles in this review used soils that were contaminated in the laboratory.

Natural Attenuation

Natural attenuation relies solely on unmanaged processes to decrease PAH concentrations. PAHs can dissipate through non-biological means such as irreversible sorption, inorganic oxidation, and volatilization, but biologically mediated degradation is the primary mechanism. In a greenhouse experiment using field PAH-contaminated and aged soil, dissipation after 180 days using natural attenuation removed 42% of the 16 priority pollutants, with the 2- and 3-ring compounds decreasing the most (48%) [7]. Seven field sites were monitored for natural attenuation of PAHs over a period of 25 years [8]. Degradation rates initially were rapid and declined with time, but decreases of at least 70% in all measured PAHs were observed in all sites at the end of the 25-year period. Greater plant diversity in an unmanaged system was associated with 38% smaller concentrations of PAHs than systems with limited plant diversity [9]. Soil from a 150-year-old manufactured gas plant (MGP) was treated in a series of microcosms comparing natural attenuation, bioaugmentation, and bioslurries [10]. Despite the aggressiveness of the active approaches, natural attenuation was nearly as effective in removing PAHs (86%) as bioslurry and bioaugmentation (90%).

Biostimulation

Modification of the contaminated environment to enhance the ability of existing bacteria to degrade PAHs is called biostimulation. Soil modifiers include water, nutrients, and electron donors in anaerobic processes. Recent advances have focused on recycling of high-carbon wastes to increase microbial activities and provide greater opportunity for PAH dissipation. Sewage sludge and sludge compost were added to moderately contaminated soils (< 600 μg total PAH [kg soil]$^{-1}$) and up to 65% degradation was observed after 126 days [11]. Actinomycetes and gram-positive bacteria were found to be the most important variables. Spent mushroom substrates were added to aged PAH-contaminated soil (total PAH ≤ 2.5 mg kg^{-1}), and PAH dissipation ranged from 2.5% in the unamended check samples up to 41% in fresh mushroom compost + soil after 60 days of incubation [12]. Adding pea straw enhanced PAH degradation (45%) compared to natural attenuation (27%) for the first 30 days of experimentation, but both methods had similar endpoints by day 102 (64%) [13]. Twelve types of biochar were added to contaminated soil from a coking facility, and the most effective material resulted in 58% degradation of the PAHs after 180 days [14].

Biostimulation and most other forms of bioremediation involve the action of microorganisms on PAHs. The individual species of bacteria and fungi have been heavily reviewed, and knowledge of the participating species can provide important insights into bioremediation strategies [4, 5, 15]. The details of pathways of PAH degradation also have been summarized [3, 6].

Composting

One of the least operationally complex forms of bioremediation is composting, a controlled process in which biodegradable, usually organic, materials are mixed with water and nutrients to enhance biological transformations. The organic material can be contaminant free, and PAH dissipation occurs coincidentally, or the organic material may be contaminated with the target PAHs. In examining strategies for biochar composting, dissipation of PAHs was measured concurrently, and decreases in PAH concentrations after 180 days were small (3–21%), possibly due to limiting nitrogen concentrations [16]. Oil sludge with > 500 mg total PAH kg^{-1} was composted with three animal manures for 10 months; total PAHs decreased 77–99% [17]. Adding ammonium persulfate, a chemical oxidant, prior to composting significantly increased the efficiency of PAH removal [18]. Using cattle manure and wheat straw for the composting of PAH-contaminated MGP soil, known to be resistant to treatments, did decrease PAH concentrations [19]. Composting of crude oil sludge was found to be highly effective for high molecular weight PAHs (nearly 100% dissipation) but somewhat less efficient for low molecular weight compounds [20]. The authors also characterized genes of certain fungi and bacteria associated with PAH degradation. Contaminated, rural sewage sludge was composted with sawdust, reducing the total PAH concentrations by 94% after 30 days [21].

