



*Assessment of Phytoremediation as an In-Situ Technique  
for Cleaning Oil-Contaminated Sites*

Prepared by:

C.M. Frick, R.E. Farrell and J.J. Germida

Department of Soil Science

University of Saskatchewan

Saskatoon, SK Canada

S7N 5A8

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Requests for reprints of this report should be addressed to:

PTAC  
Petroleum Technology Alliance Canada  
17<sup>th</sup> Floor, One Palliser Square  
125 9<sup>th</sup> Avenue S.E.  
Calgary, Alberta T2G 0P8  
Canada

(403) 268-7520  
tsuch@ptac.org

Dr. R.E. Farrell  
University of Saskatchewan  
Department of Soil Science  
51 Campus Drive  
Saskatoon, Saskatchewan S7N 5A8  
Canada

(306) 966-2772  
farrell@sask.usask.ca

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## EXECUTIVE SUMMARY

Phytoremediation, the use of plants and their associated microorganisms for the *in situ* treatment of contaminated soils, is a steadily emerging technology with potential for the effective and inexpensive cleanup of a broad range of organic and inorganic wastes. Based on a review of the relevant literature, we provide examples of the phytoremediation of petroleum hydrocarbons and discuss the key mechanisms as well as the special considerations involved in petrochemical phytoremediation. The benefits, limitations, and costs of phytoremediation compared to alternative approaches – including natural attenuation, engineering and bioremediation – also are discussed.

Initial indications are that phytoremediation is effective at degrading and containing petroleum hydrocarbons in soil as well as transferring these compounds from soil to the atmosphere. The literature suggests that the degradation of petroleum hydrocarbons by microorganisms in the rhizosphere of plants is the primary loss mechanism for these compounds. Based on available information, it appears that phytoremediation of petroleum hydrocarbons is quicker but more expensive than natural attenuation and, conversely, slower but less expensive than most engineering techniques and traditional bioremediation methods.

Preliminary screenings indicate that there are native and introduced plants that could be used in phytoremediation efforts in the Prairie and Boreal Plains Ecozones. Little published information exists, however, on the application of phytoremediation to oil-contaminated sites in Canada. Likewise, only a handful of studies examine in detail the specific mechanisms of petrochemical phytoremediation.

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## 1. INTRODUCTION

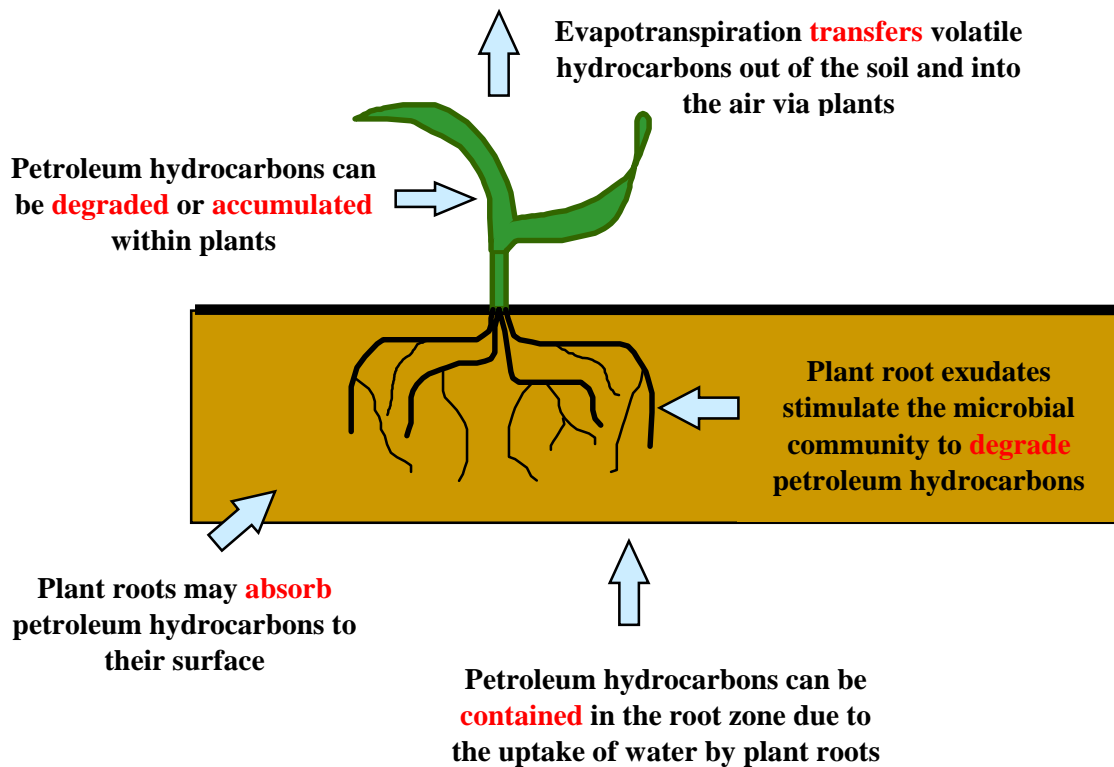
Petroleum hydrocarbons are naturally occurring chemicals used by humans for a variety of activities, including the fueling of vehicles and heating of homes (Committee on *In Situ* Bioremediation *et al.*, 1993). Natural gas, crude oil, tars and asphalts are types of petroleum hydrocarbons ultimately composed of various proportions of alkanes (e.g., methane, ethane, propane), aromatics (e.g. benzene, toluene, ethylbenzene, and xylene, collectively known as BTEX), and polycyclic aromatic hydrocarbons (PAHs; e.g., naphthalene, phenanthrene, anthracene, benzo[*a*]pyrene) (Lyons, 1996; Committee on *In Situ* Bioremediation *et al.*, 1993; Mackay, 1991).

