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REVIEW ARTICLE

Petroleum-contaminated soil: environmental occurrence and remediation strategies

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Abstract
Soil is an environmental matrix that carries life for all living things. With the rise of human activities and the acceleration of population, the soil has been exposed in part to pollution by the discharge of various xenobiotics and persistent pollutants into it. The disposal of toxic substances such as polycyclic aromatic hydrocarbons (PAHs) alters soil properties, affects microbial biodiversity, and damages objects. Considering the mutagenicity, carcinogenicity, and toxicity of petroleum hydrocarbons, the restoration and clean-up of PAH-polluted sites represents an important technological and environmental challenge for sustainable growth and development. Though several treatment methods to remediate PAH-polluted soils exist, interesting bacteria, fungi, and their enzymes receive considerable attention. The aim of the present review is to discuss PAHs' impact on soil properties. Also, this review illustrates physicochemical and biological remediation strategies for treating PAH-contaminated soil. The degradation pathways and contributing factors of microbial PAH-degradation are elucidated. This review also assesses the use of conventional microbial remediation compared to the application of genetically engineered microorganisms (GEM) that can provide a cost-effective and eco-friendly PAH-bioremediation strategy.

Keywords PAH degradation · Soil treatment · Remediation · Fungi

Introduction

Due to the dynamic increase in industrialization, urbanization, and the increasing demand for energy, pollution with persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), poses a serious threat to all forms of aquatic and terrestrial life (Mojiri et al. 2019).

Petroleum hydrocarbons (PHs) contain hazardous chemicals such as benzene, toluene, ethylbenzene, xylene, and naphthalene, which can be harmful to all parts of the ecosystem, especially the land (Sarkar et al. 2005; Liu et al. 2017; Kuppusamy et al. 2017).

PAHs exhibit persistence in soils; their solubility and biodegradability decrease as their molecular weight and the number of benzene structures increase, making them more toxic (Meador 2008). The US Environmental Protection Agency (USEPA) has classified PAHs as priority pollutants due to their persistence, high toxicity, mutagenicity/carcinogenicity, and teratogenicity for human beings (Rengarajan et al. 2015; Polidoro et al. 2017).

Oil spillage is a serious threat to all parts of the ecosystem (Sarkar et al. 2005). During extraction, transportation, storage, and distribution operations, crude oil and its refined products are frequently spilt, causing soil pollution (Macaulay and Rees 2014).

Soils contaminated with POPs associated with petroleum, such as PAHs, present high potential health risks because of their ability to enter the food chain and their affinity for accumulation in living organisms (Bastami et al. 2013; Honda and Suzuki 2020).

Generally, PAHs can be found in high concentrations in contaminated soil, indicating a potential environmental hazard. PHs alter soil biological properties, affecting microbial diversity and enzymatic activities as well as its physicochemical characteristics (Czarny et al. 2020; Dos-Santos and Maranho 2018).

Therefore, soil contamination emphasizes the need for effective environmental remediation and restoration strategies to preserve the ecosystem. Generally, soil petroleum contamination can be remediated with physical, chemical, thermal, and biological approaches (Kuppusamy et al. 2017).

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The first two methods have limitations such as high costs, inefficacy, and altering the natural ecosystem (Verma and Haritash 2019). As an alternative, bioremediation offers an eco-friendly process for the removal or reduction of petroleum pollutants in environments using selective microbial flora (Patel et al. 2020).

In this context, mycoremediation, which is fungal-based remediation, is a promising technique for the clean-up of contaminated soil (Kumar and Gopal 2015; Li et al. 2020).

Ligninolytic fungi and their enzymatic oxidative system (especially laccases and peroxidase) have proven their potential in the remediation of several complex aromatic pollutants such as dyes, PAHs and aromatic compounds. For instance, white-rot fungi (WRF) are among the most studied species for their potential to degrade a wide range of xenobiotic compounds, such as PAHs (Daâssi et al. 2021). Moreover, fungi isolated from PAH-contaminated soil can reduce petroleum pollution (Das and Chandran 2010).

Thus, the main purpose of this review is to demonstrate the physicochemical and biological remediation strategies for treating PAH-contaminated soil. The degradation pathways and contributing factors of the microbial PAHs-degradation will also be elucidated.

The impact of PAHs contamination on the soil properties

Petroleum-contaminated soil contains several types of hydrocarbon, including aliphatic (straight-chain) and aromatic (cyclic) structures that may change soil properties, such as texture, moisture, conductivity, total organic carbon, etc. Moreover, halogenated hydrocarbons with nonorganic elements like fluoride, bromide, iodide, or chloride (e.g., carbon tetrachloride), are frequently reported as environmentally persistent, toxic, and hazardous soil pollutants (Klamerus-Iwan et al. 2015; Kuppusamy et al. 2017).

Soil matrix properties and functions are closely related to the different activities occurring in the soil and to xenobiotic structures, like PAHs, associated with petroleum. PAHs' chemical stability, hydrophobicity, and resistance to microbial degradation mean that spilt oil may damage the biological and physicochemical properties of the soil it pollutes.

Physicochemical properties

Petroleum is considered the major source of PAHs that may be absorbed by soil particles due to their high hydrophobicity and thus replace water molecules, reducing the oxygen and water infiltration in the petroleum-polluted soil (Sakshi and Haritash 2019). According to Terytze et al. (1995), PAHs are characterized by a strong sorption affinity to soil organic matter.

Further, soil geotechnical characteristics may be affected by hydrocarbon contamination, such as permeability, hydraulic conductivity, and compaction, as well as the biological properties (biomass and enzymes) of the soil matrix (Zahermand et al. 2020).

Petroleum polluted areas are characterized by a lower self-purification capacity that reduces the indigenous microbes involved in soil purification processes (Hreniuc et al. 2015).

Biological properties

The presence of hydrocarbons in the soil affects its biological properties and impoverishes microbial diversity (Labud et al. 2007). The persistence and the toxicity of some PAH compounds may inhibit soil microbial communities. Alrumman et al. (2015) reported that oil contamination in the soil matrix influences soil enzymatic activities and microbial biomass carbon, and so biological functions.

Also, certain essential soil functions may be lost due to the high toxicity of such persistent aromatic hydrocarbon structures (Khomarbaghi et al. 2019). Furthermore, spilt oil may instigate anaerobic conditions and asphyxia in soil pores, with their consequent impacts on microbial activities (Sutton et al. 2013).

Petroleum-contaminated areas suffer tremendously from the drastic impact of the toxicity as well as the concentration induced by PAHs' high molecular weight. Klamerus-Iwan et al. (2015) demonstrated a significant decline of microbial biomass and enzymatic activities (urease and dehydrogenase) in soil polluted by chainsaw oil.