Soil-Slurry Bioreactors

The soil-slurry bioreactor approach to bioremediation is ex situ and not directly transferable to large-scale remediation projects. Valuable information is provided concerning mechanisms and interactions among many variables. Five coal-tar-contaminated soils were treated in a simple slurry bioreactor for 35 days, after which 80–90% of the total PAHs had degraded [22]. Combinations of algae (*Chlorella* spp.) and *Rhodococcus wratislaviensis* in slurries of aged, PAH-contaminated soil degraded up to 75% benzo[*a*]pyrene, 80% pyrene, and nearly 100% phenanthrene in 30 days [23]. Two bacterial strains (*Rhodocccus erythropolis* and *Pseudomonas stuzeri*) were added to soil slurry [24]. After 15 days, total PAHs declined from 330 to < 75 mg kg^{-1}. To evaluate only physical processes of removal in spiked-soil slurries, mercury was added to the reactors and monitored [25]. After 8 h, removal of the PAHs varied from 0.5 to 82% and was strongly correlated with PAH vapor pressure. Slurries were bioaugmented with a consortium of indigenous bacteria, resulting in some enhancement during a 410-day experiment on coal-tar-contaminated soil [26].

Phytoremediation

Phytoremediation of PAHs in soils is the use of higher plants to enhance biodegradation by rhizosphere microorganisms. Unlike phytoremediation of other organic contaminants (such as pesticides or chlorinated solvents), PAH uptake by higher plants is minimal, and degradation of PAHs within the plants is not an important pathway of PAH bioremediation [27]. Early phytoremediation studies aimed to optimize nutrient additions and to screen for plant species best suited for degradation. Hundreds of studies were published between 2018 and 2023 with recent advances combining phytoremediation and other bioremediation approaches (e.g., bioaugmentation), studying PAHs in metal co-contaminated soils, while others are continuing the "traditional" approach of testing individual plant species. Several publications investigated the use of a mix of *Festuca* L. species [28, 29] with decreases in total PAH concentration of 75%; a mixture of *Medicago sativa* and *Bromus inermis* [30] decreased individual PAHs from 13 to 61%; and the single species (*Cynodon dactylon*; [31]) showed a 56% decrease in total PAHs. Co-contamination with heavy metals is thought to complicate the phytoremediation process due to the potential toxicity of the metals to plants and microorganisms, and recent research focused on co-contamination with cadmium [31, 32] or multiple metals [33, 34]. PAH removal exceeded 50% when nitrogen was added to stimulate plant growth [32] or when a sequence of remedial steps was taken [33]. Although nutrient addition is a routine aspect of phytoremediation, other amendments also can be useful. The combination of starch with a *Fusarium* species significantly enhanced PAH degradation beyond 50% for all compounds [30]. Bioaugmentation in conjunction with phytoremediation has attempted to take advantage of the ideal rhizosphere conditions for added microbes. *Mycobacterium* species were identified in the past as having excellent PAH degradation potential and were recently examined further [35, 36]. Phytoremediation in the presence or absence of *Mycobacteria* increased PAH degradation (52% total removal) compared to the unvegetated control (41%), and the contribution of the *Mycobacteria* was not as great as that of phytoremediation. In addition to monitoring the dissipation of PAHs, bacterial community structure was examined and identified the most important degraders [28, 34, 37]. Trends in degradation of PAHs in response to phytoremediation do not follow any particular trend in the selected studies. The lowest degradation (42%) was seen in a 60-day treatment of agricultural soil contaminated by irrigation with wastewater [37], whereas the highest degradation was observed for some of the more recalcitrant matrices: steel mill waste (90%, [34]) and petroleum-associated waste (> 75%, [28, 29, 36]). The duration of the experiment was not correlated with the percentage of the PAHs degraded.

Bioaugmentation

Degradation can be enhanced by the addition of specific microorganisms, combinations of two or three specific species, or adding an entire consortium of microorganisms, and this process is called bioaugmentation. The concept of bioaugmentation for PAH degradation has existed for decades, and new microbes are frequently tested. Bioaugmentation by wood-degrading fungi is one of the most studied areas and will be given its own section in this review. In recent years, several studies examined PAH degradation through bioaugmentation with individual bacterial species including the *Burkholderia cepacian* complex [38], *Paracoccus* sp. [39, 40]. Three species were introduced simultaneously (*Bacillus* spp., *Pseudomonas* sp., *Acinetobacter* sp.) [41]. These studies were generally successful in removing PAHs but often required additional amendments, and overall biodiversity decreased in the amended soils. Soil-borne, saprotrophic fungi were amended to PAH-contaminated soil, and up to 44% degradation of benzo[*a*]pyrene was observed after 9 days [42]. Some researchers recognized the benefits of adding a consortium of microorganisms, either specifically cultured (methylotrophic, [43]; PAH-degrading isolates, [44]) or indigenous [45]. The approach to introducing exogenous degraders into aged, contaminated soil was modified to overcome initial problems of ecological adaptation [45], resulting in increased bioremediation efficiency (over 50% PAH degradation vs. 10% by biostimulated control). The approach was generally successful, but the degree of degradation was dependent upon the specific PAH in question. Degradation of PAHs in response to bioaugmentation does not appear to be related to the contaminant matrix: the smallest rate of degradation (25%; [42]) and highest rate (99%, [44]) were observed in MGP soils, and a modest rate of degradation (56%, [39]) was found in a contaminated agricultural site.