During the past century, industrialization has resulted in an ever-increasing reliance on petrochemicals. This, in turn, has resulted in the contamination of a significant number of sites with petroleum and petroleum-byproducts (Bauman, 1991). Indeed, it is estimated that in Saskatchewan alone there are several hundred sites contaminated with petroleum hydrocarbons (Carlson, 1998). In today's era of heightened environmental awareness and government regulation, efforts to cleanup these sites represent both a commitment to responsible stewardship of our limited natural resources and good business. Today, environmental managers can choose from a variety of approaches to remediate petroleum-contaminated soil and groundwater. These approaches range from intensive engineering techniques to *natural attenuation*, a "hands-off" approach relying entirely on natural processes to remediate sites with no human intervention.

Phytoremediation is the *in situ* use of plants and their associated microorganisms to degrade, contain or render harmless contaminants in soil or groundwater (Cunningham *et al.*, 1996) (Figure 1.1). In essence, phytoremediation employs human initiative to enhance the natural attenuation of contaminated sites and, as such, is a process that is intermediate between engineering and natural attenuation. Because phytoremediation depends on natural, synergistic relationships among plants, microorganisms and the environment, it does not require intensive engineering techniques or excavation. Human intervention may, however, be required to establish an appropriate plant-microbe community at the site or apply agronomic techniques (such as tillage and fertilizer application) to enhance natural degradation or containment processes.

Phytoremediation has been used effectively to remediate inorganic and organic contaminants in soil and groundwater. Various plants, including canola (*Brassica napus* L.), oat (*Avena sativa*), and barley (*Hordeum vulgare*), tolerate and accumulate metals such as selenium, copper, cadmium and zinc (Banuelos *et al.*, 1997; Ebbs *et al.*, 1997; Brown *et al.*, 1994). Alamo switchgrass (*Panicum virginatum*) accumulates the radionuclides Cesium-137 (<sup>137</sup>Cs) and Strontium-90 (<sup>90</sup>Sr), compounds present in nuclear fallout from weapons testing and reactor accidents (Entry and Watrud, 1998). Hybrid poplar trees (e.g., *Populus deltoides* x *nigra*) reduce the concentration of nitrate (a plant nutrient and water contaminant) in surficial groundwater (Schnoor *et al.*, 1995; Gatliff, 1994) and degrade the herbicide atrazine from

contaminated soils (Burken and Schnoor, 1997). Forage grasses inoculated with bacteria degrade individual chlorinated benzoic acids as well as mixtures of these compounds (Siciliano and Germida, 1998a); chlorinated benzoic acids arise out of the degradation of polychlorinated biphenyls (PCBs) and chlorinated herbicides. Of particular interest here is the fact that various plants, together with their associated microorganisms, have been found to increase the removal of petroleum hydrocarbons from contaminated soil (Aprill and Sims, 1990; Qiu *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*, 1996; Reynolds and Wolf, 1999; Schwab *et al.*, 1995; Pradhan *et al.*, 1998; refer to Section 2 for more details).