Remediation strategies of PAH-contaminated soils

Remediation of PAH-contaminated soils is a global issue that poses risks to public health. The reclamation or remediation of petroleum-polluted soils is important to remove pollutants from the environment and it can be done with several methods involving the removal, isolation, or alteration of the contaminant. Today, there are several techniques for soil reclamation, including physical, chemical, thermal, and biological remediation methods (ex-situ and in situ) (Table 1).

In-situ remediation occurs at the contaminated site and offers several advantages. It involves lower risk, lower cost, and limited human involvement, and the environmental surroundings can help in the remediation process to transform the contaminants.

Table 1 Remediation strategies for petroleum-contaminated soil

	References
Remediation strategies for Petroleum contaminated soil	
Bioremediation	
Phytoremediation	Lu et al. (2019)
Rhizoremediation	Rostami et al. (2021)
Biostimulation	Wu et al. (2016)
Bioaugmentation	Patel et al. (2020)
Microbial electrochemical system	Hao et al. (2020)
Chemical remediation	
Plasmas oxidation	Liu et al. (2019)
Flotation	Yen et al. (2011)
Physical-remediation	
Ultrasonication	Copik et al. (2021)
Electro Kinetics	Pourfadakari et al. (2021)
Vapor extraction	Cao et al. (2021)
Thermal desorption	Wei et al. (2022)
Biochar adsorption	Bianco et al. (2021)

Alternatively, ex-situ remediation processes occur off-site and the contaminant is transferred to another location for treatment. It is costly but occurs under a control system, with more human involvement and direct exposure to contaminants.

There are regulatory constraints for ex-situ remediation. Some of these remediation techniques are solvent extraction, UV oxidation, photochemical or photocatalytic degradation, bioremediation, and phytoremediation. The selection of suitable remediation techniques for petroleum-polluted soils depends on many factors such as the type and structure of contaminant, the future use of contaminated soil, the soil type and properties, the budget, etc.

Chemical treatments

Chemical oxidation reactions have been widely used to degrade oil or PAH-contaminated soils by the addition of oxidants to the soil to oxidize contaminants (Tsai and Kao 2009; Rivas 2006).

The chemical method of treating polluted soil involves oxidizing agents such as ozone (Shin et al. 2005; Yu et al. 2007); Fenton's reagent uses hydrogen peroxide as an oxidant (Flotron et al. 2005). Alternative oxidants like persulfate/Fe(II), peroxymonosulfate (PMS), persulfate, H_2O_2, and permanganate can also be used for the chemical oxidation treatment of diesel and fuel oil (Do et al. 2009; Yen et al. 2011). The chemical treatment converts hazardous contaminants into less toxic or non-hazardous compounds (Verma and Haritash 2019). Furthermore, the combined microbial and chemical treatment of PAHs can be a cost-effective strategy for further application to contaminated sites.

Liao et al. (2018) evaluated chemical oxidation combined with microbial remediation for treating PAH-contaminated soil. Their results demonstrated a complementary improvement in the impact of microbial PAH degradation yields after chemical oxidant peroxidation.

Biological treatments (bioremediation)

Biological treatment is based on the use of living organisms and their derivatives. The effectiveness of bioremediation is often a function of the microbial population or consortium and how it can be enriched and maintained in an environment (Babu et al. 2019).

Generally, the biological method employs the natural potential of microbes, including bacteria and yeasts (bioremediation), algae (phyco-remediation), plants (phytoremediation), or fungi (mycoremediation) to biodegrade PH pollutants.

Bioremediation strategies for petroleum-contaminated soil

Natural attenuation strategy

The simplest bioremediation strategy is natural attenuation. This control method requires only the natural degradation processes occurring through the native microbial population. However, this approach is not always successful and requires extensive long-term monitoring (Table 2). It can be used to restore areas with low contamination levels (Pilon-Smits 2005). Approximately 25% of all petroleum-contaminated land has been remediated with natural attenuation (Stroud et al. 2007). Guarino et al. (2017) reported that PAH-contaminated soil reached a 57% reduction of the total petroleum hydrocarbons (TPH) through natural attenuation. Similarly, Erkelens et al. (2012) reported that previously remediated hydrocarbon-contaminated soil showed a 70% increase in the remediation of trinitrotoluene (TNT) compared with the control.

Bioaugmentation

Bioaugmentation is the enhancement of the intrinsic microbial population at the contaminated site by supplementing microbes to degrade the pollutants (indigenous or exogenous microorganisms). This approach is often used to handle high concentrations of spilt oil, when natural degrading microbes are absent or insufficient (Crawford 2006).

Table 2 Bioremediation technologies used for hydrocarbon-contaminated environments

Technology	Key point	Advantages	Disadvantages
Natural attenuation	Using indigenous microorganisms and natural conditions	Cheapest technology	Requires extensive long-term monitoring not always successful
Bioaugmentation	Addition of hydrocarbon degrading-microorganisms	Using high biomass of PAHs-degrading microbes	Changes the natural microbial structure, poor adaptation of bioaugmented-biomass to the contaminated site
Biostimulation	Addition of nutrient	More efficient than natural attenuation	Not always successful
Phytoremediation	Using plants and their associated microorganisms	Supports hydrocarbon- native microorganism within plant root	Toxicity of pollutants to the plant

Hydrocarbon compounds can delay or inhibit microbial proliferation and activities, so for effective in-situ biodegradation, bioaugmentation is important (Purohit et al. 2018).

The use of native microorganisms guarantees that the organisms have a higher tolerance to the toxicity of aromatic hydrocarbons and are resistant to the local environmental variations (Kumar and Gopal 2015; Ezekoye et al. 2018). Exogenous microbes are useful to address more complex hydrocarbon structures whose rates of intrinsic biodegradation will be slower than the degradation of simple hydrocarbons.

Therefore, bioaugmentation approaches are necessary to enhance the performance of the indigenous microbial populations severalfold through introducing microbes with specific metabolic activities for effective in-situ remediation of polluted areas (Kiamarsi et al. 2020).

Conventionally, pollutant bioavailability, the survival of microorganisms, and their enzymatic catabolic activities are important for bioaugmentation (Heinaru et al. 2005) (Fig.1).

For instance, bioaugmentation with the soil-isolated fungi *Penicillium funiculus* and *Aspergillus sydowii* strains in hydrocarbon-polluted soil resulted in a reported 16% increase of the TPH compared to the treatment conducted with bioaugmentation treatment without the addition of fungi (Mancera-López et al. 2008).

