

## The Influence of Plants in the Remediation of Petroleum Hydrocarbon Contaminated Sites

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Petroleum hydrocarbon contamination is an environmental concern. Of the various hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) are a major worry because they cause many health problems including cancer and the inflammation of tissue in humans. So, it is necessary to remediate contaminated sites. Sites with diffuse low to medium level pollution can be remediated with the use of biological techniques, such as phytoremediation. Phytoremediation is a low input biotechnology approach: it relies on the knowledge that natural attenuation by biodegradation and physicochemical mechanisms will decrease the pollutant concentration. Limited PAH uptake by plant roots also takes place. This is influenced by both the organic content of the soil in which the plants are grown and the plant root lipid contents. Plant responses to growth in soils contaminated with petroleum hydrocarbons need to be taken into account, if plants are to be used to clean up petroleum hydrocarbon contamination. Different plant species show various stress responses and adaptations to survive the stress conditions caused by hydrocarbon pollution. The responses of plants also vary with the type and amount of the contaminant as well as duration of exposure. Phytoremediation can be feasible if appropriate plant species are selected. They must show sufficient morphological plasticity to survive stress situations induced by hydrocarbon-contamination, have an extended rhizosphere and appropriate root exudate patterns, positively influence the growth of hydrocarbon-degrading microorganisms in contaminated soil, and should also limit the uptake of toxic molecules through various adaptations to the root ultrastructure and cell wall components. Plants chosen should also be native to the area to be remediated so that they will be tolerant to the soil and environmental conditions. Additionally, plants that require little attention are preferable because cost is an important factor. Plants with deep, fibrous roots and fast growth, such as grasses, are generally considered useful in phytoremediation. Petroleum hydrocarbons are prevalent in our environment due to industrial activities such as gasification and liquefaction of fossil fuels (gaswork sites), and accidental oil spills, with at least 350,000 contaminated sites in Western Europe. Petroleum hydrocarbon-based products make the largest part of this contamination. Petroleum crude oil is comprised of a complex mixture of hydrocarbons such as cycloalkanes, normal alkanes, isoalkanes and aromatic compounds including polycyclic aromatic hydrocarbons (PAHs) and other organic compounds such as Nitrogen, Sulphur, Oxygen compounds (NSOs). Many of these petroleum components are toxic, mobile and environmentally persistent. Of the various petroleum hydrocarbons, PAHs are a major worry because of their carcinogenic and/or mutagenic potential, ubiquitous nature and environmental persistence, and the occurrence of these components in food poses a threat to human health. Soil is the major repository of PAHs and heavy urban traffic and residential

and communal heating increase the PAH burden in soil. PAH concentrations are found to be higher in urban soils and roadside soils whilst very high concentrations have been reported for contaminated sites such as old gas works sites. Remediation of old industrial sites becomes necessary in meeting the demand for urban housing, office and leisure space. Remediation of polluted soils and waters is desirable as the polluted sites present a potential resource to solve the food and/or fuel shortages of the world. These metabolites bind to and disrupt DNA and RNA, leading the way to tumour formation. Benzofluoranthenes, benzo (a) pyrene, benzo (a) anthracene, dibenzo (a, h) anthracene and indeno (1, 2, 3-cd) pyrene are the most potent carcinogens among the PAHs and should therefore be targeted on this basis. But, their lower bioavailability means that they actually present less of a danger than the more mobile pollutants (i.e., contaminants with lower KOW). So, it is beneficial to the wider environment to reduce these toxic hazards caused by PAHs, especially low molecular weight (LMW) PAHs. Engineering techniques based on physical, chemical and thermal processes have been used for remediation purposes, but these methods are very expensive and not always effective. Another option is landfilling of contaminated soil, but this is also expensive and becomes an increasingly greater problem as landfill sites are now in short supply and apart from this, it is not deemed a sustainable approach to solving the problem. Phytoremediation is a low input biotechnology approach: it relies on the knowledge that natural attenuation by biodegradation and physicochemical mechanisms will decrease the pollutant concentration, and is particularly beneficial where sowing seeds may be the only intervention. Planting PAH-contaminated sites gives other important benefits such as protection against wind erosion, reduction of surface water run-off, reinforcement of soil by roots and aesthetically pleasing impacts on the area: it can also maintain the livelihoods of the communities living in these environments. However, phytoremediation as a remediation technique has many bottlenecks. Plant roots are instrumental in stimulating the proliferation of PAH-degrading microorganisms within the dynamic region of their rhizosphere; hence they are of huge importance in phytoremediation. Yet, the presence of petroleum hydrocarbons in soil poses many challenges to plant root growth such as water stress, chemical toxicity, mechanical impedance and nutrient deficiency as reported in many studies. Plants have to overcome these challenges in order to grow in the stressful conditions so that phytoremediation would be a success. The main objective of this review is to identify the shortcomings with regard to the use of plants in remediation of hydrocarbon-polluted sites and finding solutions to overcome these limitations. Most plants produce one or more orders of lateral root branches that vary in branching patterns. These higher order lateral roots are generally thinner, shorter and do not live as long as those of lower orders. Root axes which originate directly from