**Figure 1.1. Phytoremediation mechanisms: degradation, containment, or transfer of petroleum hydrocarbons in soil via interactions with plants and microorganisms.**

The objective of this report is to evaluate the effectiveness of phytoremediation as a tool for cleaning up soils and groundwaters contaminated with petroleum hydrocarbons – particularly those associated with well site spills, pipeline ruptures and flare pits. This objective was achieved by reviewing the relevant literature on phytoremediation, as well as the literature



concerning alternatives to phytoremediation. Information was collected and summarized regarding the role of both plants and microbes in the phytoremediation of petroleum hydrocarbons. Grasses, herbs, shrubs, as well as deciduous and coniferous trees were the general types of plants considered. Bacteria, protozoa, and fungi were the microorganisms considered. Literature was reviewed regarding organisms in terrestrial and wetland ecosystems together with those that influence groundwater (e.g., deciduous trees and microbes). Special attention was given to plant species that may be useful in reclaiming oil-contaminated sites in the Prairie and Boreal Plain Ecozones of Western Canada.

Although the report focuses on petroleum hydrocarbons, due consideration has been given to metals, pesticides, and salts, which can also be found in combination with petroleum hydrocarbons at contaminated sites. Organic chemicals, such as polychlorinated compounds and pesticides, are used occasionally as examples to illustrate processes or mechanisms that may be important in the phytoremediation of petroleum hydrocarbons. Changes over time in the interactions among contaminants, plants and microorganisms also are considered.

The basic outline of this report is as follows: **Section 2** provides examples of the phytoremediation of petroleum hydrocarbons. Mechanisms for phytoremediation of petroleum hydrocarbons are presented in **Section 3**. **Section 4** discusses environmental factors that influence phytoremediation. **Section 5** explores special considerations relating to phytoremediation, which include the establishment of appropriate plant and microbial communities; effects of various contaminant concentrations; biotransformation, bioaccumulation, and the disposal of contaminated biomass; mixtures of contaminants; and techniques used to enhance phytoremediation. **Section 6** briefly describes alternatives to phytoremediation and, in **Section 7**, phytoremediation is compared to these alternatives. **Section 8** concludes this report by summarizing the situations where phytoremediation is most effective. The references cited in this report are listed in **Section 9**.

As you read through this report, you will notice that some words have been *italicized*. With the exception of scientific names of plants and microbes, italics are used to indicate a word that has been defined in the glossary which can be found at the end of this report (**Section 10**).

The application and effectiveness of phytoremediation depend, to a certain extent, on the nature of the compounds to be remediated. As a result, **Appendix A** has been included to provide information on the types and behavior of petroleum hydrocarbons from well site spills, pipeline ruptures and flare pits.

A database (PhytoPet) has been produced as a companion to this report. The purpose of the PhytoPet database is to serve as an inventory of plant species that tolerate or phytoremediate petroleum hydrocarbons in terrestrial, wetland, and aquatic environments. The type of information found in the database is summarized in Table 1.1. Information in the database was used to conduct a preliminary assessment of the potential application of phytoremediation to the Prairie and Boreal Plain Ecozones of Western Canada. This preliminary assessment can be found in **Appendix B**.

Finally, **Appendix C** is a listing of information relating to the phytoremediation of petroleum hydrocarbons that can be found on the Internet.

**Table 1.1. Information in PhytoPet - a database of plants that phytoremediate or tolerate petroleum hydrocarbons**

<b>General Information</b>	<b>Experimental Information</b>	<b>Plant Information</b>
Common name of plant	Laboratory or field experiment	Family of plant as well as synonyms of common and scientific names
Scientific name of plant	Initial contaminant concentration	Growth form (fern, grass, herb, shrub, tree)
Cultivar, strain, or code, (including transgenic variants)	Length of Experiment	Morphology (type of root or shoot system, nitrogen fixation)
Hydrocarbon of concern	Post-experiment contaminant concentrations and/or plant condition	Growth duration (annual, biennial, perennial)
Interaction of plant and hydrocarbon: phytoremediate or tolerate	Soil characteristics	Primary habitat (terrestrial, aquatic, wetland)
Mechanism involved in phytoremediation (degradation, rhizosphere effect, containment, transfer, unknown)	Age of plant at 1 <sup>st</sup> exposure (seed, post-germination, mature)	Habitat description (including soil texture and topography)
Types of microorganisms associated with the plant	Requirements for phytoremediation (specific nutrients, addition of oxygen)	Salinity tolerance
	Contaminant storage sites in the plant (root, shoot, leaf, no storage)	Western Canadian occurrence
		North American occurrence
		World range
		Cultural information
		Natural history notes
		Other species of significance in the genus