Meanwhile, few reports on bioaugmentation with mixed fungal–bacterial systems exist (Ellegaard-Jensen et al. 2014). Other recent studies have reported the use of living mono-fungus or mixed-fungal cultures (Ezekoye et al. 2018), fungal-bacterial consortia (Ellegaard-Jensen et al. 2014), or fungal-yeast mixed cultures (Fedorak et al. 2011) that could enhance biodegradation efficiency, especially on high concentrations of oil. Additionally, bioremediation of PAHs with high molecular weights usually requires the cooperation of more than a single species to metabolize a broad range of hydrocarbon substrates (Shabir et al. 2008). Therefore, assemblages of associated species with a variety of overall metabolic activities are required to enhance the rate of PH degradation (Zhong et al. 2007).

Atlas and Cerniglia (1995), suggested that although fungi can metabolize some hydrocarbons, they do not have the enzymes required to transform co-oxidation products. This removal value can be increased up to two-fold with biostimulation treatment, but the PAH removal rate was 16, 7, and 8 times higher with bioaugmentation treatments using *Rhizopus* sp., *P. funiculosum,* and *A. sydowii*, respectively.

Yet, although some studies showed effective degradation in the initial phase of bioaugmentation treatment, slow rates were observed over time, probably due to the organisms' competitiveness (Sabaté et al. 2004) and nutrient depletion (Table 2).

Biostimulation

Biostimulation is one of the adapted remediation techniques for increasing the efficacy of the bioremediation of crude oil or PAHs in contaminated areas (Garon et al. 2004).

This approach consists of stimulating the growth and activities of the intrinsic microbial population in the crude oil-contaminated site by adding amendments of nutrients (nitrogen, phosphorous, carbon, and organic biostimulants), and oxygen (as an electron acceptor).

According to Breedveld and Sparrevik (2000), the addition of inorganic nitrogen and phosphorous stimulated microbial growth and improved the PAH degradation efficiency in the creosote-contaminated soil of wood preserving plants in Norway.

For instance, amending crude oil-contaminated soil with nutrients (nitrogen, phosphorus, and potassium) considerably improved the biodegradation efficiency, with 62% removal of hydrocarbons compared to unamended contaminated soil where a 47% removal rate was recorded (Chaineau et al. 2005).

Trichoderma asperellum H15 was reported to degrade PAHs in contaminated soil treated with a sugarcane bagasse biostimulation treatment. After 14 days, *T. asperellum H15* had adapted to PAH-contaminated soils in microcosms and achieved up to 78% phenanthrene degradation in soils contaminated with 1000 mg Kg^{-1} (Zafra et al. 2015).

In addition, some researchers reported that the effectiveness of mycoremediation may be stimulated by generating an optimal balance of physical factors, such as aeration,

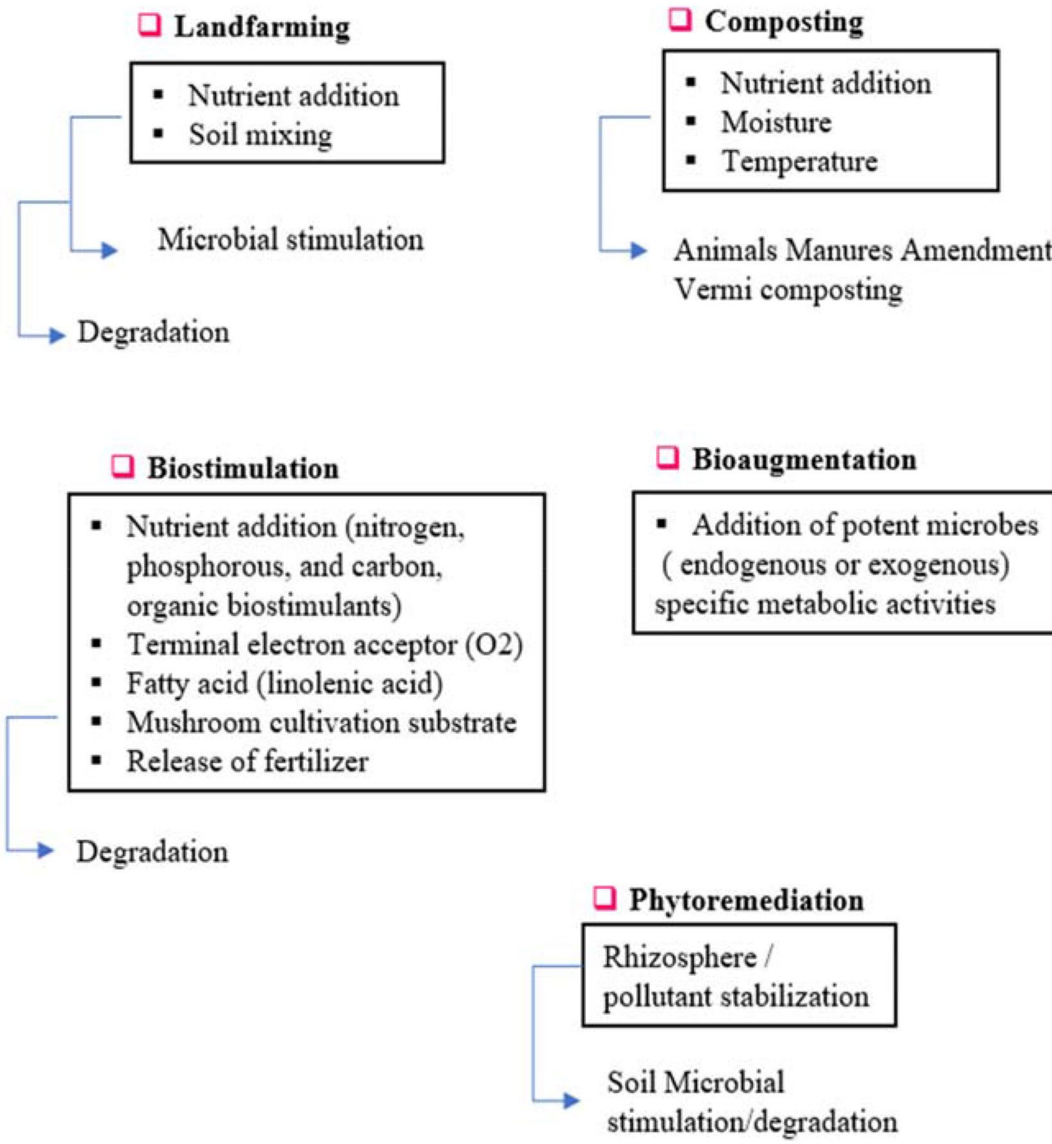

Fig. 1 Application of biological techniques for PAHs remediation in soil

temperature, and buffering environmental pH by altering the redox and electro-kinetic states of contaminated samples (Tongarun et al. 2008).

