Phytoremediation of hydrocarboncontaminated soil: Principles and applications

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Phytoremediation of hydrocarbon-contaminated soils: principles and applications

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1. INTRODUCTION

1.1. Common Target Contaminants

Total petroleum hydrocarbons (TPH) comprise a diverse mixture of hydrocarbons that occur at petrochemical sites and storage areas, waste disposal pits, refineries and oil spill sites. TPHs are considered persistent hazardous pollutants, and include compounds that can bioconcentrate and bioaccumulate in food chains [1], are acutely toxic [2], and some such as benzene [3] and benzo[a]pyrene are recognized mutagens and carcinogens [4]. Since this group includes chemicals that have physical and chemical characteristics that vary over orders of magnitude, TPHs are divided into two categories (Fig. 1). Gasoline range organics (GRO) corresponds to small chain alkanes (C6-C10) with low boiling point (60-170°C) such as isopentane, 2,3-dimethyl butane, *n*-butane and *n*-pentane, and volatile aromatic compounds such as the monoaromatic hydrocarbons benzene, toluene, ethylbenzene, and xylenes (BTEX). Diesel range organics (DRO) includes longer chain alkanes (C10–C40) and hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAH).

Whereas most of these contaminants do have natural sources, concentration and release of contaminants through anthropogenic activities has led to significant contamination of soil and groundwater. The extent of petroleum hydrocarbon contamination throughout the United States is reflected by the large number of Superfund sites and Leaking Underground Storage Tanks (LUST) sites that contain these contaminants (Fig. 2 and 3). These sites often contain high concentrations of contamination. However, individual contaminants behave differently. Some contaminants such as BTEX compounds are highly mobile in the environment, while others such as PAHs tend to bind strongly to soil particles near the source or remain entrapped within an organic phase.
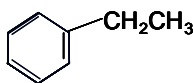
Gasoline Range Organics (GRO)
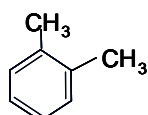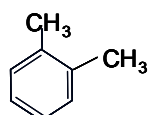
i) BTEX
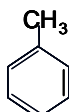
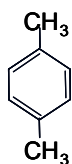Benzene   Toluene   Ethylbenzene

o-Xylene   m-Xylene   p-Xylene

ii) Common Oxygenates
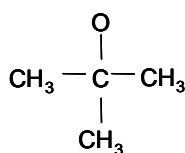
MTBE   Ethanol

Figure 1a. Examples of common Gasoline Range Organics (GRO)

Diesel Range Organics (DRO)

i) Non-Halogenated Semi-Volatile Organic Compounds (SVOC)

$C_{10}H_{22}$
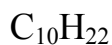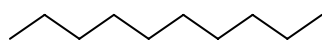
ii) Polycyclic Aromatic Hydrocarbons (PAH)
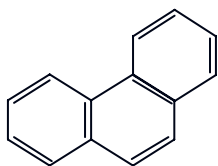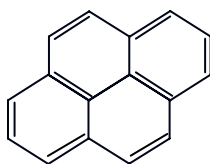
Phenanthrene   Pyrene   Benzo(a)pyrene

Figure 1b. Examples of common Diesel Range Organics (DRO).

Since hydrocarbon spills at different sites represent different mixtures, it is very difficult to find a single, efficient method of cleanup. Current treatment techniques usually involve excavation and *ex situ* treatment of the source material and the contaminated soils. However, residual contamination often exceeds regulatory limits by a relatively small margin, and occurs over extensive

areas [5]. The large volume of soil affected precludes *ex-situ* treatment due to economical constraints and requires the use of relatively inexpensive remediation schemes, such as phytoremediation.

Research and application of phytoremediation for treatment of petroleum hydrocarbon contamination over the past fifteen years has provided much useful information that can be used to design effective remediation systems and drive further improvement and innovation. This chapter will attempt to provide a strong foundation for understanding phytoremediation of petroleum hydrocarbon contaminated sites from principles to practice.

Figure 2. United States Superfund sites containing petroleum hydrocarbon contamination for FY1982 to FY1999 (834 total projects, [6]).

Figure 3. Total United States underground storage tank corrective actions (FY 1992 to FY 2003, [7]).

1.2. General Scope of Phytoremediation

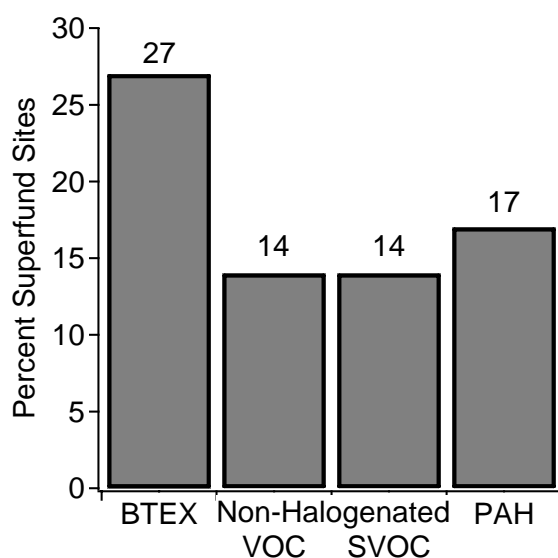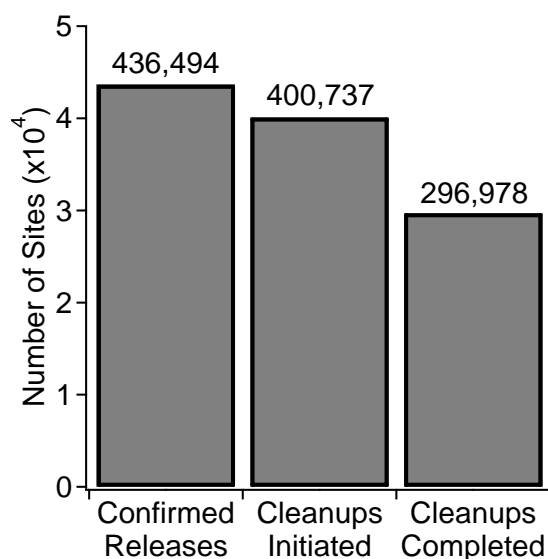Phytoremediation is a biological technology process that utilizes natural plant processes to enhance degradation and removal of contaminants in contaminated soil or groundwater. Broadly, phytoremediation can be cost-effective for

   a) Large sites with shallow residual-levels of contamination by organic, nutrient, or metal pollutants, where contamination does not pose an imminent danger and only "polishing treatment" is required; and
   b) Where vegetation is used as a final cap and closure of the site [8].

Advantages of using phytoremediation include cost effectiveness, aesthetic advantages, and long-term applicability (Table 1). Furthermore, the use of phytoremediation as a secondary or polishing *in situ* treatment step minimizes land disturbance and eliminates transportation and liability costs associated with offsite treatment and disposal. Increasing public and regulatory acceptance are likely to extend the use of phytoremediation beyond current applications.

2. PHYTOREMEDIATION MECHANISMS

Phytoremediation utilizes physical, chemical, and biological processes to remove, degrade, transform, or stabilize contaminants within soil and groundwater. Hydraulic control, uptake, transformation, volatilization, and rhizodegradation are important processes used during phytoremediation (Fig. 4) and are discussed below.

2.1. Hydraulic Control

Phytoremediation applications can be designed to capture contaminated groundwater plumes to prevent off-site migration and/or decrease downward migration of contaminants, as illustrated in Fig. 5. Trees and grasses act as a solar "pump" removing water from soils and aquifers through transpiration.

Contaminant plume capture relies on the formation of a cone of depression within an aquifer due to uptake of water by plants and subsequent transpiration.

Table 1

Advantages and disadvantages of phytoremediation over traditional technologies such as pump and treat of contaminated groundwater and soil excavation and above-ground treatment.

