Research Article

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

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Abstract

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.

Introduction

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 [1]. Petroleum hydrocarbon-based products make the largest part of this contamination [2]. 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 [3].

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 [4]. Soil is the major repository of PAHs [5] 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 [5].

Remediation of old industrial sites becomes necessary in meeting the demand for urban housing, office and leisure space [6]. 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. Also, following ingestion by organisms, PAHs can be metabolically transformed into mutagenic, carcinogenic and teratogenic agents such as dihydrodiol epoxides at the site of entry. 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 [5]. But, their lower bioavailability means that they actually present less of a danger than the more mobile pollutants (i.e., contaminants with lower K_{OW}). So, it is beneficial to the wider environment to reduce these toxic hazards caused by PAHs, especially low molecular weight (LMW) PAHs [6].

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 [7,8]. 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. Biological techniques such as phytoremediation are considered attractive due to their cost effectiveness and the

benefit of pollutant mineralization to carbon dioxide and water [6,7]. 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 [6]. 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 [6]: 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 [9,10]; 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 [11-14]. 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.

Contaminant exposure pathways and entry of contaminants into wildlife food chain

Plants growing in PAH-contaminated soils can potentially take up PAHs via i) uptake of PAHs in the soil solution through root tissues, ii) absorption of PAHs to the root surfaces, iii) foliar uptake of PAHs which have volatilised from the soil surface, and iv) absorption of PAHs from the atmosphere to leaf surfaces [15,16] (Figure 1).

Many studies indicated if PAHs are taken up within plant tissues they are sequestered in either cell wall following conjugation with glucose and or other cell wall components or vacuoles. Little is known about the bioavailability of bound metabolites, but there may be a need to prevent the movement of PAHs into wildlife food chains [17].

Uptake of organic pollutants by plants

Collins *et al.* [16] documented that uptake of organic pollutants by plants from contaminated soils depends on many factors:

Abiotic

• Physicochemical properties of the molecule, i.e., log K_{ow} and molecular weight

• Soil components (clays, iron oxides, organic matter)

Biotic

• Transpiration rates

• Types and amounts of lipids in root cells

• Enzyme complement
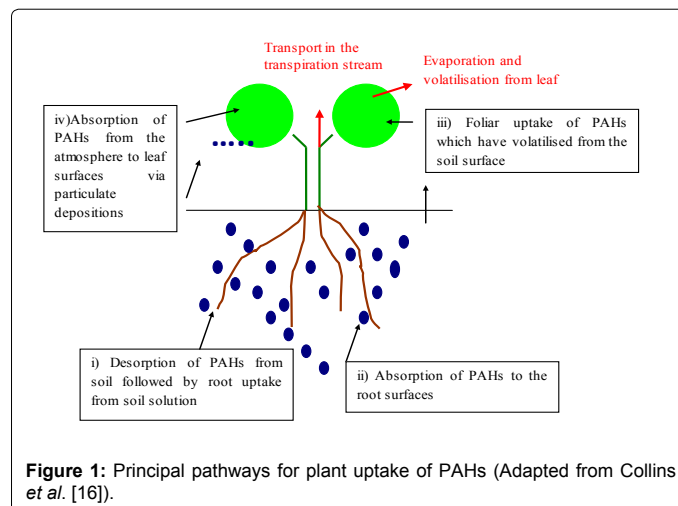
• Root exudates

• Growth dilution

Abiotic factors

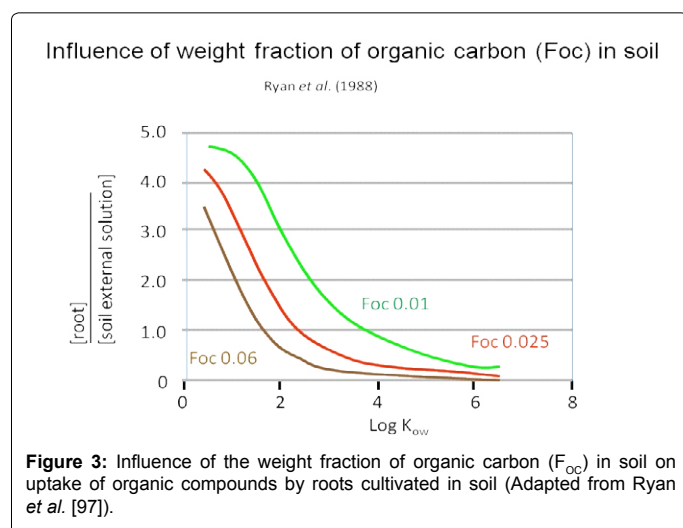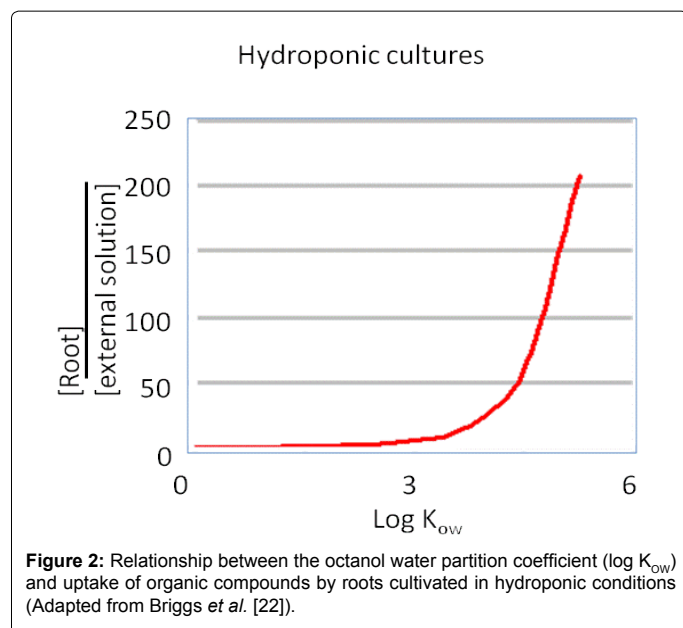**Physiochemical properties of the organic pollutant:** The hydrophobicity of a molecule is measured and classified according to its octanol-water partition coefficient (log K_{OW}), which describes its capacity to dissolve into an organic solvent (i.e., octanol) relative to an aqueous solvent (i.e., water). Generally, the hydrophobicity and environmental stability of a PAH molecule rises with an increase in size and the number of aromatic rings it possesses [18]. PAHs with more than three rings are often referred to as high molecular weight (HMW) PAHs, and those with three or less as low molecular weight (LMW) PAHs. Some PAHs are regarded as mobile pollutants, i.e., contaminants that can be leached with water [19]. Apart from naphthalene, a 2-aromatic ring, linear PAH, PAHs are practically insoluble in water and are very slow to degrade [6,7,20]. Naphthalene does not persist, and degrades rapidly to simpler, generally non-toxic compounds [6].