Many studies showed the influence of the combined biostimulation-bioaugmentation approach on the efficiency of remediating PAH-contaminated soil.

Biostimulation is more effectively used in combination with bioaugmentation methods (Tyagi et al. 2011). The kinetic efficiency of biostimulation was found to be relatively slow compared to the bioaugmentation process (Wu et al. 2016).

The biodegradation processes that occur during bioremediation are affected by several factors including bioavailability, hydrocarbon structures and natures, environmental conditions, and the presence of hydrocarbon-degrading microorganisms (Stroud et al. 2007). These factors and their effects on the bioremediation of hydrocarbon-contaminated areas are summarized in (Fig.2)

Factors affecting PAH-degradation

Biotic factors include the potential and metabolic activities of petroleum-degrading microorganisms in the environment, species abundance, diversity, competitiveness, prior exposure to PAHs, co-metabolism or interaction, biosurfactant production, metabolic pathways, and substrate affinity, among others (Peixoto et al. 2011). Hence, the efficiency of microbial remediation is affected by the

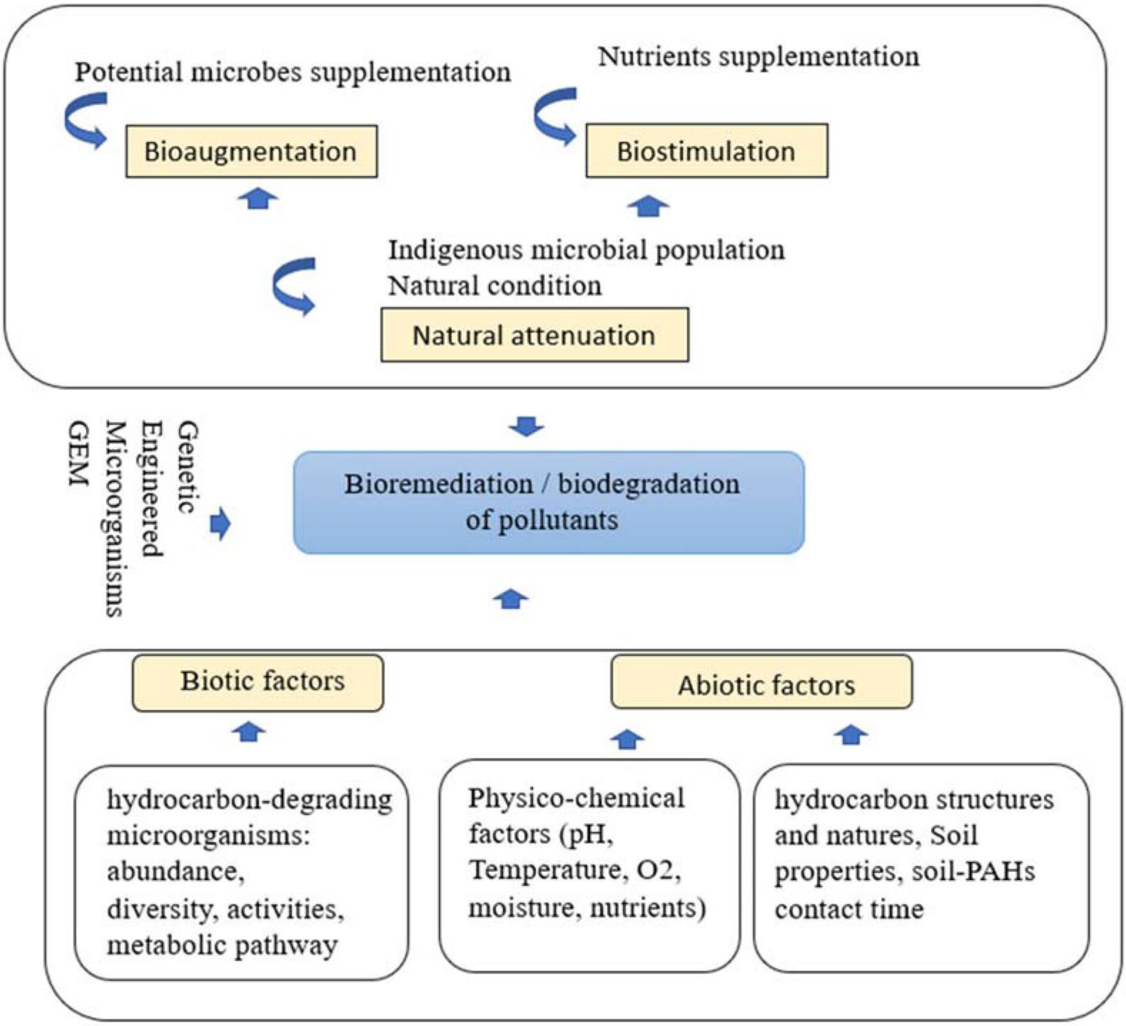

Fig. 2 The influence of biotic and abiotic factors in the remediation of pollutants

nature, structure, and bioavailability of pollutants, as well as by the degrading potential of microbes against petroleum pollutants (Kong et al. 2018).

Furthermore, PAH-degradation depends upon the amount, nature, and properties of PAHs, such as their bioavailability, hydrophobicity, molecular weight and structures, toxicity, and volatilization.

Additionally, several environmental factors are interconnected; for example, temperature variation affects PAH solubility and microbial activity (Mehetre et al. 2019). In soil PAH remediation, many factors can influence degradation yields, including soil properties such as texture, permeability, moisture content, nutrient quality, organic matter content, microbial diversity, and quantity (Chen et al. 2015).

The main biotic and abiotic (environmental) factors that may affect the effectiveness of spilt-oil decontamination are illustrated in the following sections:

Biotic factors

Biotic factors describe the potential of hydrocarbon-degrading organisms and their metabolic activities during PH degradation processes in contaminated sites.

Oil-degrading bacteria

Several researchers have reported the potential of bacteria to degrade low molecular weight PAH structures (<C3) (Xu et al. 2021). However, other studies revealed the persistence of higher molecular weight hydrocarbons (C20-icosane) after the bacterial degradation of petroleum hydrocarbons. Biodegradation processes can occur aerobically and anaerobically to convert PAHs into CO_2 and H_2O).