Advantages	Disadvantages
Relatively low cost	Longer remediation times
Easily implemented and maintained	Climate dependent
Several mechanisms for removal	Effects to food web might be unknown
Environmentally friendly	Ultimate contaminant fates might be unknown
Aesthetically pleasing	Results are variable
Reduces landfilled wastes	
Harvestable plant material	
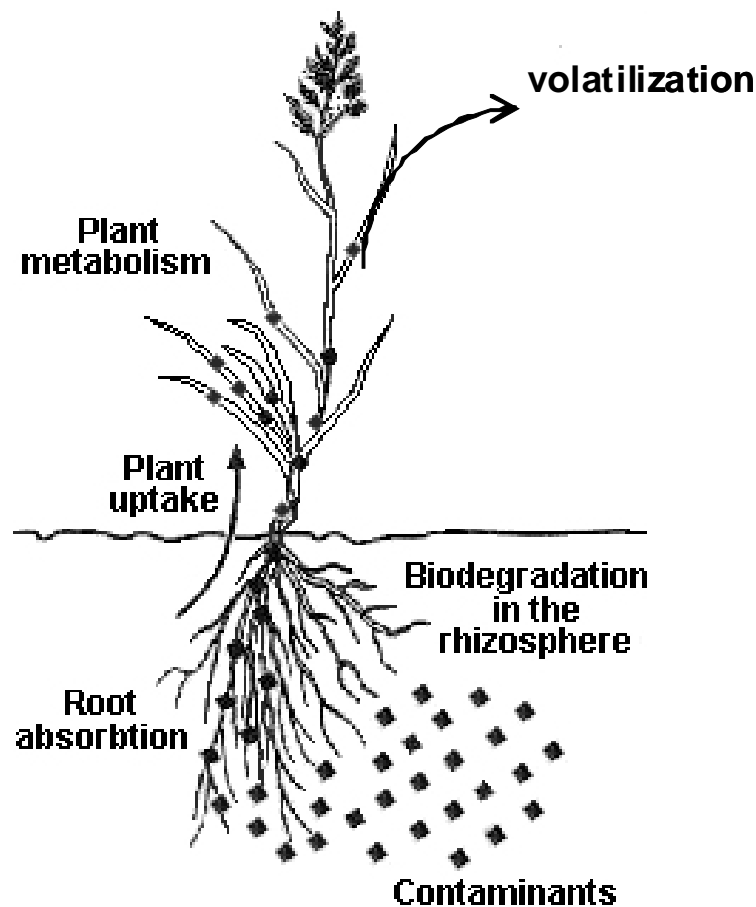
Figure 4. Schematic of different mechanisms of contaminant removal by plants [8].

The key to forming a successful barrier against plume migration is for trees to be rooted into a shallow water table aquifer.   Phreatophytes, deep-rooted plants including hybrid poplars and willows are most often used for hydraulic control. When planted densely (more than 600 trees per acre), poplars and willows usually reach optimum working conditions after 3-4 years during canopy closure when almost all the direct sunlight is intercepted.

     The application of phytoremediation requires that the bottom of the aquifer be confined by materials of low hydraulic conductivity such as clay, shale, or rock (hydraulic conductivity $< 10^{-6}$ cm/s) and does not "leak" water vertically down to another unit.   However, plume capture is not limited to shallow aquifers, as poplar trees planted in well casings have been used to tap water tables at a depth of 10-m [10].
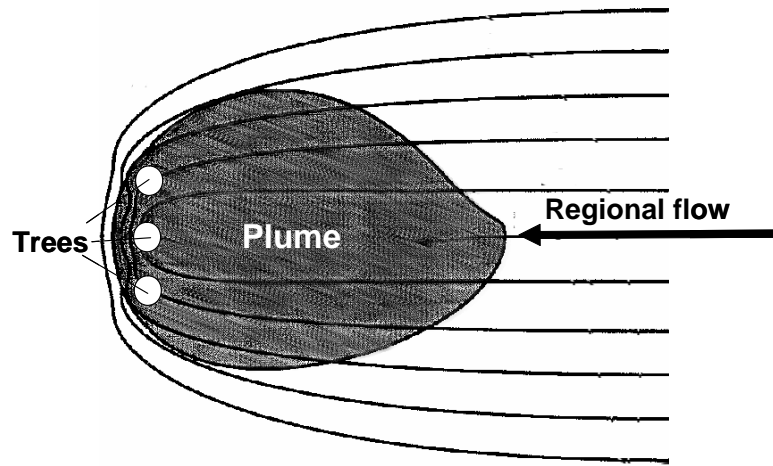
Figure 5. Plan view of trees planted on a line (similar to an interdiction well field) to capture a shallow groundwater plume (Modified after [9]).

Downward migration of contaminants due to percolation of rainwater can also be controlled with phytoremediation. Within the upper region of an aquifer, grasses with dense, fibrous root systems are used to transpire water and limit percolation of contaminants through the vadose zone and to intercept rainwater that may discourage tree root penetration through the water table.

2.2. Uptake, translocation, and transformation

Moderately hydrophobic (log K_{ow} = 1.0 to 3.0) hydrocarbons, including BTEX, can be removed from soil and groundwater through direct plant uptake. The transpiration stream concentration factor (TSCF), an indirect measure of uptake efficiency, has been used to adequately predict whether contaminants will be taken up by plants (Fig. 6). Briggs [11] proposed a bell-shaped relationship between TSCF and contaminant hydrophobicity, indicated by the logarithmic of the octanol-water partitioning coefficient (log K_{ow}). This relationship was developed for pesticide uptake by barley plants, and is given by equation (1) below. Burken and Schnoor [12] adapted this equation to describe the uptake of a wide variety of organic contaminants (including BTEX) by hybrid poplar trees. This relationship is represented by equation (2) and is depicted in Figure 6.

$$TSCF = 0.784 \exp\{-(\log K_{ow} - 1.78)^2 / 2.44\} \qquad (1)$$

$$TSCF = 0.756 \exp\{-(\log K_{ow} - 2.50)^2 / 2.58\} \qquad (2)$$
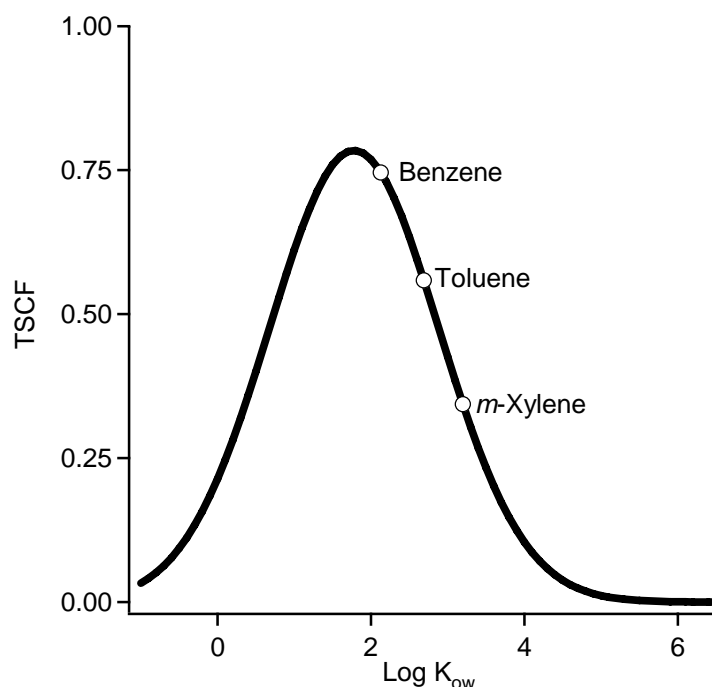
Figure 6. Estimated transpiration stream concentration factors (TSCF) for BTEX using Eq. 2.

The bell-shaped curve shown in Figure 6 reflects poor plant uptake of hydrophilic compounds (log $K_{ow} < 1$), which have little affinity for root membranes; high uptake efficiency of moderately hydrophobic hydrocarbons such as BTEX ($1.5 <$ low $K_{ow} < 3.5$); and poor uptake of hydrophobic hydrocarbons such as PAHs (log $K_{ow} > 4$), which strongly sorb to soil and are therefore, not bioavailable.

The rate of contaminant removal has been found to be a function of uptake efficiency (e.g., TSCF), transpiration rate, and the contaminant concentration in soil water, as discussed in section 5.1. Uptake efficiency varies with plant species, age, health, and physico-chemical properties of the root zone. Transpiration rate also varies dramatically and depends on the plant type, leaf area, nutrients, soil moisture, temperature, wind conditions, and relative humidity.

