# Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soils

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Rhizoremediation of PHCs is facilitated through a process known as the 'rhizosphere effect', in which plants exude a myriad of organic compounds into their root-surrounding zone (the rhizosphere), resulting in an increase in abundance and activity of certain rhizospheric microbes, which in turn can degrade or metabolize hydrocarbon contaminants.

Keywords: phytoremediation ; PGPR ; hydrocarbon-degrading bacteria ; contaminated soils ; alkanes ; PAHs

### 1. Introduction

Industrial activities, including mining and extraction of oil and gas, as well as chemical inputs into agricultural production systems, have led to different degrees of environmental contamination worldwide. Petroleum hydrocarbons (PHCs) are among the major pollutants that can pose a serious environmental threat. PHC products have adversely affected various ecosystems, causing disturbing damage to natural habitats with serious economic consequences <sup>[1]</sup>.

Petroleum hydrocarbons are heterogeneous organic mixtures composed of carbon and hydrogen atoms arranged in varying structural configurations and have different physical and chemical properties <sup>[2]</sup>. These compounds consist mainly of hydrocarbons and fewer numbers of other non-hydrocarbon constituents, such as nitrogen, oxygen, and sulfur <sup>[3][4]</sup>. They are broadly classified into two major fractions: aliphatic hydrocarbons and aromatic hydrocarbons (**Figure 1**). Prior to processing, PHCs are composed, on average, of ~57% aliphatic hydrocarbons, ~29% aromatic hydrocarbons, and ~14% asphaltenes and other polar compounds containing nitrogen, oxygen, and sulfur <sup>[5]</sup>. Aliphatic hydrocarbons include both linear or branched-chain hydrocarbons, which may be unsaturated (alkenes and alkynes) or saturated (alkanes) <sup>[6]</sup>. Aromatic hydrocarbons include monocyclic (i.e., benzene, toluene, phenol, etc.) and polycyclic aromatic hydrocarbons (PAHs) (**Figure 1**). PHCs are the most common pollutants in soil and ground water worldwide. The ever- increasing dependency of modern society on fuel for energy generation in many vital sectors, such as electricity , heat , industry, and transportation has resulted in the extensive exploitation of PHCs <sup>[2]</sup>. Although environmental transition actions have been taken in many countries, dependency on petroleum will last for some decades, contributing to organic pollution risks.



Figure 1. Schematic diagram showing the classification of PHCs.

Soil contamination with PHCs is an international issue, and the magnitude of soil pollution is hard to quantify. For example, in Australia, around 80,000 sites are estimated to be contaminated by PHCs <sup>[7]</sup>, whereas in Canada around 22,000 federal-owned sites are identified as being contaminated by PHCs <sup>[8]</sup>. In Europe, PHC contamination was observed in at least 342,000 sites <sup>[9]</sup>. These organic contaminants also pose serious health risks to humans and other organisms in addition to their adverse impact on the soil microflora, leading to environmental quality degradation. For instance, some aromatic substances, such as BTEX and PAHs, are notorious mutagens and carcinogens that can enter

our food chain together with lipophilic compounds <sup>[10]</sup>, and they have been linked with probable causes of bladder, kidney, liver, lung, and skin cancers. This explains the growing concern with these contaminants and the urgent need to use all possible means to protect the environment and to find the appropriate techniques to remediate polluted soils.

Various chemical, physical, and thermal conventional techniques have been used to remediate soils contaminated with PHCs. These conventional methods, which can contain, destroy, or separate the pollutants, include a wide range of both in situ and ex situ cleanup technologies, such as asphalt batching, biopiles, chemical oxidation, excavation, hydrolysis, incineration, photolysis, pump and treat, multi-phased slurry reactors, soil vapor extraction, soil washing, and thermal desorption. However, these methods have particular limitations. First, their cost is often prohibitive; for example, it can cost between USD 480 and 813 per m 3 for extraction <sup>[11]</sup>. Second, chemical procedures only work for specific organic compounds, and they most often destroy soil microbial communities. Third, these methods do not often result in a complete degradation of the pollutants <sup>[2][12]</sup>. Finally, PHC-contaminated soil contains numerous classes and types of toxic organic compounds, which make the choice of the proper method a challenging task. Hence, phytoremediation is a more recent and promising green-biotechnology that is perceived as an environmentally friendly, more cost-effective, and less destructive approach to cleanup contaminants in the environment.

# 2. Phytoremediation

Phytoremediation is a remediation technique that relies on the ability of plants and their associated microbiota to accumulate, degrade, sequester, or stabilize harmful environmental contaminants <sup>[13][14]</sup>. Over the past two decades, the deployment of plants (and their associated microbiota) to remediate a wide spectrum of inorganic and organic pollutants in soil and water environments has been carried out. This technique has been applied to remediate various types of pollutants such as chlorinated solvents <sup>[15]</sup>, explosives <sup>[16]</sup>, heavy metals <sup>[17]</sup>, landfill leachates <sup>[18]</sup>, pesticides <sup>[19]</sup>, PHC <sup>[20]</sup>, radionuclides <sup>[21]</sup>, and salts <sup>[22]</sup>. Although phytoremediation is still very much in its infancy, its application has been adopted by a growing number of companies. For example, the phytoremediation market has grown continuously at a rapid rate, with an estimated value of USD 32.2 billion in 2016 and is expected to reach USD 65.7 billion by 2025 <sup>[23]</sup>.