Wood Decaying Fungi

Wood decaying fungi have captured the interest of the bioremediation field for decades. "White rot" fungi were the first group of these fungi studied in depth, but the research has expanded to include all fungal genera involved in the degradation of wood. Some of the advances have combined the fungi with other approaches. (Note that nearly all the studies using wood decaying fungi used soil that was contaminated in the laboratory.) Laboratory-contaminated soils amended with 9 to 17% crude oil were inoculated with the bacterium, *Ochrobactrum intermedium*, and the wood decaying fungus, *Pleurotus ostreatus* [46]. The greatest removal of PAHs (84%) occurred when the organisms were added together, but they were also effective when added individually (70% removal). *Pleurotus dryinus* was used as an inoculant in a study that found that high concentrations of soil organic matter had little impact on PAH degradation in laboratory-spiked soils [47]. Degradation of phenanthrene was nearly 100% in all treatments, and benzo[*a*]pyrene degradation was approximately 50% even in the presence of 12% soil organic matter. Additions of *Crucibulum leave* in combination with phytoremediation were compared to phytoremediation alone, and the combined treatments were generally more effective in removing PAHs from laboratory-contaminated soil [48]. A 180-day pot experiment contrasted various combinations of *Crucibulum leave* and phytoremediation against natural attenuation in a field-contaminated soil and found that the phytoremediation + bioaugmentation treatments were the most effective [7]. In this study, natural attenuation degraded 42% of the total PAHs, phytoremediation 45%, and wood decaying fungi 39%. The fungi were most effective for the higher-ring PAHs.

Surfactants

Enhanced degradation of highly sorbed compounds by some microorganisms is achieved by increasing the lability of the contaminants through the exudation of surface-active agents. The impact of surfactants on PAH bioremediation has been studied by adding surfactant compounds, bioaugmentation with organisms known to exude surfactants, or biostimulation to optimize surfactant production. Surfactants have been combined with other forms of bioremediation. Fundamental aspects of surfactant production, critical micelle concentrations, and solubilization were quantified to help optimize the process [49]. In the presence mono-rhamnolipids, 20% of the PAHs were desorbed, and a total of 80% had dissipated after 360 h. Addition of rhamnolipids was investigated in untreated soil with high levels of PAH contamination (4370 mg kg^{-1}) from creosote and in soils that had been previously bioremediated [50]. Dissipation of the PAHs in the soils was high for total PAHs (> 85%) and pyrene (> 87%) but smaller for benzo[*a*]pyrene (mean of 58%). The efficacy of bioaugmentation (*Arthrobacter globiformis*) was compared to no treatment and enhancement with two levels each of a synthetic surfactant (Tween 80) and a rhamnolipid [51]. After 150 days, the control (absence of all additions) was the least effective (28%), and all surfactant treatments enhanced rates of PAH dissipation (36% for rhamnolipid; 30% for *A. globiformis* alone; 29% for Tween 80). Rhamnolipid additions were combined with phytoremediation and solarization [52] or biostimulation with lipids [53]. The combination of solarization + phytoremediation + biosurfactant was the most effective treatment with 95% PAH reduction [52]. Treatment of a contaminated smelting plant soil with phytoremediation, lipids, and ryegrass removed 40% of the total PAHs [53].