Once the organic xenobiotic enters the plant system, it is partitioned to different plant parts through translocation. Unlike microbial species that metabolize organic contaminants to carbon dioxide and water, plants use detoxification mechanisms that transform parent chemicals to non-phytotoxic metabolites. The detoxification mechanism within plants is often described using the "green liver" concept [13, 14]. Once a contaminant enters the plant, any number of reactions within the following series may occur.

- *Phase I - Conversion*
- *Phase II - Conjugation*
- *Phase III – Compartmentation*

Conversion reactions include oxidations, reductions, or hydrolysis that the plant uses to begin detoxification. Conjugation reactions chemically link the Phase I products to glutathione, sugars, or amino acids and thus, the plant alters the solubility and toxicity of the contaminant. Once conjugated, xenobiotics can be removed as waste or compartmentalized. During compartmentation, chemicals are conjugated and segregated into vacuoles or bound to the cell wall material (hemicellulose or lignin). Phase III conjugates are often described as "bound residues" because chemical extraction methods do not recover the original contaminants.

Trichloroethylene (TCE), which is not a hydrocarbon but is one of the more studied volatile organic compounds, has been shown to degrade to trichloroethanol, trichloroacetic acid, and dichloroacetic acid in hybrid poplars [15]. However, overall mass balances have been poor, indicating that other processes or further transformations that result in bound residues may be occurring [16]. Whereas Burken and Schnoor (1996) demonstrated that BTEX compounds translocate to the leaves, not much is known about the fate of BTEX compounds or other hydrocarbons in plants [17].

In general, the ultimate fate of phytotransformed contaminants with respect to C-cycling between a plant and its environment remains unclear. Concern centers on whether transformed contaminants will pose a threat to human or ecological health. Products of conversion reactions could be more toxic than the parent contaminants when consumed by animals or potentially leached to the environment from fallen leaves [18]. Release of contaminants from conjugated complexes or compartmentalization could occur in the gut of a worm, snail, or butterfly [8]. This raises the potential of re-introducing the pollutant into the food chain. Therefore, a thorough understanding of pathways and end products of enzymatic processes within a plant is required if phytoremediation is to be applied successfully and accepted widely.

2.3. Phytovolatilization

The natural ability of a plant to volatilize a contaminant that has been taken up through its roots can be exploited as a natural air-stripping pump system. Phytovolatilization is most applicable to those contaminants that are treated by conventional air-stripping i.e., contaminants with a Henry's constant $K_H > 10$ atm m^3 water·m^{-3} air, such as BTEX, TCE, vinyl chloride and carbon tetrachloride. Chemicals with $K_H < 10$ atm m^3 water·m^{-3} air such as phenol and PCP are not suitable for the air-stripping mechanism because of their relatively low volatility.

Volatile pollutants diffuse from the plant into the atmosphere through open stomata in leaves. Radial diffusion through stem tissues has also been reported [19-21]. For example, methyl-tert-butyl ether (MTBE) can escape through leaves, stems, and the bark to the atmosphere [22-23]. Tree core samples of

hybrid poplars exposed to TCE also showed radial diffusion from the stem [24] rather than transpiration from leaves [24, 25] as the main dissipation mechanism. Generally, the concentration of VOCs in the xylem decreases with increasing distance from the roots [24].

Once released into the atmosphere, compounds with double-bonds such as TCE and perchloroethylene (PCE) could be rapidly oxidized in the atmosphere by hydroxyl radicals. However, under certain circumstances (e.g., poor air circulation) phytovolatilization may not provide a terminal solution. For example, MTBE is long lived in the atmosphere and can pose a risk to shallow groundwater during precipitation [26]. In such cases, simple mass balance models can be utilized to determine if phytovolatilization poses a significant risk to humans and/or the environment [20, 24, 27]. Nevertheless, the rate of release of VOCs from plant tissues is generally small relative to other emissions [27]. Thus, phytovolatilization is a potentially viable remediation strategy for many volatile organic chemicals.

2.4. Rhizodegradation

Microbial degradation in the rhizosphere might be the most significant mechanism for removal of diesel range organics in vegetated contaminated soils [28-34]. This occurs because contaminants such as PAHs are highly hydrophobic and their sorption to soil decreases their bioavailability for plant uptake and phytotransformation. Briggs (1982) first demonstrated that the lipophilicity of a pesticide determines its fate in a barley plant [11]. High K_{ow} values (an indicator of hydrophobicity) corresponded to a greater possibility that the compound would be retained in the roots (Eq. 3). Burken and Schnoor (1998) published similar results for the sorption of a wide range of organic contaminants to roots of hybrid poplar plants grown hydroponically (Eq. 4) [12].

$$\log (RCF - 0.82) = 0.77 \log K_{ow} - 1.52 \tag{3}$$

$$\log (RCF - 3.0) = 0.65 \log K_{ow} - 1.57 \tag{4}$$

Where the Root Concentration Factor (RCF) (L/kg dry roots) is the ratio of organic chemical sorbed on the root (mg/kg of fresh root tissue) to that in hydroponic solution (mg/L). This equilibrium partitioning coefficient has generally proved to be a good indicator of whether a plant retains a contaminant in the root, which increases the probability of microbial degradation (not withstanding significant bioavailability limitations). However, a few exceptions exist such as phenol and aniline, which bind irreversibly to the root (especially aniline) and are chemically transformed. They are not appreciably desorbed because they are covalently bound as metabolic products in plant tissue [35].
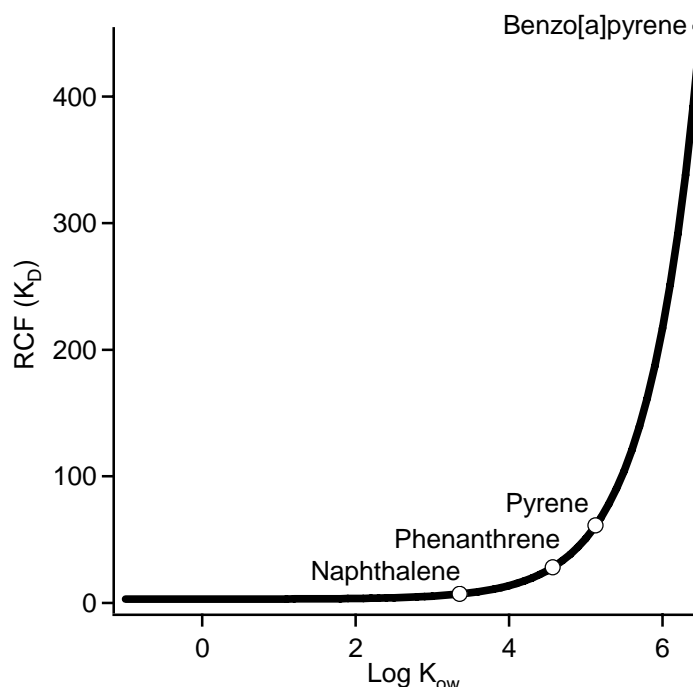
Figure 7. Estimated Root concentration factors (RCF) for PAHs using Eq. 4.

Fig. 7 uses Eq. 4 to estimate RCF values for a few common PAHs. The hydrophobic (high sorption) characteristics of PAHs and other DRO compounds result in high retention in the root zone. Fortunately, the rhizosphere of most plants promotes a wealth of microorganisms that can contribute significantly to the degradation of petroleum hydrocarbons during phytoremediation. Thus, though a plant may not directly act upon these contaminants, a plant can influence the microbial community within its root zone to a great extent.

Potential rhizosphere interactions that may be important for phytoremediation of petroleum hydrocarbons include:

1. Prolific microbial growth
2. Repression/induction of catabolic enzymes
3. Co-oxidation of contaminants
4. Changes in bioavailability
5. Chemotaxis of competent strains

Deposition of plant-derived carbon sources through root exudation, and/or root turnover provides rhizosphere bacteria with numerous organic substrates [36]. Rhizodeposition can account for release of 7 to 27 percent of the total carbon fixed during plant photosynthesis [37] and varies between plants. Commonly reported estimates are between 10 – 100 mg-C g-root material[-1] [38] of which root exudation is reported to range between 0.4 – 27.7 mg-C g-root material[-1] [39-41]. The composition and quantity of root-derived material

released into the rhizosphere varies depending on the season [42], the age of plant [42] and the health of the plant [43] but generally contains sugars (15 - 65% total organic carbon), organic acids (9 - 33% total organic carbon), amino acids (2 - 31% total organic carbon) [34,39-40] and phenolics (0.3-4 mg-C·g-root material^{-1}) [42-44]. Plant stress and age generally increase rhizodeposition.