## 2. PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS

Various plants have been identified for their potential to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons (Table 2.1). In the majority of studies, grasses and *legumes* have been singled out for their potential in this regard (Aprill and Sims, 1990; Qiu *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*, 1996). Prairie grasses are thought to make superior vehicles for phytoremediation because they have extensive, fibrous root systems. Grass root systems have the maximum root surface area (per m<sup>3</sup> of soil) of any plant type and may penetrate the soil to a depth of up to 3 m (Aprill and Sims, 1990). They also exhibit an inherent genetic diversity, which may give them a competitive advantage in becoming established under unfavorable soil conditions (Aprill and Sims, 1990). Legumes are thought to have an advantage over non-leguminous plants in phytoremediation because of their ability to fix nitrogen; i.e., legumes do not have to compete with microorganisms and other plants for limited supplies of available soil nitrogen at oil-contaminated sites (Gudin and Syrratt, 1975). The following is a brief summary of several studies on the use of plants in the phytoremediation of petroleum hydrocarbons. (Refer to Table 2.1 for Latin names of the plants if not otherwise stated.)

Aprill and Sims (1990) established a mix of eight prairie grasses in sandy loam soils to determine whether the degradation of four PAHs (benzo[*a*]pyrene, benzo[*a*]anthracene, dibenzo[*a,h*]anthracene, and chrysene) was stimulated by plant growth. The eight grasses included big bluestem, little bluestem, Indiangrass, switchgrass, Canada wild-rye, side oats grama, blue grama, and western wheatgrass. The extent of PAH disappearance was consistently greater in planted units compared to unplanted controls, indicating that phytoremediation enhanced the removal of these compounds from contaminated soil. Apparent disappearance was greatest for benzo[*a*]anthracene followed by chrysene, benzo[*a*]pyrene, and finally dibenzo[*a,h*]anthracene. This ranking correlated with the water solubility of the PAH compounds; i.e., the more water-soluble the compound the greater its disappearance from the soil.

In a three-year field-plot study, Qiu *et al.* (1997) found that prairie buffalograss accelerated the reduction of naphthalene in a clay soil compared to unplanted clay soil. The authors conducted a parallel experiment to assess the performance of 12 warm season grass species to remove various PAHs from contaminated soil. Results indicated that prairie buffalograss, common buffalograss, Meyer zoysiagrass, and Verde kleingrass accelerated the loss of the low molecular weight PAHs naphthalene, fluorene, and phenanthrene compared to an unplanted control. However, only the Verde kleingrass accelerated the loss of high molecular weight PAHs, such as pyrene, benzo[*a*]anthracene, and benzo[*a*]pyrene compared to the unplanted control.

**Table 2.1. Plants with a demonstrated potential to phytoremediate petroleum hydrocarbons<sup>1</sup>**

western wheatgrass ( <i>Agropyron smithii</i> ) <sup>2</sup>	big bluestem ( <i>Andropogon gerardi</i> ) <sup>2</sup>
side oats grama ( <i>Bouteloua curtipendula</i> ) <sup>2</sup>	blue grama ( <i>Bouteloua gracilis</i> ) <sup>2</sup>
common buffalograss ( <i>Buchloe dactyloides</i> )	prairie buffalograss ( <i>Buchloe dactyloides</i> var. <i>Prairie</i> )
bell rhodesgrass ( <i>Chloris gayana</i> )	Bermuda grass ( <i>Cynodon dactylon</i> L.)
carrot ( <i>Daucus carota</i> ) <sup>4</sup>	Canada wild-rye ( <i>Elymus canadensis</i> ) <sup>2</sup>
tall fescue ( <i>Festuca arundinacea</i> Schreb.)	Arctared red fescue ( <i>Festuca rubra</i> var. <i>Arctared</i> )
soybean ( <i>Glycine max</i> )	duckweed ( <i>Lemna gibba</i> )
annual ryegrass ( <i>Lolium multiflorum</i> )	ryegrass or perennial ryegrass ( <i>Lolium perenne</i> L.)
alfalfa ( <i>Medicago sativa</i> L.)	Verde kleingrass ( <i>Panicum coloratum</i> var. <i>Verde</i> )
switchgrass ( <i>Panicum virgatum</i> ) <sup>2</sup>	bush bean ( <i>Phaseolus vulgaris</i> L.)
Poplar trees ( <i>Populus deltoides x nigra</i> ) <sup>3</sup>	winter rye ( <i>Secale cereale</i> L.)
little bluestem ( <i>Schizachyrium scoparius</i> ) <sup>2</sup>	Indiangrass ( <i>Sorghastrum nutans</i> ) <sup>2</sup>
sorghum ( <i>Sorghum bicolor</i> ) or sudangrass ( <i>Sorghum vulgare</i> L.)	Meyer zoysiagrass ( <i>Zoysia japonica</i> var. <i>Meyer</i> )

