Pollution, 2(3): 233-246 , Summer 2016
DOI: 10.7508/pj.2016.03.001
Print ISSN 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Plant-Aid Remediation of Hydrocarbon-Contaminated Sites

Daryabeigi Zand, A.[1] and Hoveidi, H. [2*]

1. Assistant Professor, Graduate Faculty of Environment, University of Tehran, Iran
2. Assistant Professor, Graduate Faculty of Environment, University of Tehran, Iran

Received: 9 Feb. 2016 Accepted: 10 Apr. 2016

ABSTRACT: Phytoremediation is an emerging green technology that uses plants and their associated microbes to remediate different environments contaminated with various pollutants. Phytoremediation, as an effective soil remediation technology, has gained popularity in the past ten years both in developed and developing countries. The main goal of the current article is to improve the understanding of phytoremediation of organic pollutants with emphasis on hydrocarbons. To design phytoremediation systems and also enhancement of their efficiency, either in laboratory or in field experiments, there is a serious need for better knowledge of phytoremediation mechanisms and also of factors affecting phytoremediation. In addition to phytoremediation applications, advantages, and limitations, its mechanisms and related new developments have been discussed in this article.

Keywords: contaminated soil, hydrocarbons, phytoremediation, plant.

INTRODUCTION

Over the past centuries, rapid growth of population, modern agricultural activities, waste disposal, mining, industrialization, etc., have significantly contributed to extensive soil contamination (Singh and Jain, 2003). Organic pollutants like petroleum hydrocarbons have had significant share of soil pollution, particularly in the recent century. Oil products have been disposed of in the environment for hundreds of years, assuming that the environment will adequately absorb them; however, this is no longer the case and accumulating pollutants are now affecting the health of living organisms (Escalante-Espinosa *et al*., 2005). Throughout the industrial world, petroleum is the primary source of fuel. As with any large scale industrial process, petroleum production can lead to contamination of soil and groundwater. Major causes of soil contamination with oil products include leaking storage tanks and pipelines, land disposal of petroleum waste, and accidental or intentional spills.

During the last decade, concerns about hydrocarbons in the environment have considerably increased. Among them, total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs) are of great interest as the accumulation of these compounds in soil might lead to significant risks to human through different exposure pathways (soils ingestion, etc.) (Denys *et al*., 2006). The development of methods to remediate soils contaminated with toxic pollutants and other organic residues has been an area of intense

[*]Corresponding Author Email: hoveidi@ut.ac.ir, Tel.: +989121995673

research interest for several decades (James and Strand, 2009; Macek *et al*., 2000). Various physical, chemical, and biological processes have been employed for effective remediation of contaminated soil; however, the remediation strategy depends on the nature of the contaminant(s). In situ microbial remediation (bioremediation) has been attempted, but it is often difficult to generate sufficient biomass in natural soils to achieve an acceptable rate of movement of tightly bound hydrophobic pollutants like PAHs to the microbes where they can be degraded (Huang *et al*., 2004).

On the other hand, soils contaminated with different organic compounds can be treated by thermal desorption, soil washing, incineration, etc. Physical removal of contaminated soil and washing of those soils with solvents are expensive. In general, due to the fact that almost all of the engineering-based remedial technologies are expensive and disruptive, there is a growing interest in developing new remediation technologies that are environment friendly, less expensive, and efficient. Phytoremediation, use of plants for remediation, is one such highly appealing technology (Mitton *et al*., 2016; Marrugo-Negrete *et al*., 2013; Ali *et al*., 2013; Chigbo *et al*., 2013; Cofield *et al*., 2008; Rezek *et al*., 2008; Chekol *et al*., 2004). There is a serious need to enhance the knowledge of phytoremediation of oil-contaminated soils. The main goal of the present study is to improve the understanding of phytoremediation of organic pollutants with emphasis on hydrocarbons.

Phytoremediation: Applications, Advantages, Limitations

Phytoremediation is an emerging green technology that uses plants and their associated microbes to remediate soil, sediment, surface water, and groundwater environments contaminated with toxic metals, organics, and radionuclides (Pilon-Smits, 2005). It has been proposed as a ‘‘green biotechnology’’, facilitating the elimination of environmental pollutants. It has gained a significant amount of public attention, especially in recent decade. Phytoremediation combines low costs with efficient erosion control and biodegradation of a wide range of organic pollutants, thus reducing the risk that these substances represent for human health. Phytoremediation can be a promising technology for the clean-up of petroleum-contaminated soils (Merkl *et al*., 2005). This technology makes use of plants and their associated soil microorganisms, soil amendments, and agronomic techniques to remove or render harmless environmental contaminants.

The major targets of phytoremediation are organic pollutants and toxic heavy metals that persist in soil for hundreds of years at smelter and mining sites, gas manufacturing plant sites, refineries, ammunition waste sites, landfills, nuclear waste dumps, over-fertilized farmlands, and agricultural, industrial, and municipal wastes (Singh and Jain, 2003).

Phytoremediation of both organic and inorganic contaminants can be affected by various environmental factors like temperature. The positive influence of temperature increase on plant growth in a phytoremediation study has been reported in the literature (Castro *et al*., 2004). In addition, plants’ uptake and metabolism of cyanide was studied in another phytoremediation study in response to temperature variation. Ten different temperatures were applied, ranging from 11°C to 32°C. It was observed that changes in temperature can have significant effect uptake and metabolism of cyanide by plants; however, accumulation of cyanide did not increase with temperature (Yu *et al*., 2007).

Phytoremediation of organic pollutants exploits the synergy between soil micro-organisms and plant roots to increase the rate of degradation of recalcitrant and potentially toxic compounds. Positive effects have thus been demonstrated for a wide range of molecules ranging from BTEX (benzene, toluene, ethylbenzene, and xylenes) and simple aliphatics in light crude oil and fuel (Abhilash *et al*., 2009) to more complex and highly recalcitrant pollutants like polycyclic aromatic hydrocarbons (Xiao *et al*., 2015; Liu *et al*., 2014a; Johnson *et al*., 2005), explosives, and pesticides (Van Aken, 2009; Thompson *et al*., 1998).