The availability of simple organic carbon sources that can be used for growth promotes rhizosphere microbial populations which have been reported to be 4- to 100- fold greater than that observed in surrounding bulk soils [33, 45-48]. Selection of competent microorganisms during phytoremediation has been hypothesized. Miya and Firestone (2000) [28] observed greater percentages of phenanthrene degrading bacteria in rhizosphere soil than bulk soils and suggested the rhizosphere selected for PAH degraders. Siciliano et al. (2003) observed a higher frequency of catabolic genes in tall fescue rhizosphere than in bulk soil [49], suggesting that gene transfer or another mechanism of selection exists in the rhizosphere. However, the presence of contaminants in these experimental systems likely provided a strong selective pressure for competent strains [50]. Investigation of competent degraders within the rhizosphere of uncontaminated soil has not been reported; such studies are needed to provide conclusive evidence for selection of specific degraders through plant influence.

Induction of microbial aromatic degradation has also been hypothesized due to the deposition of phenolic compounds that are structurally analogous to known inducers of enzymes responsible for degradation of aromatic contaminants [51-52]. Gilbert and Crowley (1997) demonstrated induction of polychlorinated biphenyl (PCB) degradation in *Arthrobacter* sp. strain B1B, a gram-positive organism, using spearmint products and identified *l*-carvone as the compound responsible [52]. Interestingly, *l*-carvone was not a growth substrate for *Arthrobacter* sp. strain B1B, and it inhibited growth of the bacteria on fructose. Induction of PAH degrading enzymes by plant root products has not been demonstrated in the literature.

In a screening test of inducers of naphthalene dioxygenases potentially released by plants [53], none were detectable in root extracts at concentrations required for catabolic gene induction. Furthermore, Kamath et al., (2004) and Rentz et al. (2004) observed inhibition of catabolic enzyme activity on a per cell basis following exposure to environmentally relevant concentrations of plant root products (exudates and turnover) [53-54]. This was attributed to the presence of organic acids, carbohydrates, and amino acids, known repressors of aromatic catabolism within soil bacteria. However, both studies concluded that proliferation of competent genotypes through growth could compensate for the interference that labile substrates exert on the expression of PAH catabolic genes. Currently, little information concerning the expression of other catabolic enzymes during petroleum hydrocarbon phytoremediation is available.

Several researchers have suggested that co-oxidation of high molecular weight (HMW) PAH within the rhizosphere [37,47-48] is an important mechanism for phytoremediation. Generally, HMW PAHs do not serve as carbon and energy source for microbial populations during degradation. The use of plants as a method to "inject" growth substrates to contaminated soil could overcome this limitation to degradation [28]. Soil experiments with plants and root exudates (pyrene, 4-rings) have shown degradation of HMW PAH and co-oxidation was implied. However, oxidation or metabolism of HMW PAH has not been demonstrated using a well-defined system. Co-oxidation and cometabolism is likely an important process within the rhizosphere with the availability of a wide array of growth substrates, although no studies have assessed the importance of this mechanism compared to other processes.

The bioavailabilitiy of hydrophobic contaminants may also be altered with the root zone environment. Exudation of organic acids could promote contaminant desorption from soil and solublization, but re-sorption to roots [55] may compete with microbial utilization. For carcinogenic and highly hydrophobic benzo[a]pyrene, sorption to roots could prove to be an acceptable end-point with respect to human and environmental risk. However, no studies have assessed the potential of this attenuation mechanism.

Chemotaxis of competent bacteria towards the rhizosphere may also enhance rhizoremediation. Ortega-Calvo et al. (2003) demonstrated chemotaxis of PAH-degrading rhizosphere bacteria towards root exudates [56]. Interestingly, these bacteria were also attracted to naphthalene and phenanthrene, but repelled by anthracene and pyrene.

4.5. Summary of mechanisms

The different mechanisms discussed above could be utilized for the remediation of a wide variety of contaminants (Table 2). Phytoremediation could therefore be applied for the remediation of numerous contaminated sites. However, not much is known about contaminant fate and transformation pathways, including the identity of metabolites. Little data also exists on contaminant removal rates and efficiencies directly attributable to plants under field conditions. Therefore, further research is required before a tree can be designed as an engineered reactor system and optimized for efficiency at the field-scale.

Table 2

Potential clean-up mechanisms during phytoremediation of hydrocarbon-contaminated sites based on physical properties of the target pollutants such as octanol-water partitioning coefficient (K_{ow}) and Henry's dimensionless constant (K_H

Contaminants	Sources	K_{ow}[*]	K_H[*]	Potential Removal Mechanisms
Gasoline Range Organics (GRO)				
BTEX	Refineries, LUST, Fuel spills	135-1585	<0.01	Hydraulic Control Phytovolatilization
Gasoline Oxygenates	LUST	~10	~5×10^{-4}	Hydraulic Control Phytovolatilization
Diesel Range Organics (DRO)				
PAH	Coal-gasification, petroleum distillation, wood preservation, waste disposal	>10^4	<2×10^{-5}	Rhizoremediation

3. PILOT STUDIES

While numerous studies have been carried out at the lab-scale, very little has been published about field scale implementation of phytoremediation. Nedunuri et al. [57] investigated total petroleum hydrocarbon (TPH) removal at several field sites contaminated with crude oil, diesel fuel, or petroleum refinery wastes, at initial TPH concentrations of 1,700 to 16,000 mg/kg. Plant growth varied by species, but the presence of some species led to greater TPH disappearance than with other species or in unvegetated soil. At a crude oil-contaminated field site near the Gulf of Mexico, an annual rye-soybean rotation plot and a St. Augustine grass-cowpea rotation plot had significantly ($P < 0.05$) greater TPH disappearance than did sorghum-sudan grass or unvegetated control plots, at 21 months. At a diesel fuel-contaminated Craney Island field site in Norfolk, Virginia, the fescue plot had significantly ($P < 0.10$) greater TPH removal than did an unvegetated plot. At a refinery waste site, statistical analyses were not presented due to the short time since establishment of the plots, but Nedunuri et al. (2000) reported that qualitatively, the vegetated plots had greater TPH removal than the unvegetated control plots. After investigating the potential to use phytoremediation at a site contaminated with hydrocarbons, the Alabama Department of Environmental Management granted a site, which involved about 1500 cubic yards of soil of which 70% of the baseline samples contained over 100 ppm of total petroleum hydrocarbon (TPH). After 1 year of vegetative cover, approximately 83% of the samples contained less than 10-ppm TPH [58].

4. FIELD SCALE CONSIDERATIONS

Design of a phytoremediation system varies according to the contaminant/s, the conditions at the site, the level of clean up required, and the plant/s that are used. Nevertheless, it is possible to specify a few design considerations that are a part of most phytoremediation efforts. These include:

- ♦ Site Treatability
- ♦ Plant selection and planting density
- ♦ Irrigation, agronomic inputs and maintenance
- ♦ Cost Estimation
- ♦ Mathematical Modeling
- ♦ Clean-up time required
- ♦ Analysis of failure modes

4.1. Site Treatability

4.1.1. Source Removal
For phytoremediation to succeed, it is very important to physically remove the source of contamination (e.g., excavation of highly-contaminated soil and/or extraction of free phase). The presence of a continuous source can be detrimental to the health of the plants and can extend the life of the phytoremediation project indefinitely.

4.1.2. Depth of Contamination
Phytoremediation is most effective at sites with shallow (i.e., root accessible) contaminated soils where contaminants can be treated in the rhizosphere and/or by plant uptake. Roots of phreatophytic trees can be expected to grow at least 3 meters into a soil profile, and it is possible to encourage rooting to a depth of 5 meters or more using the tree-in-a-well concept [10]. On the other hand, roots of some grasses (alfalfa, switchgrass, tall fescue) can reach soil depths of only 0.25-0.4 m. Buffelgrass roots to a depth of 0.75 m but has been observed to have dense rooting pattern within 0.3 m from the topsoil layer. Hawaiian plants, Milo and Kou which were used to remediate saline soils contaminated with TPHs, rooted to a depth of more than 1.5 m by growing through the brackish water table into a zone of concentrated contaminants [59].