Phytoremediation is an innovative technique that has gained broad public acceptance, not only because it is an environmentally friendly approach but also as it requires less maintenance efforts, minimize site disturbance, and costeffective process, which is powered by solar energy. However, phytoremediation still remains a marginal option for in situ soil remediation <sup>[24]</sup>. As any other technique, phytoremediation has some limitations that affect its efficiency, performance, and time consuming. For example, phytoremediation efficiency varies with environmental conditions, such as soil physiochemical properties, contaminant level, and seasonal temperature fluctuations <sup>[13][25][26]</sup>.

Phytoremediation efficiency is dependent on many factors, including plant selection  $^{[27]}$ ; environmental parameters such as nutrient status, contaminant concentration, and bioavailability; soil pH, etc.  $^{[25]}$ , in addition to the composition and activity of plant-associated microbiomes. Plants and their associated microbiomes facilitate pollutant uptake from the environment via different processes, including degradation, extraction, stabilization, transformation, and volatilization  $^{[13]}$  [14]. The type of plant and pollutants plus the environmental conditions are key factors for determining the way in which phytoremediation techniques can be applied. Generally, phytoremediation technologies are divided into five different categories (**Table 1**). The phytoremediation method suitable for petroleum hydrocarbon-contaminated soil is called rhizoremediation  $^{[28]}$ , which is defined as the breakdown of organic pollutants by using plants and their root-associated microbiota.

Category	Mechanism	Target Pollutants	Region of Activity	Reference
Phytoextraction	Uptake and concentrate contaminants	Metals (e.g., Cd,Ni), radionuclides (e.g., Pu)	Shoot tissue	[21][29]
Phytostabilization	Immobilization and sequestration of contaminants	Primarily metals (e.g., Cu, Zn, Pb)	Root tissue	[30]
Phytotransformation	Enzymatic actions	Chlorinated solvents, ammunition waste, herbicides, monoaromatic hydrocarbons	Plant tissue	[ <u>15][30]</u>
Phytovolatilization	Uptake and evapotranspiration	Volatile organics (e.g., TCE, toluene, MTBE),	Shoot tissue	[14]

Table 1. Phytoremediation mechanisms whereby plants remediate polluted soils.

Category	Mechanism	Target Pollutants	Region of Activity	Reference
Rhizoremediation	Breakdown of organic pollutants by using plants and root-associated microbiomes	PHC (e.g., diesel), pesticides (e.g., dimethomorph)	Root	[ <u>19][28]</u>

Adapted and modified from [14][28][29][30].

Rhizoremediation of PHCs is facilitated through a process known as the 'rhizosphere effect' <sup>[31]</sup>, in which plants exude a variety of organic compounds into their root-surrounding zone (the rhizosphere), resulting in an increase in abundance and activity of certain rhizospheric microbes, which in turn can degrade or metabolize hydrocarbon contaminants <sup>[32]</sup>. Understanding the plant–microbiome partnerships, and the underlying processes that govern and control PHC degradation, is a priority challenge in rhizoremediation research nowadays <sup>[2]</sup>[33][34].

# 3. The Rhizosphere Microbiota and root exudates

In addition to shaping the microbial communities in the rhizosphere, root exudates have other functions that benefit the plant itself. Through root exudation, plants can change the soil physicochemical properties, contributing to nutrient assimilation, reducing the growth of competitor plant species, increasing the abundance of certain beneficial microbes, and regulating the microbiome composition in the rhizosphere <sup>[35][36]</sup>.

Since the beginning of phytoremediation research, many plant species have been tested for their potential to enhance rhizoremediation of PHCs <sup>[37]</sup>. Plants enhance the degradation of PHCs principally by the unique properties of the plant itself and by providing optimal conditions for microbial proliferation in the rhizosphere <sup>[38]</sup>. In general, selection of plants suitable for rhizoremediation of PHCs should be based on the following criteria: tolerance to a broad range of PHCs, speed of growth, root morphology, ability to grow in many soil types, and the root exudate profile <sup>[38][39][40]</sup>. Additionally, plants should not be selected based solely on the contaminant uptake efficiency; their ability to stimulate microbial activity and abundance also should be considered <sup>[41][42]</sup>.