<sup>1</sup> Sources: Aprill and Sims (1990); Biederbeck *et al.* (1993); Bailey and McGill (1999); Carr (1919); Chaineau *et al.* (1997); Duxbury *et al.* (1997); Edwards *et al.* (1982); Edwards (1988); Epuri and Sorensen (1997); Ferro *et al.* (1997); Ferro *et al.* (1994); Gudin and Syrratt (1975); Gunther *et al.* (1996); Jordahl *et al.* (1997); Lin and Mendelsohn (1998); Longpre *et al.* (1999); McLean *et al.* (1999); Moore *et al.* (1999); Nichols *et al.* (1997); Pradhan *et al.* (1998); Qiu *et al.* (1997); Radwan *et al.* (1995); Reilley *et al.* (1996); Reynolds and Wolf (1999); Reynolds *et al.* (1999a); Rogers *et al.* (1996); Schwab *et al.* (1995); Schwab *et al.* (1998); Schwab and Banks (1994); Walker *et al.* (1978); Watkins *et al.* (1994); Wild and Jones (1992); Wiltse *et al.* (1998); Xu and Johnson (1995).

<sup>2</sup> Aprill and Sims (1990) evaluated the phytoremediation potential of these plants for the group as a whole, not as individual species. Switchgrass, however, also was investigated as an independent species by Reilley *et al.* (1996). Likewise, switchgrass and little bluestem were investigated as independent species by Pradhan *et al.* (1998).

<sup>3</sup> Jordahl *et al.* (1997) reported that hybrid poplar trees (*Populus deltoides x nigra*) had 5 times more BTX degraders in rhizosphere compared to bulk soil, indicating the potential for phytoremediation by these species. The ability to phytoremediate petrochemicals was not specifically investigated in the 1997 study.

<sup>4</sup> Wild and Jones (1992) found that carrots accumulated PAHs in their peels to a maximum value of 200 ug total PAHs per kg dry weight in a laboratory setting. As with many other plants listed here, since the evaluation was conducted in the laboratory, small-scale field validation of the results should be conducted prior to application to larger scale remediation efforts.

**Table 2.2. Plants with a demonstrated potential to tolerate petroleum hydrocarbons<sup>1</sup>**

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crested wheatgrass ( <i>Agropyron desertorum</i> )	tilesey sage ( <i>Artemisia tilesii</i> )
oat ( <i>Avena sativa</i> )	canola ( <i>Brassica rapa</i> ) <sup>1</sup>
water sedge ( <i>Carex aquatilis</i> )	round sedge ( <i>Carex rotundata</i> )
rock sedge ( <i>Carex rupestris</i> )	carrot ( <i>Daucus carota</i> )
bering hairgrass ( <i>Deschampsia beringensis</i> )	quackgrass ( <i>Elytrigia repens</i> or <i>Agropyron repens</i> )
tall cotton-grass ( <i>Eriophorum angustifolium</i> )	soybean ( <i>Glycine max</i> )
sunflower ( <i>Helianthus annuus</i> )	barley ( <i>Hordeum vulgare</i> )
birdsfoot trefoil ( <i>Lotus corniculatus</i> )	black medick ( <i>Medicago lupulina</i> )
alfalfa ( <i>Medicago sativa</i> L.)	<i>Melilotus altissima</i>
reed canary grass ( <i>Phalaris arundinacea</i> )	reed grass ( <i>Phragmites australis</i> )
Jack pine ( <i>Pinus banksiana</i> )	field pea ( <i>Pisum arvense</i> )
alpine bluegrass ( <i>Poa alpina</i> )	<i>Psoralea bituminosa</i>
<i>Robinia pseudacacia</i>	Arctic willow ( <i>Salix arctica</i> )
Snow willow ( <i>Salix reticulata</i> )	three-square bulrush ( <i>Scirpus pungens</i> )
<i>Senecio glaucus</i>	<i>Spartina alterniflora</i>
<i>Spartina patens</i>	alsike clover ( <i>Trifolium hybridum</i> )
red clover ( <i>Trifolium pratense</i> )	white clover ( <i>Trifolium repens</i> )
wheat ( <i>Triticum aestivum</i> ) <sup>1</sup>	cattails ( <i>Typha latifolia</i> )
fababean ( <i>Vicia faba</i> )	<i>Vicia tetrasperma</i>
maize ( <i>Zea mays</i> L.)	