Optimizing irrigation patterns can also facilitate biodegradation of contaminants by creating an "expanded rhizosphere" due to translocation of organic root exudates and inorganic nutrients to relatively deep soil layers. Phytoremediation can therefore influence soils to the depth where irrigation water reaches, even if the roots are sparse in the contamination zone.

4.1.3. Soil composition and quality

Soil quality is another important factor for determining successful germination, growth and health of plants. Heavily contaminated soils have a tendency towards poor physical conditioning which is unsuitable for vigorous growth of vegetation and rhizosphere bacteria. It is therefore critical to use amendments to improve the quality of soil before planting. Common limitations are poor moisture-holding capacity, insufficient aeration, low permeability and nutrient deficiencies. Agronomic soil analysis and preliminary greenhouse or pilot scale experiments can help identify these constraints. For example, nutrient analysis of contaminated soils from a site at the Unocal Bulk Storage Terminal at Superior, Wisconsin [54] indicated general deficiencies in nitrogen, phosphorus, potassium, and zinc. To decrease the soil pH, an addition of sulfur was also recommended. This information was subsequently used in greenhouse treatability studies, from which a formula of 50 lb/ac phosphorus, 225 lb/ac zinc, and 50 lb/ac potassium was identified as optimum for growth of native grasses.

Organic amendments such as aged manure, sewage sludge, compost, straw, or mulch can be used to increase the water-holding capacity of a contaminated soil. Soil pH can be increased and decreased by the addition of lime and sulphur respectively.

4.1.4. Weather

Phytoremediation might be best suited for tropical countries where plant growth occurs all year round. In temperate climates, the active contribution of phytoremediation is restricted to the growing period only. Winter operations may pose problems for phytoremediation when deciduous vegetation loses its leaves, transformation and uptake cease, and soil water is no longer transpired. However, a combination of grasses can be used to prolong the growing period.

4.2. Plant Selection Criteria

Plants should be selected according to the needs of the application, the contaminants of concern and their potential to thrive on contaminated soil. Design requirements should include the use of native plants to avoid introduction of invasive species. Apart from this, vegetation should be fast growing, hardy, easy to plant and maintain. The main aim is to ensure that roots expand throughout the entire contaminated zone. In temperate climates with shallow contaminated aquifers, phreatophytes, such as *Populus* sp. (hybrid poplar, cottonwood, aspen) and *Salix* sp. (willow) are often selected because of fast growth, deep rooting ability down to the surface of groundwater, large transpiration rates, and the fact that they are native throughout most of the country. Among tropical plants tested for use in Pacific Islands, three coastal trees, kou (*Cordia subcordata*), milo (*Thespesia populnea*), and kiawe (*Prosopis*

pallida) and the native shrub beach naupaka (*Scaevola serica*) tolerated field conditions and facilitated clean-up of soils contaminated with diesel fuel [59].

Grasses are often planted in tandem with trees at sites with organic contaminants as the primary remediation method. They provide a tremendous amount of fine roots in the surface soil, which is effective at binding and transforming hydrophobic contaminants such as TPH, BTEX, and PAHs. Grasses are often planted between rows of trees to provide for soil stabilization and protection against wind-blown dust that can move contaminants off-site. Legumes such as alfalfa (*Medicago sativa*), alsike clover (*Trifolium hybridum*), and peas (can be used to restore nitrogen to poor soils. Fescue (*Vulpia myuros*), rye (*Elymus sp.*), clover (*Trifolium* sp.) and reed canary grass (*Phalaris arundinacea*) have been used successfully at several sites, especially petrochemical wastes. Once harvested, the grasses can be disposed off as compost or burned.

Plant tolerance to high contaminant concentrations is also a very important factor to keep in mind. The phytotoxicity of petroleum hydrocarbons is a function of the specific contaminant composition, its concentration, and the plant species used. Major adverse effects typically include reduced germination and growth if contaminant concentrations are sufficiently high. In general, TPH values of 15 percent or greater can result in significant reductions in plant growth and in some cases mortality. Compared with uncontaminated soil, soils with 2% TPH reduced alfalfa yields by 32 percent [61]. Production of biomass by ryegrass was reduced 46 percent at a soil concentration of 0.5 percent (5000 mg/kg) hydrocarbons [47]. It was found that plants pre-grown in clean soil and subsequently transplanted to the contaminated soil grew nearly as well as the control, showing that toxicity was associated with germination and/or early plant growth. Similarly, poor rooting of ryegrass compared to legumes appeared to adversely affect the removal of TPH from Gulf War-contaminated soils [62]. Also, although the germination of sunflower seeds and beans was greater than that of maize, vegetative growth was greater for maize than beans, demonstrating that germination and later plant growth may be affected differently [63].

Aged spills tend to be much less phytotoxic than fresh ones, possibly because of the lower bioavailability of toxic compounds in the aged spills. However, the speciation of petroleum hydrocarbons is also very important in determining phytotoxicity. A fuel oil with 30 percent aromatics resulted in LC_{50} germination (oil concentration lethal to 50 percent of test plants) values of 7 percent (70,000 mg/kg) for sunflower seeds. The volatile fraction can prove most toxic to plants. Aromatic volatile petroleum hydrocarbons such as benzene have been used as herbicides in the past years, illustrating their phytotoxicity when applied to plant leaves [64]. In contrast, no phytotoxic effects were observed in hybrid poplar trees exposed to a simulated groundwater containing a mixture of VOCs including BTEX, chlorinated aliphatics, and alcohols at a total concentration of

169 mg/L [65]. Reduction of the volatile fraction may be accomplished through management, such as by tillage of the soil. If initial efforts at plant establishment at a site fail, replanting the area may ultimately lead to success as concentrations or bioavailability of the more phytotoxic components decline.

Solution-phase concentrations of hydrocarbons are also important, particularly for aquifer remediation applications of phytoremediation. Additional components with phytotoxic effects include various unsaturated hydrocarbons and acidic hydrocarbons such as alicyclics with carboxylic acid groups (naphthenic acids) [64].

A screening test and knowledge from the literature of plant attributes is essential for selection of plants. Most experts recommend a mixture of grasses or legumes to address surface soils contaminated with petroleum hydrocarbons. However, design engineers should work in interdisciplinary teams that include a botanist and/or agricultural specialist to identify and select plants that will grow well at the site. Preliminary greenhouse studies should also be used to identify plants that can thrive and enhance transformation of contaminants of concern to non-toxic or less toxic products.

The U.S. Department of Agriculture also provides two databases on plants (http://Plant-Materials.nrcs.usda.gov/   and   http://plants.usda.gov/).   For information specifically pertaining to plants used for phytoremediation of petroleum hydrocarbons, the Phytopet database compiled by the Department of Soil Science, University of Saskatchewan in co-operation with Environment Canada is available at http://www.phytopet.usask.ca.

4.2.1. Time scale of clean-up

Degradation of organics may be limited by mass transfer, i.e., desorption and mass transport of chemicals from soil particles to the aqueous phase may become the rate determining step. Therefore, phytoremediation may require more time (see Section 4) to achieve clean-up standards than other more costly alternatives such as excavation or ex-situ treatment, especially for hydrophobic pollutants that are tightly bound to soil particles. In many cases, phytoremediation may serve as a final "polishing step" to close sites after more aggressive clean-up technologies have been used to treat the hot spots.

4.2.2. Plant Density

Planting density depends on the application. Louis Licht, Ecolotree® Inc., (http://www.ecolotree.com), pioneered the use of hybrid poplar trees as riparian zone buffer strips, landfill caps, and at hazardous waste sites. For hybrid poplar trees, 1000-2000 trees per acre are typically planted with a conventional tree planter at 12-18 inches depth or in trenched rows 1-6 ft deep. The poplars are planted simply as "sticks", long cuttings that will root and grow rapidly in the first season. Several phreatophytes in the Salix family, such as willow and

cottonwood, can be planted in a similar manner.   Poplars have the ability to root along the entire buried depth. If a row conformation is used, the trees may be spaced with 2 ft between trees and 10 ft between rows. Hardwood trees and evergreens may require a lower planting density initially.