Soil composition: Organic chemicals such as PAHs can be sorbed or bound to several components in soil such as clays, iron oxides, and especially organic matter. Competitive sorption between plant lipids and soil organic carbon results in less bioavailability of the organic chemicals for plant roots at a higher soil organic matter (SOM) content [15,21]. Briggs *et al.* [22] for example examined the uptake of organic compounds by roots cultivated in hydroponic conditions and showed that molecules with a high log K_{ow} were taken out of solution by binding to roots (Figure 2).

Soil-plant transfer of persistent organic chemical residues like PAHs also depends on soil moisture content [22]. Normally, PAH uptake by crops is favoured under moist conditions in soils with low organic matter content. In dry soils with high organic matter content, chemical residues are strongly bound by soil, retarding uptake by plant roots [23].

An increase in log K_{OW} of the organic contaminant retards the uptake of organic chemicals by crops growing in soil [15] (Figure 3). Soil-crop bio concentration factors (BCFs) were reported to decrease with increasing log K_{OW} for PAHs up to about 4.5 (above this log K_{OW} value no changes were discernible). Here, BCF was measured by dividing contaminant concentration in peel and core of the roots by contaminant concentration in soil [23,24]. Several researchers including Karickoff [25] have found empirical relationships between the lipophilicity of a chemical and its affinity to sorb to soil organic matter. Non-mobile PAHs with a high log K_{OW} [e.g., benzo (a) pyrene-a 5 aromatic ring, branched PAH molecule] tend to partition in the organic humic phase [26], or are retained in the soil as a result of sorption, cation exchange, or precipitation processes, and are

Figure 1: Principal pathways for plant uptake of PAHs (Adapted from Collins *et al.* [16]).

Figure 2: Relationship between the octanol water partition coefficient (log K_{ow}) and uptake of organic compounds by roots cultivated in hydroponic conditions (Adapted from Briggs *et al.* [22]).

Figure 3: Influence of the weight fraction of organic carbon (F_{OC}) in soil on uptake of organic compounds by roots cultivated in soil (Adapted from Ryan *et al.* [97]).

thus far less bioavailable to soil organisms and plants. On the other hand, more water-soluble and more volatile low molecular weight compounds such as acenapthene, fluorine and phenanthrene are more susceptible to crop uptake [23]. It should however be noted that, stable PAHs originating from decades of industrial pollution will have a far lower bioavailability as a result of sorption to soil organic matter for long periods of time compared to those added experimentally [27,28]. Hence, when predicting uptake of experimentally added PAHs and evaluating their bio-phytoremediation aspect, a correction factor for sorption of PAHs to soil organic matter needs to be considered. On the other hand, in sand and water media, the PAH molecules are readily bio available, due to the absence of SOM that PAHs preferentially bind to, in these media.

Biotic factors:

Roots: The root is an important feature in phytoremediation, because plant roots are in direct contact with the contaminants in their surrounding soil. The presence of hydrocarbon contaminants modifies the soil structure [13,14] and soil structure influences root growth [29].

Roots modify biological activity, structure, water status and mineral constituents of the surrounding soil [30,31], but will also be modified themselves by the contaminants.

Plant roots have several functions in the terrestrial ecosystem such as:

a. Feeding soil organisms with carbon substrates and contributing to soil organic matter through root exudation and root death

b. Providing a habitat for mycorrhizal fungi and rhizosphere organisms including PAH degrading bacteria

c. Changing soil pH, and concentrating rare elements as well as toxic PAHs within the rhizosphere

d. Regulating plant growth via sensor-networking, predominantly due to plant hormones playing a central role in sensor network [32] frequently in conjunction with other signalling molecules such as sugars and sugar-like compounds [33].

e. Absorbing soil resources comprised of water and nutrients, whilst promoting formations of hydraulic conduits and redistributions of soil water and nutrients within the root growth zone

f. Strengthening the soil via humification and stabilisation; preventing soil erosion by forming a vegetative cover

g. Providing mechanical support for the plants which are essential for keeping a stable environment, by regulating the global climate as well as promoting the sustainability of their surroundings in several ways at different levels such as molecular, cellular, organ, individual, community, regional, ecosystem and global ecosystem levels [32].

h. Playing a vital role in sustaining the agricultural environment during times of stress, via production of higher root mass: higher amounts of photosynthetic sugars are translocated to the roots under stress conditions [34,35]. The roots of some crops such as carrot and beetroot are also storage organs, providing food for humans and other animals.