Plants may serve multiple roles influencing the fate of organic contaminants in soil, simultaneously promoting the degradation of the available fractions by stimulating microbial activity, increasing the number of sites in the organic matrix available for adsorption of organics and eventual binding by contributing root matter to the soil organic matter (SOM), and/or exuding compounds that increase the bioavailability of the contaminants (Parrish *et al*., 2005). For some persistent organic pollutants, such as PAHs phytodegradation seems to be one of the most promising approaches. It occurs mostly through an increase of the microbial activity in the plant rhizosphere, allowing the degradation of organic substances, a source of carbon for soil microbes (Denys *et al*., 2006).

Phytoremediation has many advantages but also some limitations. Phytoremediation has a number of inherent technical limitations. The contaminant must be within (or must be drawn toward) the root zones of plants that are actively growing. This implies water, depth, nutrient, atmospheric, physical, and chemical limitations. Phytoremediation is limited by root depth because the plants have to be able to reach the pollutant. Root depth is typically 50 cm for herbaceous species or 3 m for trees, although certain phreatophytes that tap into groundwater have been reported to reach depths of 15 m or more, especially in arid climates (Negri *et al*., 2003).

The plants that mediate the cleanup have to be where the pollutant is and have to be able to act on it. Therefore, the soil properties, toxicity level, and climate should allow plant growth. In addition, phytoremediation may also be limited by the bioavailability of the pollutants. If only a fraction of the pollutant is bioavailable, but the regulatory cleanup standards require that all of the pollutant is removed, phytoremediation is not applicable by itself. Pollutant bioavailability may be enhanced to some extent by adding some types of soil amendments (Pilon-Smits, 2005). These imply that phytoremediation is a site-specific technology and to some extent dependent on environmental conditions.

Many plant species are quite sensitive to contaminants, including TPHs and PAHs (Liu *et al*., 2014b). Therefore, either the plants do not grow or they grow slowly on contaminated soil. If growth is slow, the plants do not produce sufficient biomass to realize meaningful rates of remediation. Furthermore, in most contaminated soils, the population of microorganisms is depressed so that there are not enough bacteria either to

Table 1. Advantages and disadvantages of phytoremediation (Huang *et al*., 2004; US EPA, 2000)

Advantage	Disadvantage
- good image, high public acceptance - inexpensive - in situ - maintains soil and stimulates soil life - can be combined with other methods and has the potential to be rapid - solar driven - high levels of microbial biomass in the soil can be achieved	- few practical experience - long-lasting - only few uses of area possible - phyto and ecotoxicity (especially for inorganic pollutants) - not applicable for all compounds

facilitate contaminant degradation or to support plant growth. Another limitation with this technology is that it has not been demonstrated conclusively at many sites and in full-scale projects (Singh and Jain, 2003). Table 1 presents some important advantages and disadvantages of phytoremediation technology.

PHYTOREMEDIATION MECHANISMS

The introduction of plants to the polluted site(s) has the potential to yield several indirect contaminant attenuation mechanisms which assist in the removal of toxic substances/management of polluted sites. However, contaminant attenuation mechanisms involved in phytoremediation are complex and not limited only to the direct metabolism of contaminants by plants (Singh and Jain, 2003). Certain indirect attenuation mechanisms are involved in phytoremediation, such as the metabolism of contaminants by plant-associated microbes and plant-induced changes in the contaminated environment. In terrestrial species, transport of contaminants to the plant is dominated by the uptake of water by roots and distribution within the plant relies on xylem or phloem transport (Macek *et al*., 2000).

Various terms, reflecting each specific attenuation mechanism, have been extensively used to better describe specific applications of phytoremediation. Mechanisms believed to affect the transport and fate of organic contaminants in plants are the following: 1. effects of the root system on physical and chemical soil conditions, 2. overall increase of microbial population and diversity, 3. supply of root exudates and litter for co-metabolic degradation processes, 4. stimulation of humification, and 5. sorption, plant uptake, and translocation (Huang *et al*., 2004). We discuss the most important phytoremediation mechanisms in this section. These include phytoextraction, phytodegradation, phytovolatilization and rhizodegradation.

Phytoextraction

Plants can extract pollutants and accumulate them in their tissues, followed by harvesting the (above ground) plant material. This mechanism is called phytoextraction (Li *et al*., 2012; Huang *et al*., 2004). This process often occurs with heavy metals, radionuclides, and certain organic compounds that are resistant to plant metabolism, by uptake and translocation of such compounds in the soil by plant tissue in a recoverable form. Such hyper-accumulation is only possible when plants grow vigorously and produce over 3 t dry matter/hectare (Singh and Jain, 2003) able to accumulate large concentrations of the contaminant(s) in the harvestable plant tissue (>1,000 mg/ kg).

After a certain time period, the plants are harvested and disposed of or processed by incineration or, in the case of organic pollutants, composted for recycling. Phytoextraction is mainly used for metals and other toxic inorganics (Se, As, radionuclides). This is not a predominant mechanism in phytoremediation of organic pollutants like hydrocarbons.

Phytodegradation

It refers to a process beyond uptake and storage of contaminants. Plants can degrade organic pollutants directly via their own enzymatic activities, a process called phytodegradation (McGuinness and Dowling, 2009; Peuke and Rennenberg, 2005). In phytodegradation as a kind of phytotransformation, contaminants are taken up from soil/water, metabolized in plant tissues, and broken up to less toxic or non-toxic compounds within the plant by several metabolic processes via the action of compounds produced by the plant (Singh and Jain, 2003; Macek *et al*., 2000).