Projects using hydraulic control are most effective at canopy closure, when transpiration is maximized (within 5-6 years).   Theoretically, this can be determined based on the amount of energy received from the sun and that required to evaporate water. For mid-latitudes during the growing season, the earth receives an average 30 million Joules per square meter per day (30×10^6 J m^{-2} d^{-1}) of solar insolation. It takes about 2.5×10^6 Joules to evaporate one liter of water. Thus, it is thermodynamically possible to evaporate 12 L m^{-2} d^{-1}. But no plant is 100% efficient, and energy is required to lift the water from the groundwater to the atmosphere with friction. Typical crops, like corn, can evapotranspire about 4-5 L m^{-2} d^{-1} during their growth period. Poplars can perform about 30% more efficiently than corn if they are rooted in the groundwater table, but they actively transpire only about 4-6 months of the year (due to seasonal changes), depending on the geographic location. Thus, the best that can be expected from a phytoremediation effort where the trees have canopied and are rooted in the groundwater table is 4.5 L m^{-2} d^{-1} x 1.3 x 6/12 x 365 days per year x ($1m^3$/1000 L) = 1.07 m/yr, which is approximately one million gallons per acre per year. Typically, evapotranspiration rates range from about 0.4-1.0 million gallons per acre per year for a good phytoremediation effort using phreatophyte trees rooted into shallow groundwater.

A high initial planting density assures a significant amount of evapotranspiration in the first year which is normally desirable, but the trees will naturally thin themselves by competition to 600-800 trees per acre over the first six years. If desirable, hybrid poplars can be harvested on a six-year rotation and sold for fuel wood or pulp and paper, and the trees will grow back from the cut-stump (coppicing trait). The dense, deep root system stays in place to sustain growth for the next year. The lifetime of hybrid poplars such as *Populus deltoides x nigra* DN-34 (Imperial Carolina) is on the order of 30 years which is usually sufficient as the design life of the project.

4.3. Agronomic Inputs

4.3.1. Irrigation
Results suggest that irrigation can enhance bioremediation of certain diesel components. For terrestrial phytoremediation applications, it is often desirable to include irrigation costs on the order of 10-20 inches of water per year, in the design. Spray irrigation is less efficient than drip irrigation as it encourages the growth of weeds that compete for nutrients with plants and hinder their delivery to the contaminated zone. Irrigation of the plants is especially important during the start of the project. However, after the first year, hydrologic modeling can be

Table 3
Macro- and Micro-nutrients required for healthy plant growth.

Macronutrients[a] (~100 ppm)	Micronutrients[b] (~1 ppm)
Nitrogen (N)	Iron (Fe)
Phosphorus (P)	Boron (B)
Potassium (K)	Zinc (Zn)
Magnesium (Mg)	Copper (Cu)
Calcium (Ca)	Manganese (Mn)
Sulfur (S)	Molybdenum (Mo)

[a]http://extension.oregonstate.edu/mg/botany/table3.html
[b]http://extension.oregonstate.edu/mg/botany/table4.html

used to estimate the rate of percolation to groundwater under irrigation conditions. Over time, irrigation can be withdrawn from the site, provided the area receives sufficient rainfall to sustain the plants.

4.3.2. Fertilizer Requirements

Contaminated soils are usually deficient in macro- and micro-nutrients (Table 3) necessary for establishing healthy vigorously growing plants and stimulating microbial contaminant degradation.

Nitrogen fertilization of motor oil-contaminated soils was found to increase the growth of corn and reduce what appeared to be nitrogen-deficient yellowing of the leaves [66]. The source of nutrients also appeared to affect the germination and growth of plants. Organic sources of nitrogen are better than inorganic sources. This is probably because organic nitrogen sources provide a slow release source of nitrogen, and also help to improve soil structure and soil-water relationships for plant growth. It was found that poultry manure increased the growth of corn in a soil containing 3 percent weight per volume crude oil more than an inorganic fertilizer containing nitrogen, phosphorus, and potassium [67]. The addition of sawdust alone improved germination by decreasing oil contact with seeds, but accentuated the adverse effect of the oil on later growth, apparently by further widening the carbon-to-nitrogen ratio [67].

With respect to TPH degradation, nutrient addition during phytoremediation has yielded mixed results. Hutchinson et al. (2001) observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments [68]. Joner et al. (2002) reported improved degradation of 3 and 4 ringed PAHs with the addition of N/P, but diminished degradation of 5 and 6 ringed PAHs [69]. Finally, Palmroth et al. (2002) observed no improved degradation of diesel fuel with nutrient amendments during phytoremediation with pine, poplar, or grasses [70].

Microbial bioremediation of TPH contaminants with nutrient addition also produced widely varying results. Diesel fuel degradation was stimulated with the addition of N/P using cold region soils [71] and P amendments stimulated

creosote degradation [72]. Breedveld and Sparrevik (2000) observed improved degradation of 4 ringed PAHs with N/P addition, but no increased degradation of 3 ringed PAHs [73]. However, Graham et al. (1999) assessed an array of N/P amendments for hexadecane biodegradation and suggested amendments above stoichiometric requirements can lead to diminished rates of degradation [74]. This potentially occurs because addition of excessive nitrogen additions results in an increase in soil salinity and this increases the osmotic stress and suppresses the activity of hydrocarbon-degrading organisms [71]. Carmichael and Pfander (1997) observed slower degradation of 3 and 4 ringed PAHs with N addition and no effects for P addition [75]. Johnson and Scow (1999) reported similar results indicating N/P addition inhibited or did not change phenanthrene degradation (3 ringed PAH) [76]. Their results showed that soil with initial low concentrations of N or P is more likely to show decreased degradation with N/P addition. Many PAH-degrading organisms are adapted to low nutrient conditions and activity may decrease with the addition of soil amendments.

Thus, addition of nutrients should be considered on a site-by-site basis and a balance should be considered between biodegradation and plant growth. Application of amendments exclusively for plant growth may result in diminished contaminant degradation, the ultimate goal of phytoremediation.

4.3.3. Oxygen requirements

Soil oxygen is required for optimal aerobic microbial degradation of petroleum hydrocarbon contaminants. Similar to nutrient deficiencies, oxygen depletion is caused by natural microbial respiration of contaminants. Within phytoremediation, plants may be a net positive or negative oxygen source [77].

Plants may improve soil oxygen through two mechanisms. First, specially adapted plants use aerenchyma, channels of reduced air resistance, to transport oxygen to the root zone, enhancing aerobic biological degradation [37, 78]; although there are no reports of aerenchyma within hybrid poplars, the subject of this report. Second, soil dewatering and fracturing increases soil porosity, allowing increased diffusion of atmospheric oxygen [6].

Plant roots can also be a net oxygen sink within petroleum-contaminated soils. Rentz et al., (2003) observed stimulation of hybrid poplar growth and increased poplar root density with the addition of Oxygen Release Compound$^{®}$ (ORC) when plants were grown in petroleum smear zone soils (high biochemical oxygen demand) [79]. Flux of oxygen into soil by plants could be offset by root turnover and root exudation that provides microbial populations with simple carbon sources that could deplete soil oxygen when metabolized [80]. Furthermore, plant roots are known to require oxygen [81]. For soils with a high biochemical oxygen demand, oxygen addition may be required to promote plant growth and stimulate microbial degradation.

Passive methods of oxygen delivery are suggested to keep costs of phytoremediation low and include the following. Perforated aeration tubes, placed next to cuttings, can supply oxygen to roots along a vertical axis [82]. Perforated ADS tubing, placed at depth prior backfilling the planting trench provides oxygen on a horizontal plane. Gravel used to backfill planting trenchs allows permeation of oxygen on vertical and horizontal axis. Finally, the use of solid peroxides (e.g. Oxygen Release Compound®) can provide oxygen to soils when in contact with water [83].