The pattern of development of a root system is termed root architecture. The recognition of different types of root architecture is important in phytoremediation as these roots morphological variations will influence the capacities of roots to stimulate the proliferation of PAH degrading microorganisms within the rhizosphere as well as to survive adverse conditions. 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 [36]. 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 adventurous root systems [37].

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 [38]. 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 adventitious root systems, several axile roots are generated from the stem throughout plant development. This type of root system is typical of grasses [37]. The root architecture of grasses is referred to as fibrous and is comprised of fine roots.

In plant-initiated remediation of PAHs or other organic contaminants, maximising root-soil contact is an important attribute. A high specific root length (SRL, length per unit mass) implies an effective and maximised root-soil contact. SRL varies between species. Generally, SRL is lower in the eudicots having coarse, woody root architecture (e.g., for apple tree, SRL is 5 m g^{-1}), than in grasses possessing fibrous root architecture (e.g., for ryegrass, SRL is 750 m g^{-1}) [38]. The amount of root in the soil and root length density (RLD), also vary among species and are generally higher for grasses as seems evident for SRL [31]. Grasses also show fast growth which is a favourable feature for phytoremediation. Grasses are favoured in the remediation of surface contamination [6,12], but deep rooted plants such as poplar are required to access contaminants found deep in the soil profile [39].

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 [40]. This guidance system constitutes both sensing and response mechanisms with which deviations from the preferred direction of growth can be corrected [40]. 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 [41]. At a smaller scale, roots generally exhibit some convolutions in response to the mechanical constraints and unfavourable factors which they experience [37].

In a study conducted by Balasubramaniyam and Harvey [42], 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 [13,14], 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 [43]. 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 Balasubramaniyam and Harvey [42].

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 [9]. 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.

The effect of transpiration on transport of organic contaminants from soil pore water across the root: The transport of water within the plant, which is known as translocation, is the major mechanism for the movement of nutrients and other components from soil pore water to plant [44]. Water and solutes are transported upward from the root into other plant parts through the xylem by mass flow resulting from a pressure gradient. This driving force is created during transpiration, where water is drawn in through the root system by capillary action to replace evaporative losses from stomata within the leaves [45].

Chemical transfers from the soil into the root are primarily mediated by the uptake of soil pore water during transpiration where contaminants can move from the root system to stems, leaves, and storage organs through the transpiration stream [45]. In order for chemicals to be taken up into plant roots to reach the xylem, they must first penetrate a number of plant tissues: the epidermis, the cortex, endodermis and pericycle, and need to be dragged along the water transportation pathway [15,46].

Water is transported into the conducting xylem vessels via the apoplastic fluxes or the symplastic stream. In the apoplastic pathway, water and molecules diffuse between cell walls and through intercellular air spaces whilst the symplastic pathway allows entry of molecules into the root cell cytosol after transport across the cell membrane or plasmodesmata (Figure 4) [9].

At the endodermis, which is a layer of cells packed with no intercellular space, separating the cortex from the central cylinder [47], 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 [48,49]. 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 [50] enter the symplastic stream at the endodermis to travel across the stele [48]. 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 [46]. Lateral roots generally appearing on a defined number of ranks on the mother root also face the internal xylem poles of the primary root [37]. 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 [46].
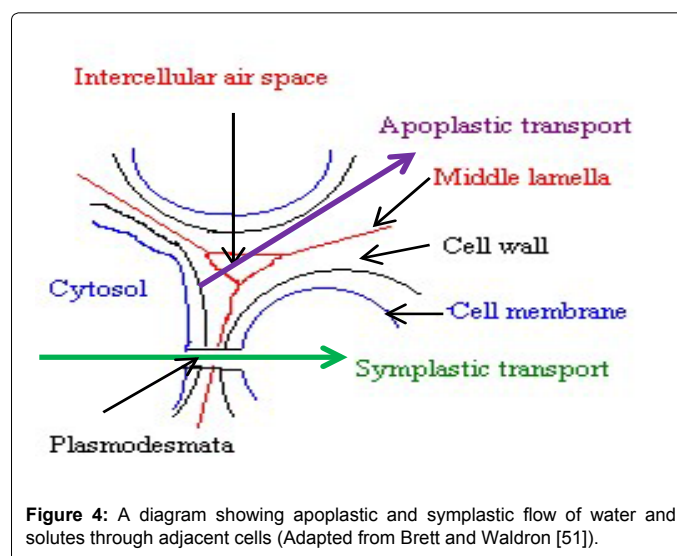

Figure 4: A diagram showing apoplastic and symplastic flow of water and solutes through adjacent cells (Adapted from Brett and Waldron [51]).

In the investigations conducted by Balasubramaniyam and Harvey [51] and Balasubramaniyam *et al.* [12], exodermal Casparian band structures and a lack of passage cells in the endodermis were shown in tall fescue grown in naphthalene-treated sand, together with extensively thickened endodermis. In the study of Balasubramaniyam [12], it has been demonstrated that hydrophobic xenobiotic compounds are unable to penetrate the inner core of the roots in the root hair zone of tall fescue adapted to grow in naphthalene-treated sand as opposed to the control roots, exemplified by the path of Nile red, apparently due to these root structural modifications.