The overall metabolic process involved in phytodegradation is in some ways analogous to human metabolism of xenobiotic chemicals; thus, a ‘green liver’ conceptual model is often used to describe phytodegradation. The uptake of hydrophobic organic chemicals is very efficient while extremely hydrophobic or hydrophilic compounds are not very good candidates for phytoremediation. Such contaminants cannot be easily translocated within the plant, as they are either bound strongly to the surface of the roots or are not absorbed by roots and are actively transported through plant membranes. Phytodegradation works well for organics that are mobile in plants such as herbicides, TNT, MTBE, and TCE (Burken, 2003).

Phytovolatilization

After uptake in plant tissues, certain pollutants can leave the plant in volatile form; this is called phytovolatilization. This is another form of phytotransformation in which volatile chemicals or their metabolic chemical compounds are released into the atmosphere through plant transpiration. This mechanism has been observed for contaminants such as VOCs, PCBs, and total petroleum hydrocarbon (Macek *et al*., 2000). Many VOCs can be volatilized passively by plants. Pollutant volatility, expressed as Henry's law constant (H_i), is a measure of a compound's tendency to partition to air relative to water (Davis *et al*., 2003).

Pollutants with $H_i > 10^{-4}$ tend to move in the air spaces between soil particles, whereas pollutants with $H_i < 10^{-6}$ move predominantly in water. If H_i is between 10^{-4} and 10^{-6}, compounds are mobile in both air and water and able to move readily from the soil via the transpiration stream into the atmosphere. Both water-mobile and air-mobile organic contaminants can diffuse passively through plants. While the fate of water-mobile organics is phytodegradation or sequestration, volatile organics can be rapidly volatilized by plants without chemical modification.

Because volatilization completely removes the pollutant from the site as a gas, without need for plant harvesting and disposal, this is an attractive technology. Volatilization may be promoted in several ways. Although volatilization of VOCs is passive, the process may be maximized by using phreatophyte species with high transpiration rates and by promoting transpiration (preventing stomatal closure through sufficient irrigation) (Huang *et al*., 2004).

Phytostimulation or Rhizodegradation

The remediation process in which the contaminant is transformed by microbes in the rhizosphere (i.e., the microbe-rich zone in intimate contact with the vascular root system of the plant) is referred to as rhizodegradation, rhizosphere bioremediation, or phytostimulation (Bisht *et al*., 2015; Cebron *et al*., 2009; Huang *et al*., 2004). Plants can facilitate biodegradation of organic pollutants by microbes in their rhizosphere. The term phytostimulation is used for hydrophobic organics that cannot be taken up by plants but can be degraded by microbes. Examples are PCBs, PAHs, and other petroleum hydrocarbons (Hutchinson *et al*., 2001).

Rhizosphere remediation occurs completely without plant uptake of the pollutant in the area around the root. The rhizosphere extends approximately 1 mm around the root and is under the influence of the plant. Plants release a variety of photosynthesis derived organic compounds in the rhizosphere that can serve as carbon sources for heterotrophic fungi and bacteria. As much as 20% of carbon fixed by a plant may be released from its roots. As a result, microbial densities are 1-4 orders of magnitude higher in rhizosphere soil than in bulk soil, the so-called general rhizosphere effect (16).

In the rhizosphere, soil redox conditions, organic content, moisture, and other soil properties are manipulated by the activity of plant roots. Rhizodegradation is significantly responsible for the enhanced removal of total petroleum hydrocarbons from soil by deep-rooted trees and other annual species. The fate of PAHs and other organic contaminants in the environment is associated with both abiotic and biotic processes, including chemical oxidation, bioaccumulation, and microbial transformation. Microbial activity has been deemed the most influential and significant cause of PAH removal.

Organic pollutants may be degraded in the rhizosphere by root-released plant enzymes or via phytostimulation of microbial degradation. The enzymatic degradation of organics can happen in both root and shoot tissue. In one approach to stimulate rhizosphere degradation, certain agronomic treatments may be employed that favor the production of general and specific exudate compounds, such as clipping or fertilization (Leigh *et al*., 2002). Inorganic fertilizer is preferred over organic fertilizer (manure) for use in phytostimulation because the latter provides an easy-to-digest carbon source that microbes may prefer to use instead of the organic pollutant. Various phytoremediation mechanisms mentioned above can occur simultaneously. Because the processes involved in phytoremediation occur naturally, vegetated polluted sites have a tendency to clean themselves up without human interference.

METABOLISM OF ORGANIC CONTAMINANTS DURING PHYTOREMEDIATION IN PLANTS

Most of the available literatures in the world present some information about phytoremediation mechanisms but they rarely discuss metabolism of organic contaminants during phytoremediation in plants (Singh and Jain, 2003). We discuss this important issue in the current section. The specific interactions of a pollutant with soil, water, and plants will vary depending on the chemical properties of the contaminant, the physiological properties of the introduced plant species, and the contaminated medium.

Organic compounds can be translocated to plant tissues (Salt *et al*., 1998) and subsequently volatilized, they may undergo partial or complete degradation, or they may be transformed to less phytotoxic compounds and bound in plant tissues. Collectively, these properties determine whether a contaminant is subjected to phytoextraction, phytodegradation, phytovolatilization or rhizodegradation, although in all cases, the process of phytoremediation begins with contaminant transport to the plant. In general, most organics appear to undergo some degree of transformation in plant cells before being sequestered in vacuoles or bound to insoluble cellular structures such as lignin.

The metabolism of certain organic contaminants such as PAHs, TCE, 2, 4, 6-trinitrotoluene (TNT), glyceroltrinitrate (GTN), and other chlorinated compounds has been documented (Singh and Jain, 2003; Esteve-Nunez *et al*., 2001; Macek *et al*., 2000). Uptake of pollutants by plant roots is different for organics and inorganics. Organic pollutants are usually manmade, and xenobiotic to the plant. Consequently, there are no transporters for these compounds in plant membranes. Organic pollutants, therefore, tend to move into and within plant tissues driven by simple diffusion, dependent on their chemical properties.