Laccase Enzymes

Laccase enzymes are naturally occurring in bacteria, fungi, and higher plants and are capable of oxidizing compounds containing phenolic substrates. Their apparent involvement in the degradation of PAHs has led to many investigations. To be effective, laccase systems must attack compounds with lower redox potential than the laccase, although mediator compounds can be introduced that enhance the oxidative potential of the enzyme. Dodor [54] optimized the laccase system for oxidation of several PAHs using two mediator compounds in combination with the laccase stabilized on kaolinite or stabilized on soil. Some PAHs interfered with the oxidation of others, and efficiency was lost as the system became more complex, but degradation efficiency ranged from 25% for benzo[*a*]anthracene and pyrene to nearly 100% for anthracene and benzo[*a*]pyrene. Degradation of pyrene (90%) and benzo[*a*]pyrene (58%) in laboratory-contaminated soil increased with immobilization of laccase, and 40 °C and pH 4 were the optimal conditions for degradation [55]. Similarly, optimal degradation was found to be between pH 5 and 7, and the mediator ABTS significantly increased PAH degradation (85% for anthracene, 70% for benzo[*a*]pyrene) [56]. Others have examined specific stabilizers, including an Fe-based compound [57] and calcium alginate beads [58]. Ferulic acid and ABTS mediators were found to be superior to coumaric acid in decreasing PAH concentrations in a field-contaminated soil to 13% of the original concentrations [59]. Two alternative laccase mediators were evaluated and found to interfere to some degree with the oxidation of PAHs in field-contaminated soils [60].

Exudates and Extracellular Enzymes

Exudates from soil microorganisms and higher plant roots are frequently involved in bioremediation of PAHs. In addition to the laccases reviewed in the previous section, other enzymes and water-soluble compounds have been identified as directly contributing to PAH degradation. The research cited in this section generally dealt with identifying exudates and enzymes rather than quantifying PAH degradation, and none of the studies used field-contaminated soil. Phytoalexins present in the apoplast of higher plants have the potential to interact with endophytic bacteria and accelerate PAH degradation. At relatively low concentrations, three phytoalexins increased removal of phenanthrene and anthracene to 50% by *Methylobacterium extorquens* [61]. Phytoremediation with *Zea maize* and mycoremediation with *Pleurotus ostreatus* resulted in increased degradation of PAHs and increased concentrations of several extracellular enzymes [62]. Remediation of PAH-contaminated soil with *Pseudomonas brassicacearum* decreased PAH concentrations in the soil, and an enzyme participating in the degradation was identified [63]. After degradation of PAHs, four *Bacillus* species were identified and found to be highly in efficient in bioremediation [64]; catechol 1,2 dioxygenase was isolated and deemed to be an important contributor to the process. Contamination of soil with coal tar increased the activities of several oxidoreductive enzymes [65]. During rhizoremediation of petroleum hydrocarbons by *Vigna unguiculata* and *Vigna radiata*, the composition of root exudates was measured as were the enzyme activities of the microbes *Micrococcus luteus* and *Bacillus cereus* [66]. A multifaceted study examined the degradation of PAHs during phytoremediation with *Salix viminalis* with or without three species of mycorrhizae; degradation ranged from 84 to 80% after 90 days [67]. Bioremediation increased in the presence of the *Salix viminalis* and increased further when mycorrhizae were present; organic acid exudates and various enzymes (including laccase) were quantified.

Changes in Microbial Communities During Bioremediation

All facets of bioremediation attempt to optimize the biological breakdown of PAHs, and a shifting microbial population is a logical consequence. Classic culture techniques, phospholipid fatty acids analysis (PLFA), genetic sequencing, and the identification of specific PAH degrader genes have been used to monitor population dynamics. Initial contamination often dramatically changes microbial populations, and further changes are observed during remediation, the magnitudes of which are dependent upon the technologies employed and subsequent land use [68]. Based upon enzymatic activities, PLFA analyses, and microbial mass, the impact of PAH contamination is strongly dependent upon the environment prior to contamination [69]. In creosote-contaminated soil containing PAHs highly resistant to degradation, bacterial strains with higher capabilities for PAH bioremediation were enhanced [70]. In laboratory-contaminated soils evaluated for bacterial diversity and metabolism using metagenomics, no correlation was observed between changes in high molecular weight PAH concentrations and specific microbial taxa, but degradation of low molecular weight PAHs appeared to be related to *Betaproteobacteria* [71]. Additions of rhamnolipid surfactants altered the community composition through selection for certain species [72]. Mixed surfactants were added to contaminated soils and enhanced biodegradation of PAHs in the first 20 days of the experiment. Microbial population parameters were analyzed in detail, but clear patterns of impacts on specific genera did not emerge [73, 74]. In another surfactant study, addition of the surfactant to the critical micelle concentration lowered microbial diversity, partially due to enhanced populations of surfactant-degrading species [75]. Although additions of rhamnolipid enhanced PAH biodegradation by increasing degrading species, the microbial richness was diminished [76]. Assessment of the bacterial community and genes responsible for PAH dissipation in polluted soils found *Actinobacteria*, *Betaproteobacteria*, and *Chloroflexi* to be the most important [77]. Analysis of a field-contaminated soil identified bacterial strains that produce biosurfactants: *Bacillus* spp., *Priesta* spp., *Pseudomonas* spp., *Enterobacter* spp., and *Kosakonia* spp. [78].