The effect of root lipids content on root uptake of petroleum hydrocarbons: Organic contaminants such as PAHs can be absorbed by the roots for subsequent storage, metabolism or translocation via the transpiration stream [52-54], and this is termed phytoextraction. Phytoextraction is considered as a minor phytoremediation pathway in the remediation of petroleum hydrocarbons [13,14]. Several investigators such as Verdin *et al.* [55], Wild *et al.* [10], Alkio *et al.* [56] and Gao *et al.* [57] have documented root absorption and uptake of PAHs. Alkio *et al.* [56] 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 [56]. Similarly, Verdin *et al.* [55] have shown intracellular accumulation of the low molecular weight PAH, anthracene, in the root cell lipid bodies of chicory (*Chicorium itybus*) grown on nutrient medium containing 140 mg L^{-1} 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.* [57] 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_{OW} 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_{OW} values of pyrene than that of phenanthrene [57]. This finding is however, contradictory to the results of the study carried out by Zohair *et al.* [24], where PAH burden in potatoes and carrots was dominated by low molecular weight compounds, and BCFs (soil-crop bio concentration factors) were shown to decrease with increasing log K_{OW}. 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.* [57], 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.* [24] could explain the differences between the two results.

The study by Gao *et al.* [57] showed that different concentrations of phenanthrene and pyrene occurred between different plant species (Figures 5 and 6). These differences were positively correlated (*p*<0.05) with root lipid contents of the plants (Phenanthrene R^2=0.79; Pyrene R^2=0.86; N=12) [57]. The contribution from plant uptake to soil PAH removal was negligible (<0.01% for phenanthrene and 0.24% for pyrene) in the study by Gao *et al.* [57]. Still, this study was suggestive of translocation from root to shoot as the major pathway of shoot accumulation of PAHs.
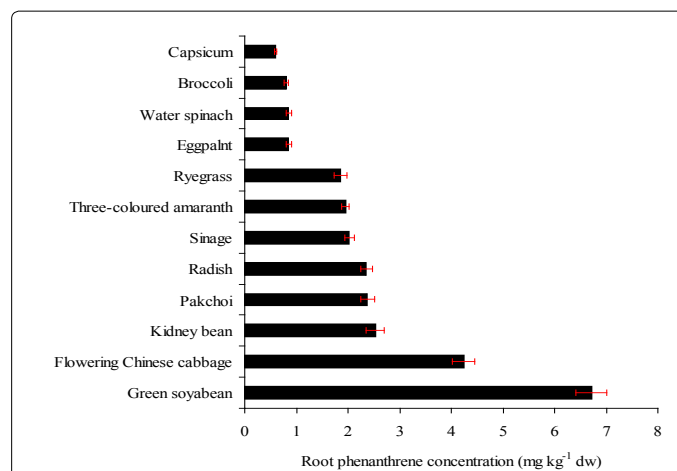
Figure 5: Root concentrations of phenanthrene (dw) for plants growing in spiked soils (soil pH 5.05, organic matter 1.45%) with initial phenanthrene concentration of 133 mg kg^{-1}, after 45 days of exposure to the PAH. Bars show standard error of the mean (Adapted from: Gao *et al.* [58]).
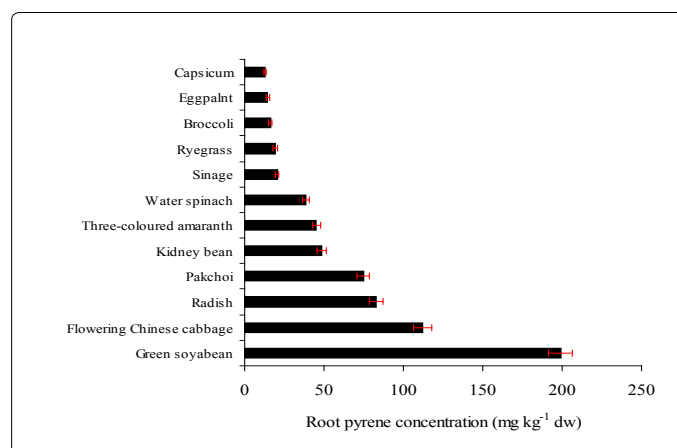
Figure 6: Root concentrations of pyrene (dw) for plants growing in spiked soils (soil pH 5.05, organic matter 1.45%) with initial pyrene concentration of 172 mg kg^{-1}, after 45 days of exposure to the PAH. Bars show standard error of the mean (Adapted from: Gao *et al.* [58]).

Chiou *et al.* [58] also suggested that plant lipids are a major factor in causing differences in plant uptake of lipophilic contaminants. Additionally, peeling carrots and potatoes removed 55.9-100% of PAH residues, depending on crop variety and contaminant properties [24]. Here, the higher lipid content in peels than that in their corresponding cores could explain the removal of organic pollutant residues by peeling [54,58]. Even though there was no correlation for PAHs with either carrots or potatoes, stronger correlations of polychlorinated biphenyls (PCBs) and organo chlorine pesticides (OCPs) in soil and their uptake by carrots than potatoes were found [24], and this could be due to the presence of oil channels in carrots [59]. Here oil channels refer to secreting ducts that secrete and store essential oils and can be found either on the surface of plants or within the plant tissue [60].