Sequestration and transportation of contaminants inside plant tissues enable plants to remediate PHC-polluted soil <sup>[43][44]</sup>. Plants can also degrade or transform organic pollutants into less toxic forms via their enzymatic machinery, or synthesizing a variety of defensive proteins and metabolites <sup>[13][45]</sup>. Therefore, plants can adapt and confront many unfavorable stressful conditions, such as PHC contamination. However, plant growth has been retarded under highly stressed conditions, e.g., PHC pollutants are expected to be lower than those under optimal conditions <sup>[46]</sup>. Therefore, plant growth may be positively enhanced by the presence of plant growth-promoting rhizobacteria (PGPR) that are able to alleviate stresses in plants via many mechanisms, such as reducing soil nutrient deficiencies (fixing nitrogen, solubilizing phosphorus, and enhancing iron uptake), synthesizing plant hormones, suppressing ethylene production via 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity <sup>[46][47]</sup>, and degrading a broad range of PHCs <sup>[2]</sup>.

Ethylene production by plants at low concentrations can be beneficial. However, when produced at high concentrations, it can stunt plant growth and development by inhibiting root growth <sup>[48]</sup>. In response to various biotic and abiotic stressor conditions, plants synthesize different enzymes, metabolites, and stress proteins to alleviate the adverse effects of stress <sup>[49]</sup>; of particular interest is ethylene. Once plants encounter stress, such as flooding, drought, or presence of toxic compounds, plant growth is inhibited because the ethylene precursor, 1-aminocyclopropane-1-carboxylate, is induced <sup>[50]</sup>. However, certain PGPR can hinder ethylene biosynthesis via production of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) that cleaves the ethylene precursor ACC into alpha-ketobutyrate and ammonia <sup>[51]</sup>, thus balancing ethylene levels and reducing its adverse impact on plant growth <sup>[51]</sup>.

## 4. Enhancing the Understanding of Mechanisms through Which Host Plants Assemble a Beneficial Microbiota, and How It Functions, under Pollutant Stress

Studies of the rhizosphere microbiota in natural and agricultural settings have generated most of our knowledge about host plant selection processes and plant-microbiome interactions taking place in the rhizosphere and how plants recruit different microbiota from surrounding environments <sup>[34][52]</sup>. For example, previous studies using 16S rRNA amplicon sequencing revealed that microbial communities in the rhizosphere and adjunct bulk soils are different; the recruitment of rhizosphere microbiota by plants is strongly dependent on the structure and composition of the bulk soil microbiome <sup>[53]</sup>, and different plant genotypes were found to select for different rhizosphere microbiomes <sup>[54]</sup>.

Over the past few years, several experiments have been conducted to optimize phytoremediation systems and improve their efficacy using high-throughput sequencing approaches. For example, Bell et al. <sup>[55]</sup> used high-throughput 454-pyrosequencing of bacterial 16S rRNA genes and the fungal internal transcribed spacer (ITS) region to compare the community structure and composition of the rhizosphere microbiome of native and non-native Salix cultivars across uncontaminated and PHC-contaminated soil. Their results indicated higher fungal sensitivity to PHC contamination than that found for bacterial communities. Additionally, certain fungal class (Pezizomycetes) reacted differently following plant introduction to soils <sup>[55]</sup>, implying the importance of plant species selection in phytoremediation with regard to their impact on plant-associated microbiomes <sup>[34]</sup>. Similarly, Hassan et al. <sup>[56]</sup> used 454-pyrosequencing of the AMF 18S rDNA gene to examine how rhizospheric AMF communities are shaped within the rhizosphere of 11 Salix cultivars introduced across non-contaminated and PHC-contaminated soil. While PHC contamination levels had a strong impact on AMF community structure, Salix planting increased the abundance of several AMF families <sup>[56]</sup>, inferring that AMF, possibly due to opportunistic associations with the plant, are involved in plant adaptation to PHC contamination <sup>[34]</sup>.

Tardif et al. <sup>[57]</sup> amplified the bacterial 16S rRNA gene and fungal ITS regions using Ion Torrent sequencing in order to characterize the variations between plant compartments (bulk soil, rhizosphere soil, roots, and stems) in the microbiome of two Salix cultivars growing under three PHC contamination levels at a former petrochemical site. PHC contamination was found to be the main factor, shaping not only the rhizosphere but also the root and stem microbiome structure <sup>[57]</sup>. Additionally, the presence of the plant offered a protective buffer zone against PHC pollution in the rhizosphere and other plant tissues, subsequently minimizing the severe effects of PHC contamination caused a shift in the microbial community composition, favoring beneficial microbiome communities such as putative PHC-degraders and PGPR <sup>[57]</sup>.

In a recent study, Mitter et al. <sup>[58]</sup> used high-throughput Illumina MiSeq amplicon sequencing of the 16S rRNA gene to characterize the bacterial root microbiome associated with annual barley and sweet clover growing in an oil sands reclamation site. Results confirmed that, consistent with previous reports, the rhizosphere compartment produced the strongest differentiation of the root microbiome community structure <sup>[53][58][59]</sup>; for example, Proteobacteria was the predominant phyla in the endosphere microbiome, whereas phyla such as Acidobacteria and Gemmatimonadetes were restricted only to the rhizosphere microbiome <sup>[58]</sup>. Additionally, host plants play a major role in shaping the root microbiome community structure <sup>[58]</sup>.

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