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<sup>1</sup> Tolerance is defined here as the ability of a plant to grow in hydrocarbon contaminated soil; it does not necessarily mean the plant is healthy. For example, although canola and wheat tolerated exposure to oil- and creosote-contaminated soil, they exhibited poor growth (Bailey and McGill 1999). Biederbeck *et al.* (1993) found similar results for wheat exposed to oily waste sludge.

<sup>2</sup> Sources: Aprill and Sims (1990); Biederbeck *et al.* (1993); Bailey and McGill (1999); Carr (1919); Chaineau *et al.* (1997); Duxbury *et al.* (1997); Edwards *et al.* (1982); Edwards (1988); Epuri and Sorensen (1997); Ferro *et al.* (1997); Ferro *et al.* (1994); Gudim and Syrratt (1975); Gunther *et al.* (1996); Jordahl *et al.* (1997); Lin and Mendelsohn (1998); Longpre *et al.* (1999); McLean *et al.* (1999); Moore *et al.* (1999); Nichols *et al.* (1997); Pradhan *et al.* (1998); Qiu *et al.* (1997); Radwan *et al.* (1995); Reilley *et al.* (1996); Reynolds and Wolf (1999); Reynolds *et al.* (1999a); Rogers *et al.* (1996); Schwab *et al.* (1995); Schwab *et al.* (1998); Schwab and Banks (1994); Walker *et al.* (1978); Watkins *et al.* (1994); Wild and Jones (1992); Wiltse *et al.* (1998); Xu and Johnson (1995).

Gunther *et al.* (1996) found that soil planted with ryegrass lost a greater amount of a mixture of hydrocarbons than soil that was unplanted. The hydrocarbon mixture included *n*-alkanes ( $C_{10}$ ,  $C_{14}$  to  $C_{18}$ ,  $C_{22}$ ,  $C_{24}$ ), as well as pristane, hexadecane, phenanthrene, anthracene, fluoranthene, and pyrene. After 22 weeks, the initial extractable hydrocarbon concentration of 4330 mg total hydrocarbon per kg soil decreased to less than 120 mg per kg soil (97% reduction) in planted soils, but to only 790 mg per kg soil (82% reduction) in unplanted soil. Larger microbial numbers and activity in the planted versus unplanted soil led the authors to conclude that plant roots enhanced biodegradation of the hydrocarbons by stimulating the soil microbes.

Results of an investigation by Reilley *et al.* (1996) indicate that grasses and legumes enhance the removal of PAHs from contaminated soils. The plants (investigated independently) included the legume alfalfa and three grasses: tall fescue, sudangrass, and switchgrass. Pyrene and anthracene were used as PAH contaminants. Planted soils had significantly lower concentrations of the PAHs than the unplanted soils, with 30 to 40% more degradation in the planted soils. Enhanced biological degradation in the rhizosphere soil appeared to be the primary mechanism of dissipation, while leaching, plant uptake, abiotic degradation, *mineralization*, and irreversible sorption were shown to be insignificant.

Reynolds and Wolf (1999) examined the phytoremediation potential of two cold-hardy plants, Arctared red fescue and annual ryegrass, planted together in soil contaminated with either crude oil or diesel. Results of the experiment indicated that contaminated soils planted with the two species had significantly lower concentrations of total petroleum hydrocarbon (TPH) compared to unplanted controls. The initial crude oil concentration for planted treatments and unplanted controls was approximately 6200 mg TPH per kg soil, while the initial diesel concentration was approximately 8350 mg TPH per kg. After 640 days, crude oil-contaminated soil planted with both species had 1400 mg TPH per kg soil (77% reduction), while the unplanted control contained 2500 mg TPH per kg soil (60% reduction). Likewise, diesel-contaminated soil planted with both species had 700 mg TPH per kg soil (92% reduction) after 640 days compared to 2200 mg TPH per kg soil (74% reduction) for the unplanted control.