The microscopic observations of the passage of Nile red across the root tissues of tall fescue by Balasubramaniyam *et al.* [12] suggest that penetration of the hydrophobic xenobiotic compounds into the central cylinder where conducting vessels are located, did not occur in tall fescue roots growing in naphthalene-contaminated sand. It has

been assumed that mobile pollutants are drawn into the rhizosphere via transpiration stream, and that a limited amount of PAH uptake by plants takes place [18], but the xenobiotic compounds may not reach the xylem vessels which represent the predominant route for the long distance transportation towards the shoots. Hence, the PAHs taken up by plant roots may not reach the shoots.

Similarly, Wild *et al.* [10] demonstrated that the radial movement of anthracene and phenanthrene did not extend beyond the cortex cells of the roots of maize and wheat grown in a contaminated sand medium, over a 56 days growth period. Wild *et al.* [10] have shown highly focussed streaming of the xenobiotic compounds within the cortex of the plant roots, but also demonstrated that the xenobiotic compounds did not pass beyond the base of the roots into the stems. Additionally, the uptake and movement of anthracene within maize cell walls was approximately 3 times slower than that of phenanthrene, presumably reflecting differences in the solubility of the PAHs tested. Also, the uptake of both anthracene and phenanthrene were approximately 3 times slower in wheat than in maize, perhaps due to differences in lipid composition of the cell walls between the two plant species [10]. Furthermore, similarly as Harms [61], Wild *et al.* [10] reported that anthracene was located predominantly within the cell walls. Meagher [62] suggested that the plants isolate toxic xenobiotic compounds from the metabolic powerhouse of the cytoplasm, by sequestering them in either cell walls or vacuoles, presumably as a protective mechanism. Hence, the passage of PAHs across the root tissues could be predominantly apoplastic.

The effect of enzyme complements on catabolism of hydrocarbons within the root tissues: The metabolic breakdown of complex molecules into simpler ones, often resulting in a release of energy is termed catabolism. Catabolism of petroleum hydrocarbons within a biological system helps to achieve complete mineralization of the compounds to non-toxic end products such as CO_2 and H_2O and is typically undertaken by rhizospheric heterotrophic microorganisms. Plants which are phototrophic do not need to catabolize/ mineralise these compounds for energy extraction purposes, but appear nevertheless to catabolize them to non-toxic intermediates that are then stored in vacuoles or excreted into the cell wall. Since hydrocarbons are virtually insoluble in water, more highly reduced than cellular molecules, and chemically unreactive, they require specialised enzymes for their initial oxidation which is an essential prerequisite before they can be incorporated into cellular material or metabolised. Oxidation often means the removal of hydrogen atoms or electrons from the substrate; however, in the case of plant oxidation of hydrocarbons, the initial step of hydrocarbon oxidation usually requires the addition of an oxygen atom from atmospheric oxygen. There are two classes of direct oxidation:

a. Those in which both atoms of a molecule of O_2 are added to the hydrocarbon, catalysed by dioxygenases

b. Those in which only one atom of O_2 is added, catalysed by mono-oxygenases [63].

The addition of an oxygen atom increases both the solubility and the chemical reactivity of the hydrocarbon [64-66]. However, plants must carry the genes which code for the synthesis of these enzymes required for the oxidation of hydrocarbons. Oxidation of hydrocarbons within plant tissues depends both on the genetic composition of the plant and the type of hydrocarbon molecules [11,63,67].

Short-chain n-alkanes ($<C_9$) are soluble in water and can be found in higher concentrations in water, therefore they are toxic to organisms. They bind to membrane lipids and proteins and thereby cause disorganisation of the plasma membrane. n-Alkanes of the C_{10}-C_{22} range are usually readily metabolised, but higher molecular weight alkanes tend to be solid waxes and are not readily biodegraded. Unsaturated compounds and branched-chain compounds are also less readily biodegraded. Monocyclic aromatic hydrocarbons can be toxic, but in low concentration can be metabolised. PAHs with two to four rings are biodegraded at the rates that decrease with increase in the number of rings the PAH possesses. PAHs with five or more fused rings resist biodegradation. At low concentrations, cycloalkanes are degraded at moderate rates, but highly condensed cycloalkanes are resistant to biodegradation [3,63].

Adhesion between the cell and a hydrocarbon droplet followed by solubilisation of the droplet by specific agents may promote the entry of the droplet into the cell. Once inside a biological system, hydrocarbons, especially n-alkanes ($>C_9$ or C_{10}–C_{22}) are initially oxidised to the corresponding alcohol (n-alcohol) via the mono-oxygenase class of enzymes [63]. Metabolic studies have shown that a reducing agent such as NADH or NADPH has to be present, and is oxidised concurrently with the hydrocarbon molecule. Sufficient activation of the rather stable O_2 molecule to be split into two atoms is achieved by linking the mono-oxygenase enzymes to one or more of several different electron-carrier systems such as cytochrome P_{450} [17]. Pairs of electrons are transferred from NADPH or NADH via electron-carrier proteins to the mono-oxygenase. The enzyme then adds a single atom from molecular oxygen to the hydrocarbon and forms the corresponding alcohol and water (Figure 7). The mono-oxygenases are termed hydroxylases since a hydroxyl group is formed. They are also called mixed function oxidases as they oxidise a wide variety of substrates [63].