The most common bacterial species mentioned in PAHs degradation processes were: Alcaligens denitrificans, Acinetobacter calcoaceticus, Pseudomonas putida, Mycobacterium sp., Pseudomonas fluorescens, Corynebacterium renale, Pseudomonas cepacia, Rhodococcus sp., Pseudomonas vesicularis, Moraxella sp., Bacillus cereus, Beijerinckia sp., Micrococcus sp., Pseudomonas paucimobilis, and Sphingomonas sp. (Aitken et al. 1998; Lu et al. 2019; Khomarbaghi et al. 2019) (Table 3).

In general, the bacterial pathway of PAHs degradation is known to incorporate three major steps, comprising the ring cleavage process (RCP), side-chain process (SCP), and central aromatic process (CAP) followed using tricarboxylic acid (TCA) to generate CO_2 and ATP for bacterial growth (Kweon et al. 2007). The RCP is stimulated by benzene-ring hydroxylation followed by the action of oxygenases, cytochrome P450 monooxygenases (CYPs) and ring-hydroxylating dioxygenases (RHDs) to generate dihydrodiol compounds. These processes are followed by dihydroxylation and then ring-cleavage dioxygenation. Next, the SCP and CAP occur to transform the aromatic compound into simple metabolites that are later degraded by the CAP. At this stage, protocatechuate 3,4-dioxygenase can add two atoms of oxygen to the metabolite protocatechuate (Peng et al. 2020) (Fig. 3A).

Oil-degrading fungi

Hydrocarbon-degrading fungi are ubiquitously distributed in various habitats, including freshwater and marine environments and the affected soil matrix. In oil-polluted areas, PAHs can be susceptible to fungal transformation when the fungi use the petroleum compounds for their growth and reproduction. Indigenous isolates are predisposed to the biodegradation of petroleum-contaminated sites (Das and Chandran 2010).

Fungi have advantages over other microorganisms in that they are characterized by a robust morphology, large hyphal network, adaptability to extreme conditions, and tolerance of high concentrations of pollutants (Selbmann et al. 2013).

The hyphae system exhibits effective absorption and transportation of PHs inside the fungal cells for further metabolism, including hydrolysis, dehalogenation, oxidation, and entry into the tricarboxylic acid (TCA) cycle (Fig.3B).

Furthermore, fungal species can produce versatile extracellular enzymes such as laccases, peroxidases, and integral-membrane enzymes like cytochrome 450 and oxidoreductases (Ostrem Loss and Yu 2018) that interact with various structures of hydrocarbons with a fairly high degree of non-specific activity (Martínková et al. 2016).

The common mechanism of PAHs transformation begins with intracellular bioaccumulation proceeding to breakdown by the cellular metabolism (Fayeulle et al. 2014).

The fungal biodegradation pathway includes two major stages. The first stage refers to the generation of oxidized intermediates, such as hydroxyl-, dihydroxy-, dihydrodiol-, and quinone compounds, followed by the second stage, which includes ring fission with methyl-, sulfate-, or organic acid groups (Fig.3B). All of these metabolites are less harmful than the initial PAH structures (Kadri et al. 2017).

Halophilic fungi

Halophilic PAH-degrading fungi are important microbial resources for mycoremediation applications. Those fungi are tolerant of saline conditions and can be used for remediation in otherwise challenging PAH-polluted environments, such as oceans and marine sediments. Several fungal species have been isolated from saline or marine environments and characterized as PAH-degrading (Lin et al. 2014; Zhou et al. 2016). Recently, some studies have highlighted the effectiveness of marine-derived fungi in PAH bioremediation. For instance, Mahajan et al. (2021) revealed the potential of *Penicillium ilerdanum* and *Aspergillus versicolor* as marine fungal isolates for PAH degradation in sediment and contaminated soil. Similarly, *Marasmiellus* sp. CBMAI

Table 3 List of PAHs-degrading bacterial strains

Bacterial Genera	PAH	Substrate or environment	References
Marinobacter hydrocarbonoclasticus, *Roseovarius pacificus*, *Pseudidiomarina sediminum*	Phenanthrene and Fluorene	Mangrove sediments	Moghadam et al. (2014)
Bacterial mixture: *Comamonas testosteroni, ATCC11996* *Pseudomonas putida* *Acinetobacter calcoaceticus, seudomonas sp,* *Rhodococcus sp*	Benzo[a]pyrene, anthracene and, phenanthrene	Polluted areas	Xu et al. (2021)
Rhodococcus sp. P14	PAHs	Marine environment	Peng et al. (2020)
Stenotrophomonas (MTS-2) followed by Citrobacter (MTS-3) and Pseudomonas (MTS-1)	PAHs contaminated soils	Manufactured gas plant	Kuppusamy et al. (2016)
Erythrobacter, *Nitratireductor*, *Acinetobacter*, *Pseudonocardia* and *Brevundimonas*	Phytane and pristane	Deep-sea hydrothermal sediments	MA et al. (2021)