An important property of the organic pollutant for plant uptake is its hydrophobicity. Hydrophobicity is usually expressed as the octanol: water partition coefficient, or log K_{ow} (the octanol: water distribution coefficient, a measure for pollutant hydrophobicity). Organics with a log K_{ow} between 0.5 and 3 are hydrophobic enough to move through the lipid bilayer of membranes, and still water soluble enough to travel into the cell fluids. If organics are too hydrophilic (log $K_{ow} < 0.5$), they cannot pass through membranes and never get into the plant; if they are too hydrophobic (log $K_{ow} > 3$), they get stuck in membranes and cell walls in the periphery of the plant and cannot enter the cell fluids (Huang *et al*., 2004).

Because the movement of organics into and through plants is a physical rather than biological process, it is fairly predictable across plant species and lends itself well to modeling. Uptake in terrestrial plants has been studied for many plant contaminant combinations and quantitative models to predict uptake rates have been documented. It should be considered that depending on the phytoremediation strategy, pollutant uptake into the plant may be desirable (e.g., for phytoextraction) or not (e.g., for phytostabilization). For either application, plant species with the desired properties may be selected (Huang *et al*., 2004).

FACTORS AFFECTING PHYTOREMEDIATION OF ORGANIC POLLUTANTS

To increase the efficiency of phytoremediation technologies, it is important that we learn more about the factors affecting phytoremediation. There are so many factors involved in phytoremediation of organic pollutants. For example the movement of an organic contaminant in soil toward plant roots depends on the chemical's relative water solubility, vapor pressure, molecular size, and charge and on the presence of other organics in the soil. The ability of soil to absorb and sequester organics is directly associated with the organic matter content of soil, the type and amount of clay present, soil structure, and the pH as well as with the age of the spill and water flux through the profile. A wide range of parameters that influence the efficiency of phytoremediation still remains to be identified. But some important factors contributing to phytoremediation of polluted sites with organics like hydrocarbons are presented in this section.

Pollutant Bioavailability

Bioavailability is important in determining both the toxicity of contaminants and their accessibility to both microorganisms and higher plants (Allard *et al*., 2000). For plants and their associated microbes to remediate pollutants, they must be in contact with them and able to act on them. Therefore, the bioavailability of a pollutant is very important for its remediation. Pollutant bioavailability depends on the chemical properties of the pollutant, soil properties, environmental conditions, and biological activity. Two important chemical properties of a pollutant that affect its movement in soils are hydrophobicity and volatility. A high log K_{ow} corresponds with high hydrophobicity. Extremely hydrophobic molecules such as PCBs, PAHs, and other hydrocarbons (log $K_{ow}> 3$) are tightly bound to soil organic matter and do not dissolve in the soil pore water. This lack of bioavailability limits their ability to be phytoremediated, leading to their classification as recalcitrant pollutants.

Additionally, in viewpoint of plant uptake, if organics are too hydrophobic (log $K_{ow}>3$), they get stuck in membranes and cell walls in the periphery of the plant and cannot enter the cell fluids (Huang *et al*., 2004). If organics are too hydrophilic (log $K_{ow}< 0.5$), they cannot pass through membranes and never get into the plant. Organics with moderate to high water solubility (log K_{ow} between 0.5 and 3) are able to migrate in the soil pore water to an extent that is inversely correlated with their log K_{ow}. In addition, the bioavailability of contaminants can be influenced by soil properties, including soil texture, organic matter content, water content, pH, and structure, in addition to properties of the contaminant and environmental factors, such as temperature and moisture (Parrish *et al*., 2005).

Soil properties

Soils exert strong effects on rhizosphere communities, having different pH, aeration, and physio-chemical characteristics that result in distinct microbial communities.

There are contrasting reports on the interaction between plant species and soil type on rhizosphere microbial communities. In some cases, the effect of soil type was greater than that of the plant species (Damastri *et al*., 1999), while in studies by Grayston *et al*. (1998) and Miethling *et al.* (2000), plant species had a stronger effect on the community composition than soil type.

Soil properties have direct and indirect influences on phytoremediation efficiency. For example, soil constituents may affect the biodegradation of PAHs by directly influencing the activity of microorganisms themselves (e.g. presence of nutrients, O2 diffusion, etc.) and not just indirectly by modifying the PAH bioavailability. Soils with small particle size (clay) hold more water than sandy soils, and have more binding sites for ions, especially cations. The concentration of organic matter (humus) in the soil is also positively correlated with cation exchange capacity (CEC), as well as with the capacity to bind hydrophobic organic pollutants. This is because humus mainly consists of dead plant material, and plant cell walls have negatively charged groups that bind cations, as well as lignin that binds hydrophobic compounds (Burken, 2003).

Plant type

A variety of plant species have been identified with the capability to enhanced rhizosphere degradation of many recalcitrant contaminants (Cofield *et al*., 2007). Various plant species used in phytoremediation studies have shown different capabilities to remove petroleum hydrocarbons in soil as presented in Table 2. Different phyto-mechanisms make use of different plant properties and typically different plant species. Plants (and plant types) are known to vary widely with respect to root parameters such as morphology, root exudation, fine root turnover, root decomposition, and associated microbial communities. If the dominant mechanism of PAHs dissipation in planted soil is associated with the activity of rhizosphere microbial communities, then it would be expected that remediation potential would also vary across plant species and life-history types.