Specific Genes and Genetic Analyses

Increased understanding of the microbial species and enzymes responsible for PAH degradation has been coupled with the quest to better predict and optimize bioremediation [79]. Various forms of genetic analysis were used by researchers cited in previous sections, particularly in monitoring shifts in microbial populations during contamination and subsequent bioremediation. The encoding genes of PAH-degrading enzymes were examined in fungal isolates [80, 81]. Genomic and transcriptomic approaches to fungal bioremediation revealed an array of inducible genes specific to degradation of PAHs [82]. Amplicon-based metagenomics were applied to a PAH-contaminated site and identified *Geobacter* spp., *Mycobacterium*, and *Sphingomonas* spp. as key components of bioremediation [83]. Six pyrene degraders were identified using DNA stable isotope probing [84]. Functional genes were identified for several PAH-degrading enzymes, including naphthalene dioxygenase (nahAc) [85–90], PAH hydratase-aldolase (pahE) [85, 86, 88], and catechol dioxygenase (xylE) [85, 88, 91].

Bioremediation by Fauna

Bioremediation is generally viewed as involving microorganisms (e.g., bacteria or fungi) or higher plants, but soil-borne animals also can contribute. In petroleum-contaminated soils spiked with up to 30,000 gm kg^{-1} total petroleum hydrocarbons (TPH), earthworms *Eisenia fetida* reduced TPH content by 50% after 200 days compared to 25% in soils without worms [92]. The accumulation of contaminant hydrocarbons in the earthworms in the contaminated soils was indistinguishable from worms clean soils. In aged, field-contaminated soils, two species of worms (*Porcellio scaber* and *Lumbricus terrestris*) were evaluated for their ability to survive in contaminated soils and to decrease contaminant concentrations [93]. The dissipation of contaminants was limited (approximately 20% after 28 days), and many of the contaminants proved to be toxic to the worms. Worms also have been used in conjunction with phytoremediation [94] and phytoremediation plus bioaugmentation [95]. A laboratory-contaminated soil had greater degradation of a single contaminant (anthracene) in the presence of plants and *Eisenia fetida* than in the absence of worms [94]. In an aged, field-contaminated soil, all remediation approaches were at least somewhat effective, but trends of the effects combinations of treatments were not clear [95]. Contaminant degradation averaged approximately 65% removal for total PAHs and individual compounds. The introduction of mussels to a constructed wetland improved removal of PAH contaminants [96].

Bioremediation of PAHs in Anoxic Environments

Degradation of PAHs is more rapid and more likely to reach complete mineralization under aerobic conditions. However, PAHs can be found as contaminants in anaerobic conditions, and increasing degradation efficiency in the absence of oxygen is an ongoing pursuit. In controlled conditions, a facultative anaerobe (PheF2, a strain of *Trichococcus alkaliphilus*) was able to nearly eliminate phenanthrene in anaerobic conditions using Fe^{3+} as the electron acceptor; the kinetics of degradation were approximately one-half those in aerobic conditions [97]. A field-contaminated soil was incubated in a glovebox in an anoxic atmosphere, and nitrate was used as the electron acceptor [98]. Additions of nitrate enhanced dissipation of 3- and 4-ring PAHs but had little impact on 5- and 6-ring compounds after approximately 1 year. Laboratory-contaminated soils were bioaugmented under anaerobic conditions, and naphthalene (85%) and benzo[*a*]pyrene (52%) were significantly degraded [99]. *Bacillus firmus* was isolated and used as a bioaugmentation source for laboratory-contaminated soil [100]. The additions of nitrate alone or nitrate in combination with *Bacillus firmus* dramatically reduced PAH concentrations over 56 days.