Further oxidation of primary alcohols proceeds via the aldehyde to the corresponding monocarboxylic acid (fatty acid); catalysed by the enzyme called dehydrogenase that removes hydrogen atoms from the hydrocarbons [63]. Subsequent metabolism of fatty acids could occur following the same pathway as that involved in lipid metabolism, such as β-oxidation pathway. This could lead to the generation of acetyl CoA, succinyl CoA and pyruvate molecules and eventually produce CO_2 and H_2O [62,63].

The ability of plants to catabolise hydrocarbons, especially aromatic hydrocarbons, is extremely limited, perhaps due to the inadequacy of the enzyme systems required for PAH-degradation within plant tissues [17,63]. Furthermore, plants do not require these compounds as a source of carbon and energy as microorganisms do. Nevertheless, metabolism of PAHs inside the plant root tissues may occur, depending on several factors such as the growth medium, the genetic composition
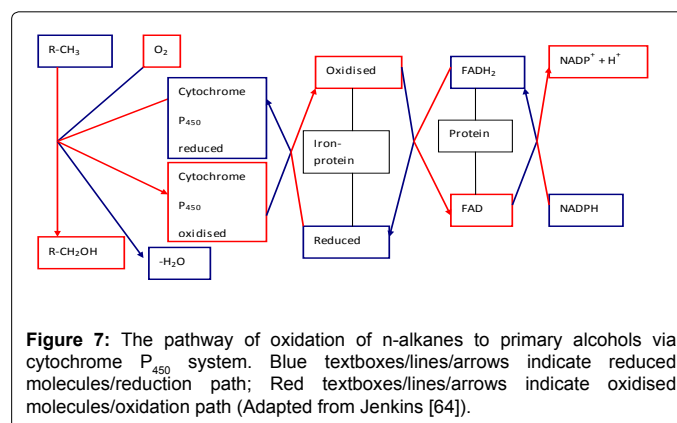
Figure 7: The pathway of oxidation of n-alkanes to primary alcohols via cytochrome P_{450} system. Blue textboxes/lines/arrows indicate reduced molecules/reduction path; Red textboxes/lines/arrows indicate oxidised molecules/oxidation path (Adapted from Jenkins [64]).

of the plant, and the concentration and physicochemical characteristics of the PAH. Wild *et al.* [10] have shown degradation of anthracene to the partial breakdown products anthrone, anthraquinone, and hydroxyanthraquinone in the zones of root elongation and branching of maize root, through in situ visualisation of the movement of these xenobiotic compounds that chemically auto-fluoresce inside living plant tissues, using two-photon excitation microscopy (TPEM). It has been suggested that the activity of most enzymes and enzyme systems change in different growth zones of the root and that the enzymatic activity is relatively high in the mature zones. The cells in the zone of elongation possess a complete profile of enzyme systems and the activity of some enzymes including those associated with PAH uptake and assimilation increase after elongation [68]. Anthracene degradation was principally observed in the mature region of the root, presumably because the enzymes involved in PAH-degradation were sufficiently developed in the mature zone of the plant roots [10].

Furthermore, investigations carried out by Kolb and Harms [69], Huckelhoven *et al.* [70], Sandermann [71] and Harms *et al.* [72] on fluoranthene, pyrene and benzo (a) pyrene in plant cell suspensions demonstrated biotransformation of the PAHs into hydroxyl-PAHs or PAH-quinones. These PAH metabolites are often considered to be conjugated with glucose, glucuronic acid or other cell wall components via the mono-oxygenase pathways [18,69,73]. According to Schnoor *et al.* [74], remediation can occur by the direct uptake of the contaminant into vegetative tissues, resulting in transformation by plant enzymes, sequestration within the plant, or transpiration through leaves.

Root exudates: Roots may modify the physical, chemical and biochemical characteristics of the soil in their vicinity (rhizosphere) by not only absorbing nutrients and other components in soil pore water, but also by releasing different compounds such as root boarder cells and root exudates [75]. Synthesizing, accumulating and secreting a diverse array of compounds are specialized processes mediated by roots in the rhizosphere. The chemicals secreted by roots into the soil are broadly classified as root exudates. Nearly 5% to 21% of all photosynthetically fixed carbon is transferred to the rhizosphere through root exudates, representing a significant carbon cost to the plant [76]. Root exudates have been traditionally classified into low-and high-molecular weight compounds. Low-molecular weight compounds such as amino acids, sugars, organic acids and phenolics comprise the majority of root exudates. Microorganisms can modify and degrade low molecular weight exudates like citric acid and phenolic compounds [77]. High-molecular weight compounds primarily include mucilage and proteins. Root exudates act as chemical messengers that communicate and initiate biological and physical interactions between roots and soil organisms, and participate in regulating the soil microbial community in the rhizosphere [76]. Therefore this remarkable metabolic feature of plant roots is important in plant-promoted biodegradation of organic compounds.

The presence of petroleum hydrocarbons in soil changes the rhizosphere environment of plant roots. Survival of any plant species in a particular growth environment depends chiefly on the ability of the plant to perceive changes in the local environment that require an adaptive response. Upon encountering a challenge, roots typically respond by secreting root exudates [78,79] which participate in root-soil contact as well as positive or negative communication with other plant roots and soil organisms [76]. The pattern of root exudates is not homogeneous between plant species, under different environmental conditions or along the root axis of a plant root [76,80].