Among studies that have evaluated multiple species within a given experiment, some have found species-specific and life-history differences with respect to enhancement of PAHs dissipation while others have not (Chen *et al*., 2000). Favorable plant properties for phytoremediation of organic pollutants in general are to be fast growing, high biomass, competitive, hardy, and tolerant to pollution. In addition, high levels of plant uptake, translocation, and accumulation in harvestable tissues are important properties for phytoextraction of inorganics. Favorable plant properties for phytodegradation are large, dense root systems and high levels of degrading enzymes. A large root surface area also favors phytostimulation, as it promotes microbial growth. Furthermore, production of specific exudate compounds may further promote rhizodegradation via specific plant-microbe interactions (Huang *et al*., 2004; Pilon-Smits, 2005).

Nutrient supply for plants and rhizosphere microbes

For successful phytoremediation, both plants and microbes must survive and grow in contaminated soil. Nutrient availability for plants is an important factor governing the success of phytoremediation and can be regulated through the addition of fertilizer (Alvarez-Lopez *et al*., 2016; Sessitsch *et al*., 2013; Hutchinson *et al*., 2001). The inorganic nutrients that are most often limiting in the bioremediation and phytoremediation of hazardous organic compounds are nitrogen (N) and phosphorus (P).

Generally, N is the growth limiting nutrient and, therefore, is needed in the highest concentration. Organic and inorganic N amendments result in

Table 2. Comparison of phytoremediation investigations results using different plant species

Compounds	Used Plants	Selected Findings	Reference
TPHs	Sorghum, Ryegrass, St. Augustine Grass	– Ryegrass and St. Augustine Grass demonstrated better performance compared to sorghum (25% more removal rate)	Nedunuri *et al*., 2000
Diesel and heavy oil	Mixture of Grasses and Legumes	– Significant reduction of TPHs in planted treatments compared to unplanted treatments	Banks *et al*., 2003
Aged petroleum Hydrocarbons	Mixture of Grasses and Legumes	– Removal rate of TPHs in planted treatments was reported to be 38 miligram per kilogram soil per month while the removal rate was reduced by half in unplanted treatments.	Siciliano *et al*., 2003
Aged petroleum Hydrocarbons	29 Vascular Plant Species	– Variation in TPHs removal between planted and unplanted treatments was not significant after 180 days phytoremediation	Kulakow *et al*., 2000
Diesel	Mixture of Grasses and Legumes	– Rhizodegradation of diesel was significantly enhanced in presence of plant species in soil. – Dissipation rate of diesel in the rhizosphere of legumes was greater than that of grasses. – Nutrient availability was shown to be the limiting factor affecting phytoremediation effectiveness.	Riser-Roberts, 1998
Oil Shale	Mixture of Ryegrass, Kentucky Bluegrass and Fescue	– Oil Concentration was declined by 93% in presence of plant species in soil after four months. – Oil removal was increased by the factor of ten in presence of plant species.	Truu *et al*., 2003
PAHs	Reed and Alfalfa	– Reed and alfalfa removed PAHs by, respectively, 68.7% and 74.5% after two years. – Microorganisms population and activity in presence of alfalfa was greater than reed.	Muratova *et al*., 2003
PAHs	Alfalfa	– PAHs degradation in soil was enhanced in planted treatments in comparison with unplanted treatments. – Concentration of higher molecular weight PAHs i.e. Indeno (1,2,3-c,d) pyrene and Dibenzo (a,h) anthracene reduced by 58% and 55%, respectively, during the phytoremediation period.	Tassi *et al*., 2004

increased plant biomass production and greater reductions of TPHs. Appropriate agronomic practices such as tillage and lime additions can also be used to improve soil physical and chemical conditions to enhance plant and microbial growth. However, the addition of mineral nutrients has given variable results. Both negative and positive effects of N and P on degradation of single PAHs have been reported.

In the case of PAHs originating from creosote pollution, Phillips *et al*. (2000) observed a positive effect only of P amendments, with no or negative effects of N or N+P, on the other hand, noted a positive effect of N+P on degradation of four-ring PAHs in a creosote polluted soil, but none on three-ring PAHs. Furthermore, due to the hydrophobic nature of oil-contaminated soils, the fertilizer solution initially accumulated at the soil surface before percolating into the soil. Plants might have damaged ('burned') by direct exposure to the fertilizer solution or by accumulated fertilizer concentrations in the upper soil layer. But generally, carbon mineralization rates increased in response to the addition of fertilizer, indicating the importance of sufficient nutrients in enhancing oily waste decomposition in soil (Hutchinson *et al*., 2001).

In general, the nutrient requirements for microbes are approximately the same as the composition of their cells. The exception to this is carbon, which is needed at higher quantities and can be supplied by the contaminant for heterotrophic microorganisms. In addition to C, heterotrophic microorganisms require inorganic nutrients to degrade organic contaminants. There are three categories of nutrients based on the quantity and essential need for them by the rhizosphere microorganism: macro, micro, and trace nutrients. For example, the macronutrients carbon, nitrogen, and phosphorus are known to comprise 50, 14, and 3% dry weight, respectively, of a typical microbial cell. Sulfur, calcium, magnesium, which are micronutrients only comprise 1, 0.5, and 0.5%, respectively, of the dry weight of a cell (Huang *et al*., 2004).

Trace nutrients, which are found in the least quantity, are not required by all organisms. The most common trace elements are iron, manganese, cobalt, copper, and zinc. Based on this approach, the optimal C: N: P mole-ratio recommended for biological remediation techniques is 100:10:1 (Hutchinson *et al*., 2001). For example, 150 mg of nitrogen and 30 mg phosphorus would be required to degrade one gram of a theoretical hydrocarbon into cellular material. Studies that have detailed the metabolic pathways of PAHs have indicated that the carbon evolution into carbon dioxide occurs at the end of the degradation. Therefore, additional carbon supplements would not be required until the degradation of the individual contaminant is almost completed.