4.4. Cost

Phytoremediation is usually less costly than competing alternatives such as soil excavation, pump-and-treat, soil washing, or enhanced extraction. Apart from costs incurred during installation of vegetation at the site, a field-scale phytoremediation project involves expenditure on design, site preparation, reporting, monitoring, and operation and maintenance. It would be prudent to include preliminary greenhouse experiments along with agronomic soil testing during the design phase to ensure vigorous plant growth at the field-site. Mathematical modeling may be necessary to demonstrate the effectiveness of the technology to regulatory agencies (See section 6).

Including all these costs, the start-up cost for phytoremediation at $10,000 – 25,000/ acre is still considerably less expensive than other competing technologies (Table 4). However, since phytoremediation usually requires five or more years, it is very important to make sure that funding for operation and maintenance is available during the life of the project.

4.5. Operation and Maintenance Issues

Operation and maintenance (O & M) is vital to ensure vigorous growth of plants. Some of the major problems in the field have been weeds, killing frosts or drought, insect or disease infestation, beaver or deer browse, and damage by voles. It has been estimated that at least 30 percent of the plants may need to be replanted in the second or third year. Phreatophytic trees are also a source of concern since there is a potential for the expanding roots to enter and restrict flow of subsurface drains and sewers and break power and communication cables and small pipelines. Further, mowing, pruning, harvesting, monitoring vegetation for contaminants, irrigation and fertilizer costs should be included in the initial estimated costs. Jordahl, *et al.* (2002) provides a good summary of key siting and O&M issues that occur during the life of a field-scale project [85].

Table 4.
Five-Year Cost Comparison of Phytoremediation by Hybrid Poplar Trees versus Conventional Pump and Treat [84]

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1. Phytoremediation

Design and Implementation	$ 50,000
Monitoring Equipment	
Capital	10,000
Installation	10,000
Replacement	5,000
5-Year Monitoring	
Travel and administration	50,000
Data collection	50,000
Reports (annual)	25,000
Sample analysis	50,000
TOTAL	$ 250,000

2. Pump and Treat (3 wells and Reverse Osmosis System)

Equipment	$ 100,000
Consulting	25,000
Installation/Construction	100,000
5-Year Costs	
Maintenance	105,000
Operation (electricity)	50,000
Waste disposal	180,000
Waste disposal liability	100,000
TOTAL	$ 660,000

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5. Mathematical Modeling

5.1. Groundwater Capture and Transpiration

One must understand where the water is moving at a site in order to estimate contaminant fate and transport. For applications involving groundwater remediation, a simple capture-zone calculation [86] can be used to estimate whether the phytoremediation "pump" can be effective at intercepting and extracting the plume of contaminants. Trees can be grouped for consideration as average withdrawal points. The goal of such a phytoremediation effort is to

create a water table depression where contaminants will flow to the vegetation for uptake and treatment or volatilization. It is important to realize that organic contaminants are not taken-up at the same concentrations that are present in the soil or groundwater. Rather, there is a transpiration stream concentration factor (a fractional efficiency of uptake) that accounts for the partial uptake of contaminant (due to membrane barriers at the root surface).

$$U = (TSCF)(T)(C) \tag{5}$$

where:
U = uptake rate of contaminant, mg/day
TSCF = transpiration stream concentration factor, dimensionless
T = transpiration rate of vegetation, L/day
C = aqueous phase concentration in soil- or ground-water, mg/L

A method for estimating the Transpiration Stream Concentration Factor (TSCF) for eq. (5) was given by eq. (1) and (2).

If the contaminant plume is not completely taken-up by the vegetation, the plume that remains could be evapoconcentrated; i.e., the mass of contaminant in the plume will be less due to uptake by vegetation, but the concentration remaining will actually be greater due to preferential uptake of water over the contaminants. This is a potential concern for phytoremediation of groundwater plumes or created wetlands, where a relatively hydrophilic contaminant can be concentrated on the downstream side of the phytosystem.

Mature phreatophyte trees (poplar, willow, cottonwood, aspen, ash, alder, eucalyptus, mesquite, bald cypress, birch and river cedar) typically can transpire 3-5 acre-ft of water per year (36-60 inches of water per year).   This is equivalent to about 600-1000 gallons of water per tree per year for a mature species planted at 1500 trees per acre. Transpiration rates in the first two years would be somewhat less, about 200 gallons per tree per year, and hardwood trees would transpire about half the water of a phreatophyte. Two meters of water per year is a practical maximum for transpiration in a system with complete canopy coverage (a theoretical maximum would be 4 m/yr based on the solar energy supplied at $40^{o}N$ on a clear day that is required to evaporate water).   If evapotranspiration of the system exceeds precipitation, it is possible to capture water that is moving vertically through soil. Areas that receive precipitation in the wintertime (dormant season for deciduous trees) must be modeled to determine if the soil will be sufficiently dry to hold water for the next spring's growth period.   The Corps of Engineers Hydrologic Evaluation of Landfill Performance (HELP) model (Vicksburg, Mississippi) and other codes have been used to estimate vertical water movement and percolation to groundwater.

5.2. Contaminant Uptake and Clean-up Time

From equation (5) above, it is possible to estimate the uptake rate of the contaminant/s. First order kinetics can be assumed as an approximation for clean-up time. The uptake rate should be divided by the mass of contaminant remaining in the soil:

$$k = U/M_o \qquad\qquad (6)$$

where:
k = first order rate constant for uptake, yr^{-1}
U = contaminant uptake rate, kg/yr
M_o = mass of contaminant initially, kg

Then, an estimate for mass remaining at any time is expressed by equation (7) below.

$$M = M_o e^{-kt} \qquad\qquad (7)$$

where:
M = mass remaining, kg
t = time, yr

Solving for the time required to achieve clean up of a known action level:

$$t = -(\ln M/M_o)/k \qquad\qquad (8)$$

where:
t = time required for clean-up to action level, yr
M = mass allowed at action level, kg
M_o = initial mass of contaminant, kg

Equations (5-8) can be applied to most sites where soil clean-up regulations are known for metals or organic contaminants.

5.3. Rhizodegradation

The Root Concentration Factor, which was previously described (eq. (3) and eq. (4)) is defined as the ratio of the contaminant in roots to the concentration dissolved in soil water (µg/kg root per µg/L). It is important in estimating the mass of contaminant sorbed to roots in phytoremediation systems. While RCF is a simple indicator of whether a contaminant will be retained on the root surface, mathematical modeling of the removal of contaminants in the rhizosphere is highly complex. The most sophisticated rhizosphere fate model available is the Pesticide Root zone model (PRZM) available from the EPA

(http://www.epa.gov/oppefed1/models/water/index.htm). It allows for the estimation of the fate of pesticides in the root zone through hydrologic and chemical transport simulation. The processes of plant uptake, surface run-off, erosion, decay, volatilization, advection, dispersion and adsorption are considered. However, for PAHs and other highly hydrophobic contaminants, factors such as microbial mobility, spatial variability, plant root growth and depth of root penetration, root turnover and rhizosphere volume are probably more important.

Current models [87] are built on a conceptual framework in which the soil-plant contaminant system is compartmentalized into multiple zones: the root itself, a series of root influenced zones (the rhizosphere), a decaying root zone and a non-root-influenced zone (the bulk soil). The essence of the system conceptualization is that each of the modeled zones is treated as a variable volume, uniformly mixed continuous reactor. The change in each zone's volume over time is determined from a pair of forcing functions that describe the specific growth and senescence rates of the plant system. Thus, as the new roots penetrate the soil and the associated microbial community is established, bulk soil will be transformed into rhizosphere soil. Similarly, as root senescence occurs, the root and rhizosphere volume will be converted into decaying root zone that ultimately returns, through humification, to bulk soil. Different growth and senescence functions can be used to simulate various grass species growth and biomass production patterns throughout an annual cycle. The model also includes the idea that the rhizosphere bacteria will face a gradient of influencing factors as the distance from roots increases.

The model is useful to identify important variables from those with only minor effects, and to extrapolate results for one geographic region to another, based on the patterns of interaction between physical and biological factors. However, it does not take into account the effect of temperature and availability of nutrients such as nitrogen, phosphorus, soil oxygen, moisture and pH on degradation rates. Also, it cannot simulate growth of multiple plant species that might be used in field-scale applications.