the shoot system are called axile roots or order 1 axes. Two main types of root system are distinguished according to the methods of root emergence: primary root systems and adventitious root systems. Primary root systems originate entirely from a single root called a radicle, which emerges soon after germination. Nodal roots successively form on the plant stem and these are called axile roots. With respect to the root system of mature plants for example in mature maize (*Zea mays* L.), the primary root is a minor constituent. The primary root and its branches constitute the tap root system or primary root system, as seen in the mustard plant. Eudicotyledons/eudicots (e.g., poplar, willow, apple) possess primary root system which also shows secondary growth, giving way to mature, thicker "woody" roots with bark and additional vascular tissue. They produce coarse roots that may live for a long time and have important roles in transport and mechanical support. The root architecture of eudicotyledons can be referred to as coarse and woody. In root system architecture, the growth direction of the roots is an important component. The main guidance system that operates in the young root of the germinating seed is that which senses gravity and this ensures that the root is directed downwards. This guidance system constitutes both sensing and response mechanisms with which deviations from the preferred direction of growth can be corrected. Some of the long roots have very specific behaviours such as vertical or horizontal growth, depending on the environmental characteristics, and this strongly determines the overall shape of the system, including such important characteristics as overall width and depth. At a smaller scale, roots generally exhibit some convolutions in response to the mechanical constraints and unfavourable factors which they experience. In a study conducted by Balasubramaniam and Harvey, the roots of tall fescue (*Festuca arundinacea*) plants showed deviations from normal root orientation responses to gravity, turning away from the oil-contaminated soil matrix back into the top layer of clean sand, during the plant acclimatization period of three months. Petroleum hydrocarbon-contamination presents adverse conditions for the growth of plant roots, and exposure to any stressor results in the excessive production of reactive oxygen species (ROS). ROS is known to play a role in gravitropism and it has been demonstrated that scavenging of ROS by antioxidants inhibited gravitropism. Hence growth in contaminated soil affects the growth direction of plant roots that possess fibrous root architecture. Importantly the plasticity exhibited by grass roots regarding growth direction seems to facilitate the establishment of grass plants in petroleum hydrocarbon-contaminated matrix as evidenced in the study by Balasubramaniam and Harvey. Furthermore, key structural components of root cells, such as cellulose, proteins, hemicellulose, lignin, pectin, and suberin, differ in proportion/volume along a developing root. It is hypothesised that the progressional changes in cellular structure from the root tip to the branching zone is likely to affect the uptake of organic chemicals including PAHs. So, a study on the structure of plant

roots exposed to the petroleum contaminants merits attention in the context of uptake of the toxic PAHs by plant roots. At the endodermis, which is a layer of cells packed with no intercellular space, separating the cortex from the central cylinder, molecules in the apoplastic stream are shunted from the apoplastic pathway into the symplastic stream since the deposition of Casparian strip and suberin lamellae in the endodermis prevents the uptake of molecules from the apoplast directly into the endodermis. Passage cells which are endodermal cells without suberin lamellae deposition would allow the substances which are in the apoplastic path and which are neither too big nor positively charged enter the symplastic stream at the endodermis to travel across the stele. Passage cells, if present, are generally situated opposite to the proto xylem poles, and solutes and water could enter the vessel elements unimpeded via the passage cells. Lateral roots generally appearing on a defined number of ranks on the mother root also face the internal xylem poles of the primary root. If Casparian bands are formed in the hypodermis, i.e., the cortex layer just beneath the epidermis, it is called exodermis. If exodermal Casparian band structures and suberin lamellae are present in a plant root, the restrictions on the apoplastic inflow of solutes occurs near the root surface. Organic contaminants such as PAHs can be absorbed by the roots for subsequent storage, metabolism or translocation via the transpiration stream, and this is termed phytoextraction. Phytoextraction is considered as a minor phytoremediation pathway in the remediation of petroleum hydrocarbons. Several investigators such as Verdin et al., Wild et al., Alkio et al. and Gao et al. have documented root absorption and uptake of PAHs. Alkio et al. have shown internalisation of phenanthrene in the root and leaf tissues of *Arabidopsis thaliana* with the use of gas chromatography-mass spectrometry (GC-MS) and fluorescence spectrometric techniques. Here plants were grown on Murashige and Skoog (MS) medium supplemented with 0.5 and 0.75 mM of phenanthrene. Similarly, Verdin et al. have shown intracellular accumulation of the low molecular weight PAH, anthracene, in the root cell lipid bodies of chicory (*Chicorium intybus*) grown on nutrient medium containing 140 mg L<sup>-1</sup> anthracene. However, it is important to note that the plants were grown hydroponically in nutrient medium in both studies, so that no competitive sorption could have occurred between PAHs and the organic carbon, as would have been the case had they been grown in soil. Hence, a correction factor for sorption of the PAHs to SOM may need to be applied when predicting PAH uptake in field conditions, when using the results of these studies. Another study carried out by Gao et al. in which 12 different species of plants were grown in phenanthrene or pyrene-spiked soil, containing 1.45% of organic matter (w/w), revealed that PAHs accumulated in the roots, with pyrene levels being higher than those of phenanthrene. Higher log K<sub>OW</sub> values denote a higher degree of hydrophobicity. Since highly hydrophobic substances can be adsorbed to lipophilic plant components easily, this is likely to have resulted from the higher log K<sub>OW</sub> values of pyrene

than that of phenanthrene . This finding is however, contradictory to the results of the study carried out by Zohair et al. , where PAH burden in potatoes and carrots was dominated by low molecular weight compounds, and BCFs (soilcrop bio concentration factors) were shown to decrease with increasing log KOW. The facts that the soil was experimentally spiked with PAHs and had only 1.45% of organic matter in the study by Gao et al. , whereas the crops were grown in organic farm soil with organic matter content ranging from 3% to 5% in the study by Zohair et al could explain the differences between the two results. The study by Gao et al. showed that different concentrations of phenanthrene and pyrene occurred between different plant s