NEW DEVELOPMENTS IN PHYTOREMEDIATION OF ORGANIC POLLUTANTS

In the recent decade, phytoremediation has gained acceptance as a remediation technology and has been acknowledged as an area of research. However, phytoremediation efficiency is still limited by a lack of accurate knowledge of many basic mechanisms such as plant processes, plant-microbe interactions, pollutant characteristics, etc. Therefore, there is a need for phytoremediation enhancement and more field studies to demonstrate and improve the effectiveness of this technology for remediation purposes. Some efforts have been made in recent years to enhance phytoremediation, particularly for persistent organic pollutants. We categorized these new developments in two different groups: phytoremediation potential to combine with other approaches and introduction of transgenic plants.

Potential to combine with other methods

One of the feasible, applicable, and recent approaches to enhance the effectiveness of soil pollution removal by phytoremediation technology is its potential to combine with some other techniques. Among several methods to enhance the efficiency of phytoremediation, the most common and influential techniques which can be combined with phytoremediation are bioremediation, land-farming, application of Plant Growth Promoting Rhizobacteria (PGPR), biosurfactant application, and/or their combination.

Introduction of transgenic plants

One new development in phytoremediation is the use of transgenic plants. Knowledge gained from plant molecular studies in recent years has led to the development of some promising transgenics that show higher tolerance, accumulation, and/or degradation capacity for various pollutants. Although plants have been found that will naturally take up and metabolize organic and inorganic pollutants, transgenic plants have enhanced phytoremediation capabilities in contaminated sites.

Recently, some researchers have used wild or genetically modified plants (GMPs) to extract a wide range of heavy metals and organic pollutants from the soil. In one experiment, plants with a profound increase in metabolism of trichloroethylene (TCE) have been engineered by introducing the mammalian cytochrome P450 2E1. This enzyme oxidizes a wide range of important pollutants, including TCE, ethylene dibromide, carbon tetrachloride, chloroform, and vinyl chloride. The transgenic plants had a dramatic enhancement in metabolism of TCE of up to 640-fold as compared with null vector control plants. It seems that transgenic plants with this enzyme can be used for more efficient phytoremediation of many sites contaminated with halogenated hydrocarbons.

Doty *et al*., (2000) developed transgenic plants able to degrade explosive nitrate esters and nitroaromatics by introducing the bacterial enzyme pentaerythritol tetranitrate reductase. Other new developments in plant genetic engineering are tailored transgenics that overexpress different enzymes in different plant parts (e.g., root-specific expression of one gene and shoot-specific expression of another) or that express a transgene only under certain environmental conditions. Transgenic plants have also been engineered to rapidly detoxify and transform some xenobiotic chemicals (Dietz and Schnoor, 2001).

To date, most of the efforts in this field have been made to remove heavy metals from contaminated soils and application of transgenic plants for organic pollutants has been limited. Initial experiments with transgenic plants have shown that they are indeed efficient in drawing metals from heavily contaminated soils. In the coming years, development of new genomic technologies may lead to the identification of novel genes important for organic pollutant remediation. The expression of these genes may then be manipulated in high-biomass species for use in phytoremediation.

However, despite potential advantages of this approach, the progress and application of this technology to tackle widespread environmental problems is being hampered by ideology-driven, restrictive legislation over the use and release of genetically modified plants in some European countries like Germany. So far, transgenic plants have mainly been tested in laboratory studies (Huang *et al*., 2004). As transgenics are being tested in the field and the associated risks assessed, their use may become more accepted and less regulated, as has been the case for transgenic crops.

CONCLUSION

Phytoremediation offers an in situ, cost-effective, aesthetic, non-invasive, and environment-friendly alternative or complementary technology for conventional remediation methods such as soil incineration or excavation and pump-and-treat systems especially for developing countries. During phytoremediation, both plants and microorganisms take part in the degradation process, either independently or through synergistic effects. Phytoremediation can be an effective remediation approach for reduction of many organic pollutants in soils. Several plant species have shown promising potential for phytoremediation of many highly toxic and recalcitrant organic compounds such as TPHs, PAHs, etc. For successful phytoremediation, both plants and microbes must survive and grow in contaminated soil. Unlike inorganic pollutants, which are immutable at an elemental level, organic pollutants can be degraded or even mineralized by plants or their associated microorganisms. Rhizospheric microorganisms can play a major role in the decomposition of many organic contaminants.

Phytoremediation has some advantages over other natural technologies like land-farming or bioremediation provided that plants can grow on the contaminated soil and attain biomass sufficient for phytodegradation and root-associated microbial degradation. For instance, one of the advantages of phytoremediation over bioremediation is the efficiency of plants at removing the larger, more strongly soil-bound persistent organic contaminants like PAHs. However, phytoremediation alone has some intrinsic limitations, especially for large-scale applications.

Although phytoremediation works effectively for a wide range of organic and inorganic pollutants, the underlying biological processes are still largely unknown in many cases. Aspects such as the role of enzymes, metabolites, microorganisms, plant selection, and genetic engineering have to be better understood. Proposals of using multi-stage phytoremediation processes seem to be attractive, since combination of processes, as mentioned in former sections, could overcome some limitations of phytoremediation alone, particularly for removal of persistent organic pollutants.

It should be considered that actual environmentally contaminated and aged soils often behave differently from laboratory-spiked soils with respect to phytoremediation. Field studies typically do not obtain the same results as laboratory scale studies do. The reason is the mass transfer limitations that occur. In addition, the bacteria and supplemental nutrients are not always able to reach the contamination in contaminated sites. Further research needs to be conducted on mass transfer limitations to address this concern in field applications. Phytoremediation efficiency is still limited by a lack of knowledge of many basic plant processes and plant-microbe interactions. There is a serious need for more phytoremediation field studies to demonstrate the real effectiveness of the technology and increase its acceptance.

Finally, one should consider that phytoremediation is still a site-specific process. The successful implementation of phytoremediation at one site for one contaminant does not guarantee similar success on a different soil type or the ability to degrade a different contaminant. As an aesthetically desirable approach, it seems that phytoremediation might be extensively used in both developed and developing countries in the upcoming years. However, there is a great need to design appropriate research on phytoremediation of various contaminants considering site characteristics and environmental factors.