Summary and Conclusions

Bioremediation of PAHs in soils has progressed steadily since 2018. The more traditional areas (e.g., bioaugmentation, phytoremediation) have expanded into finding new species of remediators, and the identifying pathways of degradation continue to be a focus. Laccase has emerged as an important extracellular enzyme, an extension of the research using wood decaying fungi. An entire book was dedicated to reporting progress on the use of wood

Table 2 Results of selected studies to compare bioremediation rates

Technology	Soil contaminant	Duration (days)	% Degradation				Reference
			ΣPAHs	3-ring	4-ring	5-ring	
Natural attenuation	Manufactured gas plant	105	86	85	71	72	10
	Sediment	8125	80	81	72	72	8
Biostimulation	Coke plant	180	43	45	29	41	14
	Sewage sludge	126	60	60	55	50	11
Composting	Manufactured gas plant	56	0	0	0	0	19
	Sewage sludge	30	94	73	100	60	21
Bioslurry	Coke plant	210	93	80	80	78	26
	Manufactured gas plant	35	84	nd	nd	nd	22
Phytoremediation	Oil field	150	80	75	80	73	29
	Agricultural wastewater	60	42	42	50	40	37
Bioaugmentation	Manufactured gas plant	30	25	30	40	22	42
	Agricultural contaminated	84	56	63	63	28	39
Wood decay fungi	Aged agricultural	180	39	37	39	43	7
	Laboratory spike	110	84	79	95	100	46
Surfactant	Wood treatment plant	20	85	86	86	35	50
	Agricultural contaminated	150	36	28	19	10	51
Lacasse enzymes	Petroleum waste	12	54	58	53	30	59
	Dredged sediments	40	nd	65	60	80	57
Vermiculture	Petrol waste	112	68	63	67	70	95
	Laboratory spiked diesel fuel	28	20 (total petroleum hydrocarbons only)				93

decaying fungi with a chapter dedicated to PAH degradation [101]. Bioaugmentation studies have recognized the vulnerability of isolated, non-indigenous species of bacteria and fungi in the harsh soil environment and have been tending toward using species naturally found in these soils with a greater probability of survival.

Combining multiple bioremediation approaches produced excellent success. Phytoremediation paired with bioaugmentation or adding surfactants enhanced degradation even for highly contaminated sites with difficult residues (e.g., coal tar or creosote). Using mediator compounds with laccase enzymes improves efficiency of decomposition, and alternative mediators have been tested. Examining the response of soil microbial communities to contamination and subsequent remediation lends insights into the potential for full ecological recovery.

In this review, the contaminant matrix was frequently included in the summary of published studies because PAHs in soils are rarely found alone or in the absence of a contaminant matrix (airborne deposition being an exception), and the nature of the matrix is critical in dictating the efficacy of bioremediation. Creosote and coal tar, for example, are particularly challenging because the PAHs are embedded in a matrix that is aged and resistant to degradation. When PAHs are applied to soil in the absence of a matrix, such as dissolving in water or acetone and spraying on the soil, the PAHs are highly labile and readily susceptible to degradation. For this reason, studies using laboratory-contaminated soils were avoided when possible in this review.

To compare the efficacy of the various bioremediation techniques, two representative studies from each approach were compiled (Table 2). For each study in Table 2, the source of contamination, the duration of the study, and the amount of PAH degradation are listed. At least one of the studies in each approach used soil with a highly recalcitrant matrix: MGP residue, coke plant waste from coal or steel industries, or wood preservatives. The second study in each method was generally from a site with lower contaminant concentrations and/or a less resistant matrix. For example, diesel fuel spill sites in agricultural soils tend to be easily remediated.

An examination of Table 2 does not reveal any obvious trends that would separate one method as being superior or inferior compared to others. The removal of total PAHs ranges from 25% degraded in an MGP site by augmentation to 94% removal by composting of contaminated sewage sludge. However, the lowest removal rate (0%) also was observed in composting.

Bioremediation continues to be one of the most promising technologies for remediating PAH-contaminated soils. The balance between moderate expense and high efficiency while preserving the soil resource combine to create an attractive cleanup alternative.

Author Contribution Schwab was responsible for all aspects of preparing this manuscript.

Data Availability All relevant data are included in the paper.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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