An investigation conducted by Gao *et al.* [81] on the impact of root exudates on desorption of phenanthrene and pyrene in soils, revealed that the addition of Artificial Root Exudates (ARE) positively influenced the desorption of phenanthrene and pyrene in test soils. Gao *et al.* [81] reported that desorption of phenanthrene and pyrene increased with ARE concentration, and particularly higher with the addition of citric and oxalic acid, but decreased with higher soil organic matter (SOM). They attributed the increased desorption of phenanthrene and pyrene in the presence of AREs to the increased dissolved organic matter (DOM) in solution and decreased SOM, and indicated the dominant influence of organic acids on the desorption of the PAHs. They also reported that ageing of the PAHs had a negative impact on desorption of the compounds. Gao *et al.* [81] implied that root exudates conditionally promote the release of PAHs from the soil, enhancing the bioavailability of the PAHs with effects varying with the concentration of root exudates in soil and ageing of the PAHs. Root exudation processes may contribute to improving plant fitness from the point of phytoremediation, because, the presence of particular root exudates can stimulate the proliferation of PAH-degrading microorganisms within the rhizosphere [11,67]. Also some root exudates can keep soils hydrated under drought and drying conditions [76] and can soften hard, resistant soils to facilitate root growth [81,82]. Since crude oil may impose mechanical impedance and water scarcity on plant roots [13,14], these features of root exudates could be useful in facilitating the root growth of plants in petroleum hydrocarbon-contaminated soils.

Growth dilution due to phytostimulation or rhizo-biodegradation: As plants grow and their biomass increase over the growing season, the chemical concentration within plant tissues relative to the flux of chemical uptake would be diluted [15]. Growth dilution could also be referred to dilution of PAH-concentration within the dynamic region of the rhizosphere due to increased microbial activities as the root which is important in promoting phytostimulation increases in terms of mass, surface area, length and excretion of root exudates over the growing season, and is of importance in the successful establishment of plants in PAH-contaminated soils. It has been suggested that plants accumulate hydrophobic compounds such as PAHs in the rhizosphere after facilitating their transport toward the roots [17,82]. Remediation predominantly occurs by the rhizosphere-stimulated microbial biodegradation of organic pollutants [10,57,67,74]. It has been demonstrated that when grown in hydrocarbon-contaminated soil, plants increase the catabolic potential of rhizospheric soil (soil within 1 mm of plant roots) by altering the composition of the indigenous microbial communities and proliferating the PAH-degrading microorganisms within the dynamic region of the rhizosphere [10]. Here microbial catabolic potential relates to several parameters such as substrate specificity, inducer specificity, number of catabolic routes and kinetics of catabolic enzymes [83].

Certain microorganisms such as bacteria (e.g., some species of the genus *Pseudomonas*), filamentous fungi (e.g., some species of the genus *Cladosporium*) and yeast (e.g., some species of the genus *Candida*) use petroleum hydrocarbons as their sole source of carbon and energy. Additionally, several species of microorganisms are capable of degrading the hydrocarbons including the aromatic hydrocarbons, bringing them back into the carbon cycle and degrading them. Similar to plants, microorganisms also possess electron-carrier systems to facilitate hydrocarbon-degradation, perhaps to a greater extent. In addition to cytochrome P_{450}, rubredoxin systems are also found in bacteria. Rubredoxin is quite different from the cytochrome P_{450} system, and only uses NADH as reductant, but produces the same end-products, the corresponding primary alcohol and water. The

hydrocarbons are finally degraded to CO_2 and H_2O by microorganisms, as they convert these hydrocarbons into the chemical constituents of the cell [63].

Depending on the chemical nature of the individual hydrocarbons, some compounds are utilised readily by many microorganisms, whereas some other compounds resist microbial attack and can be utilised by only a few microbial species. Some microorganisms are not able to assimilate certain hydrocarbons, but they can oxidise these molecules. In mixed communities of microorganisms such as soils, the partially-oxidised hydrocarbon produced by one type of microorganism can serve as a growth substrate for another, leading way to co-metabolism. Here, the presence of one microorganism influencing positively the activity of another is referred to as co-metabolism. Furthermore, the presence of a hydrocarbon molecule may induce the synthesis of two sets of enzymes-one set capable of metabolising the substrate and another capable of metabolising another substrate. This is also an example of co-metabolism [63].

The rhizosphere contains a rich organic mixture of root exudates, leaked and secreted compounds, mucilage, and sloughed dead cells, allowing for the proliferation of soil microorganisms [39,84]. An increase of rhizosphere microbial populations over nonrhizosphere populations on the order of 4 to 100 times has been documented [85-87].

In an investigation carried out by Liste and Alexander [83,88] the rhizosphere of several plant species including tall fescue and wheat, grown in soil contaminated with phenanthrene and pyrene, temporarily contained appreciably more phenanthrene or pyrene than the unplanted soils, but the PAHs in the rhizosphere were degraded with time. Liste and Alexander [82,88] suggested that the degradation of pyrene and phenanthrene in the rhizosphere was due to the catabolic activities of the rhizospheric microorganisms. Additionally, in a study conducted by Siciliano [11] the number of soil bacteria that contained genes involved in hydrocarbon degradation were found to be higher in planted treatments composed of a mixture of grasses (*Bromus hordeaceous, Festuca arundinacea, Bromus carninatus, Elymus glaucus, Festuca ruba, Hordeum californicum, Leymus triticoides,* and *Nassella pulchra*) and legumes (*Trefoil fragiferum, Trifolium hirtum,* and *Vulpia microstachys*) when compared to the unplanted control ($p<0.05$) (Table 1).