REFERENCES

Abhilash, P.C., Jamil, S. and Singh, N. (2009). Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol. Adv., 7, 474-488.

Ali, H., Khan, E. and Sajad, M.A. (2013). Phytoremediation of heavy metals- concepts and applications. Chemosphere, 91 (7), 869-881.

Allard, A.S., Remberger, M. and Neilson, A. (2000). The negative impact of aging on the loss of PAH components in a creosote-contaminated soil. Int. Biodeter. Biodegr., 46, 43-49.

Alvarez-Lopez, V., Prieto-Fernandez, A., Cabello-Conejo, M.I. and Kidd, P.S. (2016). Organic amendments for improving biomass production and metal yield of Ni-hyperaccumulating plants. Sci. Total Environ. 548, 370-379.

Banks, M.K., Schwab, P., Liu, B., Kulakow, P.A., Smith, J.S. and Kim, R. (2003). The effect of plants on the degradation and toxicity of petroleum contaminants in soil: A field assessment. Adv. Biochem. Eng. Biotech. 78, 75-96.

Bisht, S., Pandey, P., Bhargava, B., Sharma, S., Kumar, V. and Sharma, K.D. (2015). Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. Braz. J. Microbiol., 46(1), 7-21.

Burken, J.G. (2003). Uptake and metabolism of organic compounds: green-liver model. In: S.C. McCutcheon and J.L. Schooner (Eds), Phytoremediation: Transformation and Control of Contaminants, New York: Wiley, 59-84.

Castro, S., Davis, L.C. and Erickson, L.E. (2004). Temperature and pH effects on plant uptake of benzotriazoles by sunflowers in hydroponic culture. Int. J. Phytoremediation. 6(3), 209-25.

Cebron, A., Beguiristain, T., Faure, P., Norini, M.P., Masfaraud, J.F. and Leyval, C. (2009). Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated Soil. Appl. Environ. Microbiol. 75(19), 6322-6330.

Chekol, T., Vough, L. and Chaney, R. (2004). Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect. Environ. Int., 30, 799-804.

Chen, P., Pickard, M.A. and Gray, M.R. (2000). Surfactant inhibition of bacterial growth on solid anthracene. Biodegradation, 11, 341-347.

Chigbo, C., Batty, L. and Bartlett, R. (2013). Interactions of copper and pyrene on phytoremediation potential of Brassica juncea in copper–pyrene co-contaminated soil. Chemosphere, 90 (10), 2542-2548.

Cofield, N., Banks, M.K. and Schwab, A.P. (2007). Evaluation of hydrophobicity in PAH-contaminated soils during phytoremediation. Environ. Pollut., 145, 60-67.

Cofield, N., Banks, M.K. and Schwab, A.P. (2008). Lability of polycyclic aromatic hydrocarbons in the rhizosphere. Chemosphere, 70 (9), 1644-1652.

Damastri, C., Chiarini, L., Cantale, C., Bevivno, A. and Tabacchioni, S. (1999). Soil type and maize cultivar affect the genetic diversity of maize-associated Burkholderia cepacia populations. Microb. Ecol., 38, 273-284.

Davis, L.C., Erickson, L.E., Narayanan, N. and Zhang, Q. (2003). Modeling and design of phytoremediation. In: S.C. McCutcheon and J.L. Schooner (Eds.), Phytoremediation: Transformation and Control of Contaminants New York: Wiley. 663-694.

Denys, S., Rollin, C., Guillot, F. and Baroudi, H. (2006). In-situ phytoremediation of PAHs contaminated soils following a bioremediation treatment. Water Air Soil Pollut., 6, 299-315.

Dietz, A.C. and Schnoor, J.L. (2001). Advances in Phytoremediation. Environ. Health Persp., 109, 163-168.

Doty, S.L., Shang, T.Q., Wilson, A.M., Tangen, J., Westergreen, A.D., Newman, L.A., Strand, S.E. and Gordon, M.P. (2000). Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. Appl. Biol. Sci., 97(12), 6287-6291.

Escalante-Espinosa, E., Gallegos-Martınez, M.E., Favela-Torres, E. and Gutierrez-Rojas, M. (2005). Improvement of the hydrocarbon phytoremediation rate by Cyperus laxus Lam. inoculated with a microbial consortium in a model system. Chemosphere, 59, 405-413.

Esteve-Nunez, A., Caballero, A. and Ramos, J.L. (2001). Biological Degradation of 2,4,6-Trinitrotoluene. Microbiol. Mol. Boil. Rev. 65 (3), 335-352.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C. (1998). Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem., 30, 369-378.

Huang, X.D., El-Alawi, Y., Penrose, D., Glick, B. and Greenberg, B. (2004). A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Pollut., 130, 465-476.

Hutchinson, S.L., Banks, M.K. and Schwab, A.P. (2001). Phytoremediation of aged petroleum sludge: Effect of inorganic fertilizer. J. Environ. Qual., 30, 395-403.

James, C.A. and Strand, S.E. (2009). Phytoremediation of small organic contaminants using transgenic plants. Curr. Opin. Biotechnol. 20(2), 237-241.

Johnson, D.L., Anderson, D.R. and McGrath, S.P. (2005). Soil microbial response during the phytoremediation of a PAH contaminated soil. Soil Biol. Biochem., 37 (12), 2334-2336.

Kulakow, P.A., Schwab, A.P. and Banks, M.K. (2000). Screening plant species for growth on weathered, petroleum hydrocarbon-contaminated sediments. Int. J. Phytorem., 2, 297-317.

Leigh, M.B., Fletcher, J.S., Fu, X. and Schmitz, F.J. (2002). Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environ. Sci. Technol., 36, 1579-1583.