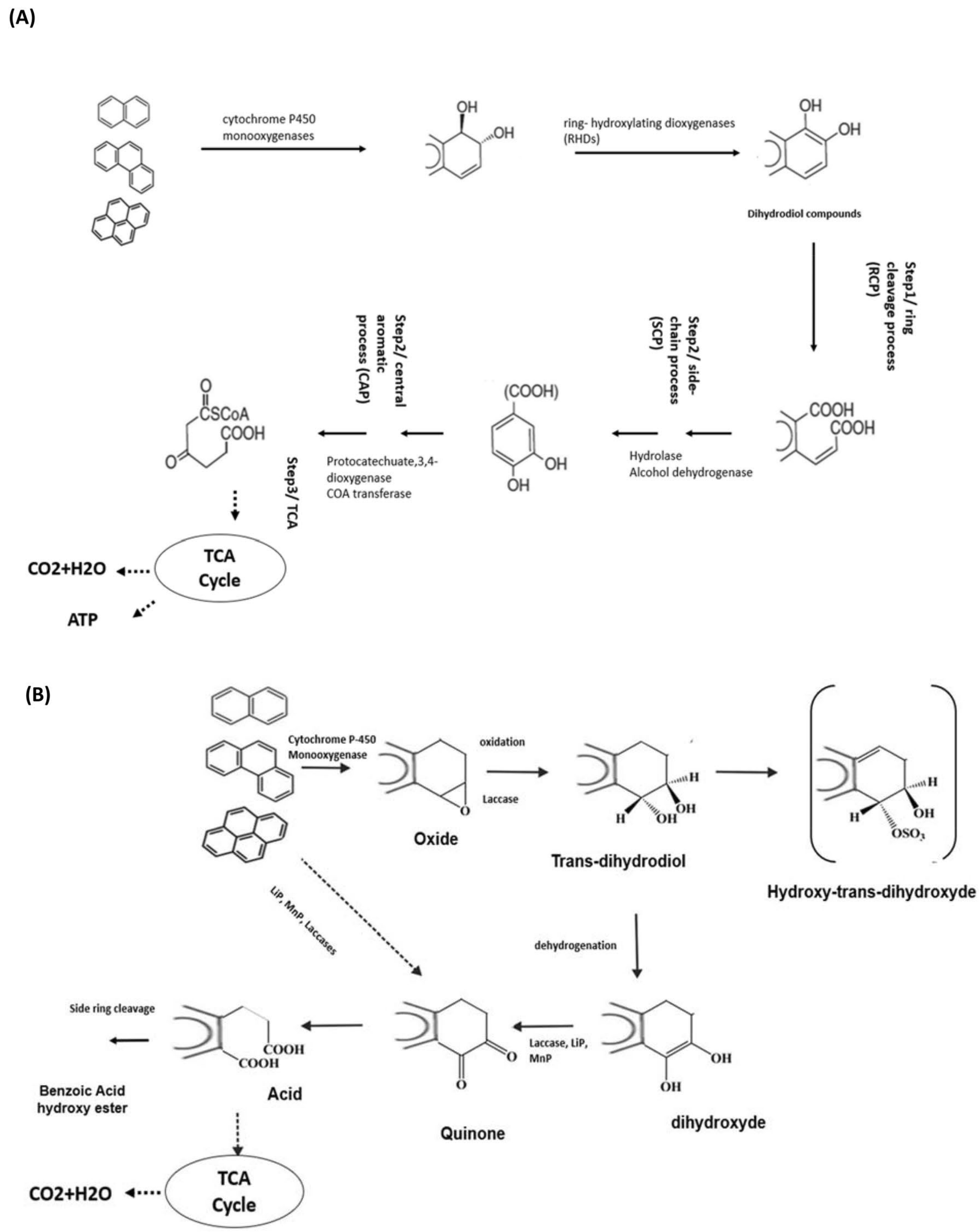

Fig. 3 Overview of PAHs degradation pathway: **A** in bacteria and **B** in fungi

1062, of marine origins, was reported by Vieira et al. (2018) as effective in the degradation/detoxification of pyrene and benzo[a]pyrene (BaP) under saline conditions. Accordingly, Passarini et al. (2018) studied the potential of selected marine-derived filamentous fungi *Aspergillus sclerotiorum* CBMAI 849 and *Mucor racemosus* CBMAI 847.

Finding the degradation pathway of halotolerant or halophilic PAH-degrading fungi is a critical step for understanding the fate of hydrocarbons in saline areas (Arulazhagan and Vasudevan 2009; Debajyoti et al. 2016).

Several PAH-degrading halotolerant and halophilic fungal strains have been isolated from hypersaline biotopes and their pathways have been investigated. For example, Feng et al. (2012) demonstrated that the halophilic isolate *Martelella* sp. AD-3 is efficient to mineralize phenanthrene by generating gentisic acid.

Soil and mycorrhizal fungi

Soil fungi are mostly considered efficient petroleum hydrocarbon-degraders and their consortia with other species ensure effective biodegradation in soil remediation. Soil fungi represent various groups, especially Ascomycota, Chytridiomycota, and Zygomycota. They are mostly non-ligninolytic saprophytes with a very good cellulose-decomposing ability. They mostly include different species in the genera *Acremonium*, *Allescheriella*, *Alternaria*, *Aspergillus*, *Beauveria*, *Cladosporium*, *Cunninghamella*, *Engyodontium*, *Fusarium*, *Geomyces*, *Microsporum*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Phlebia*, *Rhizopus*, *Stachybotrys*, and *Trichoderma* (Anastasi et al. 2013; D'Annibale Rosetto et al. 2006; Pinedo-Rivilla et al. 2009; Zafra et al. 2015) (Table 4).

Several authors have made lists containing indigenous fungi genera that can degrade a wide spectrum of PAHs, derived from petroleum polluted soil (Burghal et al. 2016).

Mycorrhizae fungi, ubiquitous soil-borne fungi, have a symbiotic association with plants' roots that can improve their nutrition, water uptake, and tolerance of environmentally stressful conditions in contaminated sites (Prasad 2017).

In the scientific literature, several studies have documented the improved phytoremediation of PAH-contaminated soil due to the symbiotic relationship between arbuscular mycorrhizal fungi (AMF), rhizobia, and plants (Yu et al. 2011; Aranda et al. 2013; Chen et al. 2018). Accordingly, Ren et al. (2017) have noted the positive effects AMF have on the phytoremediation of PAHs in contaminated soil by improving nutrient uptake and plant resistance. Indeed, despite their limited capacity to biodegrade organic pollutants, AMF may indirectly promote the microbial degradation processes by interacting with and modifying the microbial flora in polluted soil. AMF are beneficial to plant growth and survival in polluted sites (Chen et al. 2018).

Several authors investigated the application of mycorrhizal fungi genera in the bioremediation of petroleum-contaminated soil. Małachowska-Jutsz and Kalka (2010) particularly investigated the efficacy of mycorrhizal fungi associated with plant cultivation on petroleum-contaminated soil.

Ligninolytic fungi

Ligninolytic PAH-degrading fungi show promise for the restoration of petroleum-contaminated areas. These are the first to be applied in mycoremediation studies in 30% of the existing research (Chandra and Enespa 2019). White-rot fungi are so effective at degrading lignin within lignocellulosic substrates by releasing extra-cellular lignin-modifying enzymes (LME). The enzymes present in the system that are