Degradation of contaminants is principally based on the catabolic activities of microorganisms and their enzymes [7,89]. It has been reported that changes in exudate patterns of plant roots, which are induced by the presence of petroleum hydrocarbons in the rhizosphere, can cause alteration in the number and diversity of rhizospheric microorganisms [67,90], with a positive impact on contaminant degradation. The input of easily degradable root exudates such as organic acids improves physical and chemical soil conditions and increases humification and adsorption of pollutants in the rhizosphere. The root exudates also facilitate desorption of the PAHs, promoting their bioavailability [80]. The root exudates contribute to the enhanced microbial degradation of PAHs in the rhizosphere because they support larger microbial populations of PAH degraders [17].

Siciliano *et al.* [11] reported that mineralization of naphthalene was higher in the rhizosphere of tall fescue (*Festuca arundinacea*) grown in contaminated soil, than it was in the bulk soil ($p<0.05$). In contrast to this, growing rose clover (*Trifolium hirtum*) resulted in lower PAH-degradation activity in the rhizosphere compared to that in bulk soil ($p<0.05$) [11]. Other investigators have also reported a strong species

Treatments	Log colony forming units (cfu) g^{-1} soil		
	alkB positive bacteria	*ndoB* positive bacteria	*xylE* positive bacteria
Planted	6.5	6.5	6.5
Unplanted	5.5	<6	5.5

Table 1: Prevalence of soil bacteria that contained genes involved in hydrocarbon-degradation: *alkB* (gene encoding alkane mono-oxygenase), *ndoB* (naphthalene dioxygenase), and *xylE* (catechol-2, 3-dioxygenase), isolated on YTS 250 medium from planted and unplanted treatments; averaged over all the sampling points.

dependence on the ability of phytoremediation systems to promote PAH-degradation. This may be attributed to root exudate patterns but also to differences in the root architecture of the plants in question [11].

In a study carried out on benzo (a) pyrene [B(a)P] degradation using ryegrass (*Lolium perenne* L.), the extractable B(a)P concentration in the planted soil was found to be significantly lower than that in the unplanted control soil at the concentration of 50 mg B(a)P kg^{-1}, suggesting a potential of ryegrass to remediate B(a)P contaminated soil at this concentration [91]. Similarly, it was observed that the concentration of B(a)P in soil in which alfalfa (*Medicago sativa*) was grown over a period of 90 days, was significantly lower ($p<0.05$) than in unplanted soil [92]. In addition to that, Reilley *et al.* [93] demonstrated enhanced degradation of anthracene and pyrene by fescue (*Festuca* spp.) and alfalfa (*Medicago sativa*).

Also, it has been observed that PAH-degradation rates increased when alfalfa was inoculated with the arbuscular mycorrhizal fungus *Glomus caledonium* [92,94]. Moreover, anthracene degradation has been reported in the presence of chicory plants, and it was found that the degradation was higher when the roots were colonised by the arbuscular mycorrhizae, *Glomus intraradices* [56]. Studies by Binet *et al.* [95] also show the degradation of anthracene was greater in the presence of mycorrhizal ryegrass roots than in the absence of mycorrhizal colonisation.

Positive contribution of arbuscular mycorrhizal (AM) fungus in phytoremediation is possible through improved root growth by facilitating water and nutrient acquisition - important limiting factors in PAH-contaminated soil. Extra radical mycelium extending from root system can influence PAH degradation; through mycorrhiza-associated PAH degrading micro flora [95], but the AM fungus may also participate in the PAH-degradation more directly [55].

The degree of hydrocarbon-degradation was found to be greater in the rhizosphere of grasses, possibly due to the differences in cell wall components and root exudate patterns [13,14,89,96,97]. However, not all grasses contribute positively to remediation, and the fact that a plant is unaffected by contamination is not to be attributed to higher degradation of contaminants. In a study carried out by Merkl *et al.* [14], soils planted with *Brachiaria brizantha* and *Cyperus aggregatus*, that both showed morphological changes in contaminated compared to uncontaminated soil, had a higher degradation of petroleum than unplanted soil. On the other hand, no significant difference was found in the decrease of petroleum concentrations between a soil planted with *Eleusine indica,* which was unaffected by growth in contaminated soil, and its control. The reason why *Eleusine indica* did not have a significant impact on degradation is perhaps due to its small root surface area, being in itself not large enough to promote the proliferation of hydrocarbon-degrading microorganisms [14]. The characteristics of soil, the type and amount of oil contaminants, the species of plants and their root architecture, the indigenous micro flora in the soil and the environmental conditions (i.e., temperature, rainfall) all limit the

effectiveness of phytoremediation [11,13], which essentially depends on rhizo biodegradation promoting growth dilution in the rhizosphere.

Conclusion

Plants enhance the degradation of petroleum hydrocarbons in soil by supporting higher hydrocarbon-degrading microbial populations in the rhizosphere than are found in the bulk soil [11], increasing the bioavailability of PAHs, by influencing desorption of PAHs from the soil [80], and stabilisation of organic pollutants by polymerisation actions such as humification [18,90], whilst also allowing a limited amount of uptake into their cell walls and vacuoles [10,18]. The main pathway of phytoremediation is phytostimulation or rhizo biodegradation, in which the remediation of organic pollutants occurs mainly due to the catabolic activities of microorganisms proliferated by the presence of plant roots within the dynamic region of the rhizosphere [10,11]. Root architecture, root exudate patterns, cell wall components and genetic composition of the plant in question may give some plants better potential for phytoremediation [11,67].

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