6. REGULATORY ISSUES

Compliance with regulatory concerns is a critical factor when considering remediation of a site. State and federal acceptance of the technology has been slow but is the product of input by the Interstate Technology and Regulatory Cooperation Work Group (ITRC), the Superfund Innovative Technology Evaluation (SITE) program and the Research Technologies Demonstration Forum (RTDF) program of EPA. The Phytotechnologies Work Team, a part of the ITRC (www.itrcweb.org), published a Decision Tree (1999) and a Guidance Document (2001) as a first approximation for whether phytoremediation is

suitable for a particular site. The latter guidance document in combination with the USEPA document titled *"Introduction to Phytoremediation"* (EPA 600-R-99-107) should be useful in guiding industrial site managers.

Apart from the ITRC, the SITE program and RTDF were also designed to evaluate the potential of phytoremediation for field-scale purposes. Phytoremediation has been the subject of six SITE investigations and over 25 field trials by RTDF (http://www.rtdf.org). SITE is a formal program established by EPA's Office of Solid Waste and Emergency Response (OSWER) and the Office of Research and Development (ORD) in response to the Superfund Amendments and Reauthorization Act of 1986 (SARA). Consultants are responsible for operating the innovative system on site and are expected to pay the costs of the demonstration, together with site owners. EPA is responsible for project planning, sampling and analysis, quality assurance and quality control, preparing reports, disseminating information, and transporting and disposing of treated waste materials.

Under Superfund laws, EPA (1998) [88] lists nine criteria for consideration:
1. Overall protection of human health and the environment
2. Compliance with Applicable and Relevant and Appropriate Requirements
3. Long-term effectiveness and permanence
4. Reduction of contaminant toxicity, mobility, or volume
5. Short-term effectiveness (including the length of time needed to implement the technology and associated risks to workers, residents, and the environment during that time)
6. Implementability (including availability of goods and services)
7. Cost including capital, operation and maintenance, and monitoring
8. State (and federal) acceptance of the technology and its evaluation of its performance
9. Community acceptance which is addressed in the Record of Decision (ROD) Amendment (including responsiveness summary that presents public comments and responses to those comments)

Of these, phytoremediation addresses concerns about aesthetics, cost, ease of implementation and community acceptance. Phytoremediation also has an advantage over constructed remedies in the long run. Unlike other remediation technologies, the efficiency of phytoremediation increases with time until the system reaches its maximum during canopy closure. Further, since it is possible to monitor the effect of phytoremediation in mitigating vertical percolation of contaminants as well as soil erosion, it fulfills the criteria required by Risk Based Corrective Action (RBCA) as well as Monitored Natural Attenuation (MNA). For most other actions including Voluntary Programs, it is usually sufficient to

show that the cover is lush and growing and that phytoremediation meets routine (quarterly to annual) groundwater monitoring requirements.

There are certain regulatory limitations to applying phytoremediation to a site. Phytoremediation is passive technology. Meeting cleanup goals might be difficult and could require 10 years or more without a guarantee of reaching specific performance standards. Furthermore, if phytoremediation is to be used in conjunction with Monitored Natural Attenuation (MNA), it is necessary to demonstrate that the plume (or contaminated zone) is stable or shrinking and that it is not causing unacceptable risk to humans or the environment. In addition, proof that the contaminants are not in danger of moving off the site, and knowledge of the mechanism of degradation (metabolites, pathways, products) and/or immobilization/sequestration is required. The following is a list of environmental monitoring requirements that are often appropriate for phytoremediation efforts.

- Tree survival rates and replacement requirements.
- Plant (leaf area index) or root densities and replacement requirements.
- Levels of contaminants and/or metabolites measured in leaves or grasses. Quarterly groundwater monitoring for applicable or relevant and appropriate requirements (ARAR).
- Sap flow or evapotranspiration estimates to calculate volume of water treated.
- Soil gas measurements and oxygen profiles with soil depth to demonstrate aerobic degradation of aromatic constituents or gradual improvement.
- Soil corings to demonstrate that treatment is occurring at the site (heterogeneity makes this monitoring requirement imprecise and sometimes misleading)

Nevertheless, the fate of contaminants taken up by the plant or transformed in the rhizosphere is not well-understood and it can sometimes prove difficult to show that the technology reduces toxicity of the contaminants, prevents cross-media transfer of pollutants and/or reduces risks to human and ecological receptors. Furthermore, since the distribution and composition of contaminants in field-scale projects is very heterogeneous, it is almost impossible to prove that phytoremediation enhances the rate of contaminant removal at field sites.

In summary, long-term monitoring and evaluation of phytoremediation technology is still needed to demonstrate efficacy, to further define suitable plants and applications, and to gain acceptance from regulatory agencies.

7. EMERGING ETHICAL ISSUES, OPPORTUNITIES AND CHALLENGES

One emerging issue requiring consideration is the use of plants that could be genetically modified to exhibit beneficial traits for phytoremediation, such as increased water uptake for hydraulic control, drought and pest tolerance, and increased enzyme activity for faster and more complete phytotransformation of organic contaminants. A similar potential innovation is the inoculation of the rhizosphere with genetically modified organisms (GMOs) that overexpress catabolic enzymes for enhanced rhizoremediation.

The use of (microbial and/or plant) GMOs represents a research frontier with broad implications. The potential benefits of using GMOs are significant, and extend beyond improved contaminant removal efficiency and lower O&M costs. For example, GMO's might facilitate coupling phytoremediation with the production of marketable non-food (cash) crops that could be used for energy production (e.g., biomass production for fuel wood, biodiesel, or fuel ethanol) or raw materials for commercial products (e.g., pulp for paper or feedstock for cosmetic or pharmaceutical industries). Nevertheless, although GMOs have been extensively used in agriculture, little research has been conducted to assess their long-term life cycle impacts, including the consequences of increased genetic drift across species on biodiversity and biological community structure. This gives rise to much speculation and polarization regarding the consequences of *in vitro* genetic manipulation, which represents a significant political barrier to the use of GMOs in phytoremediation. Furthermore, the need for GMOs may be questionable for many projects, considering that indigenous species often perform adequately and that we have not tapped the full potential of wild species due to our limited understanding of various phytoremediation mechanisms, including the regulation of enzyme systems that degrade pollutants.

In summary, the potential benefits and risks associated with the use of genetic manipulation suggest that we need to be very cautious of GMOs, but not necessarily rule out their application in phytoremediation yet. Additional scientific input will hopefully contribute to dissipating myths, discern the benefits and consequences of using GMOs, and ensure their safe use when their application is justified.

8. CONCLUSIONS

Over the past 15 years, phytoremediation has developed into a more acceptable technology for the remediation of soils and groundwater polluted with residual concentrations of petroleum hydrocarbons. However, regulators as well as consumers are still wary about the efficiency, predictability and applications of the technique. The ITRC guidelines and decision tree has supported the use of phytoremediation for most field-scale applications. Yet, at this point there is an

urgent need for strong evidence supporting the potential of phytoremediation in protecting human as well as ecological receptors from exposure to contaminants, using rigorous methods of risk analysis. For direct application to field projects, it would be desirable if more protocols for designing preliminary greenhouse experiments reflecting field-environments and cheap innovative methods of encouraging growth of healthy plants were published. Research examining the long-term fate of contaminants in the environment would be particularly relevant. Also important is the difficult task of evaluating acceptable endpoints (e.g., humification) using standard ecological toxicity or bioavailability assays that might support phytoremediation.

Albeit, phytoremediation is an emerging technology that is based on sound ecological engineering principles. Phytoremediation is a practical and cost-effective approach with aesthetical and atmospheric-carbon-sequestration ancillary benefits, and is particularly attractive for rural areas with residual and shallow contamination. Phytoremediation also holds great potential to manage a wide variety of environmental pollution problems, including the cleanup of soils and groundwater contaminated with hydrocarbons and other hazardous substances, the attenuation of pollutants dispersing through the environment in agricultural drainage, landfill leachates, and other forms of surface runoff or sub-surface migration, and the assimilation of industrial wastewater effluents to support efforts to move towards a zero-discharge policy from industrial facilities (e.g., refineries). Although phytoremediation is not a panacea that would be universally applicable, it is rapidly achieving pedagogical maturity and it has already earned an important place in the menu of alternatives from where we select solutions for our environmental pollution problems.

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