Li, J.T., Baker, A.J.M., Ye, Z.H., Wang, H.B. and Shu, W.S. (2012). Phytoextraction of Cd-Contaminated Soils: Current Status and Future Challenges. Crit. Rev. Env. Sci. Tec. 42 (20), 2113-2152.

Liu, H., Meng, F., Tong, Y. and Chi, J. (2014a). Effect of plant density on phytoremediation of polycyclic aromatic hydrocarbons contaminated sediments with *Vallisneria spiralis*. Ecol. Eng., 73, 380-385.

Liu, R., Xiao, N., Wei, S., Zhao, L. and An, J. (2014b). Rhizosphere effects of PAH-contaminated soil phytoremediation using a special plant named Fire Phoenix. Sci. Total Environ., 473, 350-358.

Macek, T., Mackova, M. and Kas, J. (2000). Exploitation of plants for the removal of organics in environmental remediation. Biotechnol. Adv., 18, 23-34.

Marrugo-Negrete, J., Durango-Hernández, J., Pinedo-Hernández, J., Olivero-Verbel, J. and Díez, S. (2013). Phytoremediation of mercury-contaminated soils by *Jatropha curcas*. Chemosphere, 127, 58-63.

McGuinness, M. and Dowling, D. (2009). Plant-Associated Bacterial Degradation of Toxic Organic Compounds in Soil. Int. J. Env. Res. Public Health., 6(8): 2226-2247.

Merkl, N., Schultze-Kraft, R. and Infante, C. (2005). Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water Air Soil Pollut., 165, 195-209.

Miethling, R., Wieland, G., Backhaus, H. and Tebbe, C.C. (2000). Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L 33. Microb. Ecol., 40, 43-56.

Mitton, F.M., Gonzalez, M., Monserrat, J.M. and Miglioranza, K.S. (2016). Potential use of edible crops in the phytoremediation of endosulfan residues in soil. Chemosphere, 148, 300-306.

Muratova, A., Hubner, T., Tischer, S., Turkovskaya, O., Möder, M. and Kuschk, P. (2003). Plant–rhizosphere-microflora association during phytoremediation of PAH-contaminated soil. Int. J. Phytoremediation. 5, 137-151.

Nedunuri, K.V., Govindaraju, R.S., Banks, M.K., Schwab, A.P. and Chen, Z. (2000). Evaluation of phytoremediation for field-scale degradation of total petroleum hydrocarbons. J. Env. Eng., 126 (6), 483-490.

Negri, M.C., Gatliff, E.G., Quinn, J.J. and Hinchman, R.R. (2003). Root development and rooting at depths. In: S.C. McCutcheon and J.L. Schooner (Eds.), Phytoremediation: Transformation and Control of Contaminants, New York: Wiley. 233-262.

Parrish, D.Z., Banks, M.K. and Schwab, A.P. (2005). Assessment of contaminant lability during phytoremediation of polycyclic aromatic hydrocarbon impacted soil. Environmen. Pollut., 137, 187-197.

Peuke, A.D. and Rennenberg, H. (2005). Phytoremediation. *EMBO Rep.* 6(6), 497-501.

Phillips, C.J., Harris, D., Dollhopf, S.L., Gross, K.L., Prosser, J.I. and Paul, E.A. (2000). Effects of agronomic treatments on structure and function of ammonia-oxidizing communities. Appl. Environ. Microbiol., 66, 5410-5418.

Pilon-Smits, E. (2005). Phytoremediation. Annu. RePlant Biol., 56, 15-39.

Rezek, J., Wiesche, C., Mackova, M., Zadrazil, F. and Macek, T. (2008). The effect of ryegrass (*Lolium perenne*) on decrease of PAH content in long term contaminated soil**.** Chemosphere, 70 (9), 1603-1608.

Riser-Roberts, E. (1998). Remediation of petroleum contaminated soils: biological, physical and chemical processes. Boston: CRC Press.

Salt, D.E., Smith, R.D. and Raskin, I. (1998). Phytoremediation. Annu. RePlant Physiol. Plant Mol. Biol., 49, 643-668.

Sessitsch, A., Kuffner, M. and Kidd, P. (2013). The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Bio. Biochem.* 60, 182-194.

Siciliano, S.D., Germida, J.J., Banks, K. and Greer, C.W. (2003). Changes in Microbial Community Composition and Function during a Polyaromatic Hydrocarbon Phytoremediation Field Trial. Appl. Environ. Microbiol., 69 (1), 483-489.

Singh, O. and Jain, R.K. (2003). Phytoremediation of toxic aromatic pollutants from soil. Appl. Microbiol. Biotechnol., 63, 128-135.

Tassi, E., Barbafieri, M., Cervelli, S., Petruzzelli, G., Pedron, F. and Szymura, I. (2004). Phytoremediation test in PAH contaminated soil. Agrochemica. XL VIII, 73-76.

Thompson, P.L., Ramer, L.A., and Schnoor, J.L. (1998). Uptake and transformation of TNT by hybrid poplar trees. Environ. Sci. Technol., 32, 975-980.

Truu, J., Karme, L., Talpsep, E., Heinaru. E., Vedler, E. and Heinaru, A. (2003). Phytoremediation of soil oil shale waste from the chemical industry. Acta. Biotechnol., 23, 301-307.

US EPA (2000). Introduction to phytoremediation. EPA/600/R-99/107 Report, Washington DC.

Van Aken, B. (2009). Transgenic plants for enhanced phytoremediation of toxic explosives. Curr. Opin. Biotechnol., 20 (22), 231-236.

Xiao, N., Liu, R., Jin, C. and Dai, Y. (2015). Efficiency of five ornamental plant species in the phytoremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil. Ecol. Eng., 75, 384-391.

Yu, X. Z., Trapp, S., Zhou, P.H. and Chen, L. (2007). Effect of Temperature on the Uptake and Metabolism of Cyanide by Weeping Willows. Int. J. Phytoremediation., 9(3), 243-255.