Table 4 PAHs-degrading fungi assessed in different biotopes and substrates

Fungal Genera	PAH	Substrate or environment	References
Absidia cylindrospora	Fluorene	Soil	Garon et al.(2004)
Aspergillus oryzae and Mucor irregularis	Complex hydrocarbon (used engine oil)	Nigerian Crude Oil-Polluted Sites	Asemoloye et al. (2020)
Aspergillus niger	Phenanthrene, 9-fluorenone and anthracene-9,10-dione	PAHs content in waste cooking oil	Teng et al. (2021)
Alternaria sp., Penicillium spp., and Stemphylium sp.	Gulf of Mexico	Extra-heavy crude oil	Romero-Hernández et al. (2021)
Trametes versicolor and Bjerkandera adusta	Total hydrocarbons	Soil	Shahi et al. (2016)
Aspergillus sydowii BPOI	Anthracene	Mangrove soil	Bankole et al. (2020)
Bjerkandera adusta	Phenanthrene	Soil	Kadri et al. (2017)
Aspergillus terrus, Aspergillus fumigatus and Aspergillus niger, Penicillium glabrum and Cladosporium cladosporioides	Phenanthrene	Soil	Cortes-Espinosa et al. (2007)
Candida tropicalis, Rhodosporidium toruloids, Fusarium oxysporium and Aspergillus clavatus	Engine oil	Soil	Mbachu et al. (2016)
Coriolopsis gallica	PAHs	Diesel	Daâssi et al. (2021)
Trichoderma reesei	PAHs	Soil	Yao et al. (2015)
Pleurotus ostreatus	PAHs	Soil	Di-Gregorio et al.(2016)

employed to degrade lignin include lignin-peroxidase (LiP), manganese peroxidase (MnP), various H_2O_2-producing enzymes, and laccase (Pointing 2001). This ligninolytic enzymatic cluster is characterized by a low substrate specificity that allows it to act upon several classes of pollutants with structures similar to lignin. The biodegradation of complex molecules by extracellular oxidative ligninolytic enzymes has been studied in detail in *Phanerochaete chrysosporium* (Paszczynski and Crawford 1995).

In addition to *P. chrysosporium*, several other white-rot fungi (e.g., *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, and *Irpex lacteus*) are also known to degrade these compounds (Tony et al. 2011) (Table 4).

Fungi are suited for the bioremediation of crude oil in polluted sites because of their diverse metabolic activities. They can secrete a broad range of ligninolytic and non-ligninolytic enzymes to use PHs as carbon and energy sources and assimilate them into the fungal biomass (Peixoto et al. 2011).

Many organic pollutants enter fungal cells through the permeable cell membrane, where they are broken down by internal enzymes, e.g., reductive dehalogenases (Stella et al. 2017), cytochrome P450 (Ostrem Loss and Yu 2018), and nitro-reductases (Tripathi et al. 2017), into simpler metabolites. These metabolites are further metabolised through β-oxidation or entry into the TCA cycle (Varjani 2017) (Fig. 2B).

Ligninolytic enzymes from WRF containing laccase (EC 1.10.3.2), lignin peroxidase (LiP, EC 1.11.1.14), and manganese peroxidase (MnP, EC 1.11.1.13) have been investigated extensively as biotechnological tools for spilt-oil bioremediation (Cajthaml et al. 2008).

This ligninolytic enzymatic system makes WRF able to completely mineralize PAHs into CO_2 (Pointing 2001). Fungal laccases are the main enzymes involved in the bioremediation of PHs (Agrawal et al. 2018).

However, lipases have been significantly less studied for the bioremediation of PAHs (Haritash and Kaushik 2009). Ugochukwu et al. (2010) reported the presence of the enzyme lipase as an indicator of the microbial degradation of crude oil with indigenous and exogenous soil microorganisms. Among the fungal isolates, *A. niger* showed the highest lipase activity at 4.00 U/mL.

Enzyme-based remediation offers several advantages over the application of microbial cells. Enzymatic mycoremediation is simpler than using whole fungi, especially in extreme environments. Furthermore, the use of enzymes can avoid the introduction of exotic or genetically modified organisms (GMOs) to the environment.

Some advantages, including enzyme specificity and efficacity, can be improved and controlled in the laboratory (Sutherland et al. 2004). Neither whole-cell competitiveness nor toxic by-product generation occurs during enzymatic bioremediation (Setti et al. 1997). Moreover, fungal enzymatic systems have been recorded as biodegraders of hydrophobic or poorly soluble xenobiotics in aqueous solutions, like PAHs. Enzymatic oxidation can occur in the presence of organic solvents. Thus, fungal enzymatic bioremediation can present a solution to the insolubility and poor bioavailability of hydrocarbons during the clean-up process.

The potential role of fungal laccases in the oxidative transformation of PAHs in contaminated soil has been reported in the literature (Wu et al. 2008).

Despite the advantages of enzymatic bioremediation, enzymes must be stable, adapted to environmental variations, and produced at a low cost. These limitations restrict the widespread application of extracellular enzymes for oil spill remediation (Eibes et al. 2015). For instance, to overcome those limitations, restricted fungal laccase is typically studied in the ex-situ remediation of petroleum-polluted soil (Wang et al. 2018a, b; Perini et al. 2021).

Genetically engineered microorganisms (GEM)

A potent remedial technology requires microorganisms to rapidly adapt and efficiently transform pollutants in particular conditions and periods (Seo et al. 2009).

Most conventional microbial remediation shows a lack of specificity for particular pollutant structures. Thus, some authors propose the development of sustainable and multifunctional systems such as the microbial-combined system, nano-remediation technique, and genetically engineered microorganisms (GEMs). Indeed, GEMs offer high efficiency and an eco-friendly strategy for the bioremediation of a mixture of contaminants compared to other techniques. Additionally, GEMs can be efficient in the destruction of high molecular weight PAHs in polluted areas under specific conditions (Wu et al. 2021). However, ecological and environmental concerns and regulatory constraints are major obstacles for testing GEMs in the field (Menn et al. 2008).

Abiotic factors

Bioavailability

Bioavailability is one of the limiting factors for the bioremediation of PAHs in polluted sites. PAH dispersion in the soil is related to their affinity and hydrophobicity towards soil particles and organic matter in situ. Furthermore, the physicochemical properties of the soil matrix have a determinative effect on PAH partitioning in contaminated areas (Zang et al. 2021). Generally, PAH bioremediation processes are closely related to the bioavailable and bioaccessible parts of the initial target molecule for microbial uptake, passage through the cellular membrane, metabolism, and breakdown (Duan et al. 2014).

PAHs in contaminated soil diffuse in aged soil (hydrophobic zones), limiting their bioavailability for microbial uptake and reducing their desorption rates. The quantity of available PAHs in the soil is related to several factors including the soil properties (e.g., soil particle size, soil type, soil organic matter, etc.), the physicochemical properties of the compound, and the environmental conditions (Bolan et al. 2006; Duan et al. 2014; Zang et al. 2021).

Leonardi et al. (2007) studied the impact of PAH bioavailability on the mycoremediation performance of aged soil matrices. Their results showed that the WRF *Irpex lacteus* and *Pleurotus ostreatu*s interact better in the presence of mobilizing agents (i.e., Tween 20, soybean oil, Tween 80, and olive-mill wastewater) which makes PAHs more available and accessible to the cellular membranes of fungi in the soil.

Many studies have demonstrated that amending the soil contaminant with biosurfactants is an effective method for improving the bioavailability of petroleum hydrocarbons. For instance, Zang et al. (2021) reported enhancing the removal rate of PAHs in sludge by *Stenotrophomonas* sp. N5 from 27.7 to 43.1% due to the biosurfactant/phenol system, which vastly increases PAH bioavailability.

Temperature

Biodegradation of PHs occurs over a broad range of temperatures. Temperature has an imperative effect not only on pollutant chemical structures, but also on the diversity and PAH-degrading physiology of microbes (Logeshwaran et al. 2018).

For example, during in-situ remediation, the amount of degradation is enhanced by higher temperatures that ameliorate the bioavailability and solubility of petroleum hydrocarbons. However, hydrocarbon removal rates decrease with low temperatures because of the decrease in enzymatic activity (Khan et al. 2019; Kebede et al. 2021). Generally, increasing temperature decreases the soil-water partition coefficient and so enhances the rate of PAH accumulation through soil particles, which in turn decreases oxygen solubility and limits the metabolic activities of aerobic organisms (Haritash and Kaushik 2009).

In laboratory studies, the highest PAH biodegradation rates were examined at moderate temperatures (30–40 °C). For instance, Al-Dossary et al. (2020) reported that the maximum degradation occurs at 30 °C, a pH of 5.5, and with nitrogen in the form of yeast extract. Meanwhile, other reports on PAH biodegradation were demonstrated at extreme temperatures. For example, biodegradation of PHs, including PAHs, was reported in seawater at low temperatures (0–5 °C) (Brakstad and Bonaunet, 2006) while the biodegradation of petroleum hydrocarbons along with long-chain alkanes was documented at higher temperature (60–70 °C) by *Thermus* and *Bacillus* spp. (Feitkenhauer et al. 2003). Additionally, the enzymatic degradation of PAHs by fungal laccase was enhanced at 70 °C to reach 90% degradation of PAHs in spent-mushroom compost (Lau et al. 2003).

pH

pH, among other abiotic factors, is crucial for PH biodegradation. Extreme pH conditions may affect microbial activity and are expected to affect bioremediation efficiency. Additionally, pH can influence numerous cellular processes such as membrane transfer, metabolic pathways, and enzymatic reactions (Neina 2019). In this regard, the variability of pH must be controlled during biological processes. The pH of contaminated soil varies over a range of pH of 3–9 and, in some cases, reached a pH of 11 (Pawar 2015). Generally, the highest PAH-degradation yields were recorded at a neutral pH for both bacterial and fungal bioremediation processes, although fungi can interact with hydrocarbons in near-acid conditions. Pawar (2015) found that 50% of PAH degradation by bacterial mineralization occurred at a pH of 7.5 over 3 days of treatment. In the same study, *Penicillium* species were more efficient at acidic pH levels, while *Aspergillus* strains were prevalent at a pH of 7.5–8 in PAH-contaminated soil. Likewise, Kami et al. (2020) reported that *Corynebacterium urealyticu* was potent in the biodegradation of phenanthrene in contaminated soil at a pH of 7.

Moreover, soil pH was reported not only to control biological processes, but also to affect biogeochemical processes during bioremediation. Soil pH may affect the solubility, bioavailability, and mobility of organic matter, heavy metals, and trace elements, factors that determine their translocation in plants (Neina 2019).

Nutrients

Furthermore, the quality and quantity of nutrients represent an important factor for successful PAH remediation. For efficient biodegradation, the C:N:P ratio is highly critical. Many studies demonstrated the enhanced effect of a C:N:P ratio of 100:10:1 on the microbial remediation of PAHs in soil (Medaura et al. 2021).

Similarly, Premnath et al. 2021 recorded a C:N ratio of 10:2 for improving anthracene, acenaphthene, fluorene, naphthalene, and mixed PAH degradation by *Klebsiella pneumoniae*. Likewise, many types of research revealed the relationship between the addition of nitrogen and the enhancement of microbial growth and enzyme secretion during PAH degradation processes due to the involvement of nitrogen in amino acid synthesis (Xu-Xiang et al. 2006; Daâssi et al. 2021). However, a higher concentration of nutrients can also harm biodegradability and become a limiting factor affecting the process of degradation (Ghosal et al. 2016).

Oxygen

Oxygen is an essential factor in aerobic PAH degradation processes. In PAH-contaminated sites, aerobic microorganisms require molecular oxygen for hydrocarbon oxidation through the secretion of oxygenases (mono- or dioxygenases) to convert benzene rings into smaller rings of hydrocarbons (Ite and Ibok, 2019). Under the action of these degrading enzymes, several reactions, including oxidation, hydrogenation, and dehydration, mainly occur in PAH-polluted areas, leading to its final remediation. For instance, through a hydration reaction, fungal monooxygenase may oxidize the carbon on the benzene ring and then transform it into trans diols and phenols (Daâssi et al. 2021).

Typically, the first metabolic step of the degradation of aliphatic hydrocarbons is mediated by integral membrane enzymes (e.g., mono- and dioxygenases, and cytochrome 450) to oxidize the terminal methyl group into alcohol, which in turn is converted to aldehydes and fatty acids through TCA-cycle oxidation (Varjani 2017).

Likewise, the availability of oxygen in soil matrices is important for microbial survival and proliferation. Hence, aerobic bacteria or fungi were characterized by the presence of catalase activity as an indicator of microbial biotic stress tolerance and resistance to reactive oxygen species attack (Zhang et al. 2018).

Salinity

Many studies have demonstrated that salinity affects microbial growth, species abundance, species diversity, and metabolic pathways (Vieira et al. 2018; Wang et al. 2018a, b; Al-Hawash et al. 2018). Similarly, Wang et al. (2018a, b) reported the alteration of the microbial population and the negative effects of salinity on community structure and catabolic gene expression.

Enzymatic reactions are influenced by salinity and some implicated enzymes in the microbial PAH-degradation pathways exhibit lower activity under conditions with elevated salt levels. Guo et al. (2018) demonstrated that PAH dioxygenase (PDO), catechol 1, 2-dioxygenase (C12O), and catechol 2, 3-dioxygenase (C23O) exhibited lower activity at 20% salt concentration.

Conclusion

The biodegradation of PAHs and other persistent pollutants depends upon microorganisms to either transform or mineralize them into CO_2 and H_2O. This study notes that the effective removal of PAH from contaminated sites using physical, chemical, combined–bioremediation, and mycoremediation approaches appears to be the most efficient and cost-effective, environmentally friendly method of decontaminating PAH-polluted soils.

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