In a 6-month laboratory study, Pradhan *et al.* (1998) identified that alfalfa, switchgrass, and little bluestem were each capable of reducing the concentration of total PAHs in soil contaminated at a manufactured gas plant. The initial soil concentration of total PAHs for the three plant treatments and an unplanted control was  $184.5 \pm 14.0$  mg total PAHs per kg of soil. After 6 months, the concentration in the unplanted control soil was  $135.9 \pm 25.5$  mg  $kg^{-1}$ , while the concentrations in planted treatments were much lower (switchgrass =  $79.5 \pm 3.7$  mg  $kg^{-1}$ ; alfalfa =  $80.2 \pm 8.9$  mg  $kg^{-1}$ ; little bluestem =  $97.1 \pm 18.7$  mg  $kg^{-1}$ ).

Schwab *et al.* (1995) reported on the mineralization of phenanthrene from soil planted to sorghum, bermuda grass, or alfalfa. Results of the 14-day study indicated significantly higher mineralization of [ $^{14}C$ ]phenanthrene by the two warm-season grasses – sorghum (0.46% of

recovered  $^{14}\text{C}$ ) and bermuda grass (0.31%) – compared to a sterile, unplanted control (0.11%). There was no significant difference between mineralization of phenanthrene in soil planted to alfalfa (0.09%) and the control.

Finally, in a survey of 15 oil-contaminated sites in Western Europe, Gudin and Syrratt (1975) found several types of legumes growing abundantly in oil-contaminated areas. These plants included alfalfa, white clover, birdsfoot trefoil, black medick as well as *Psoralea bituminosa*, *Robinia pseudacacia*, *Melilotus altissima*, and *Vicia tetrasperma*.

### 3. MECHANISMS FOR THE PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS

There are three primary mechanisms by which plants and microorganisms remediate petroleum-contaminated soil and groundwater. These mechanisms (see Figure 1.1) include *degradation* and *containment*, as well as *transfer* of the hydrocarbons from the soil to the atmosphere (Cunningham *et al.*, 1996; Siciliano and Germida, 1998b; Sims and Overcash, 1983). The following section provides a detailed discussion of these mechanisms.

#### 3.1 Degradation

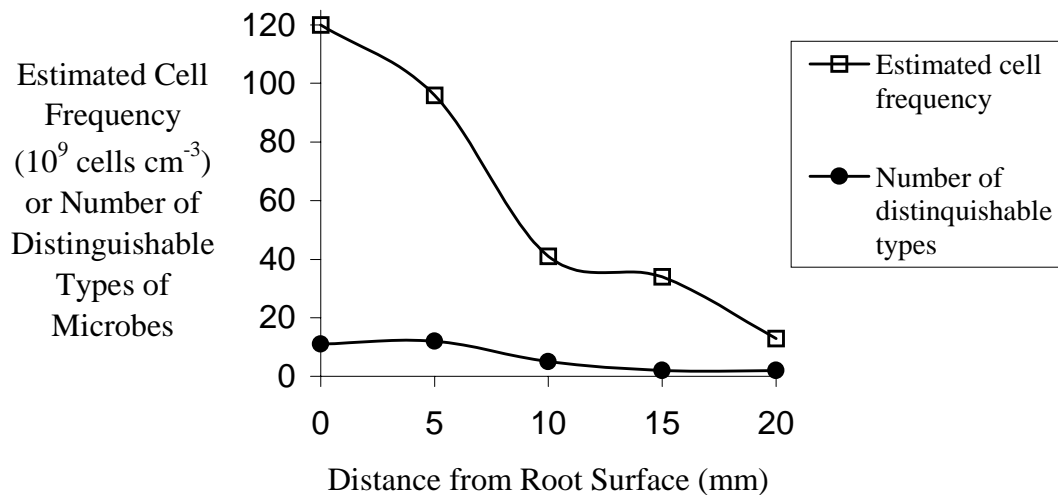
Plants and microorganisms are involved, both directly and indirectly, in the degradation of petroleum hydrocarbons into products (e.g., alcohols, acids, carbon dioxide, and water) that are generally less toxic and less persistent in the environment than the parent compounds (Eweis *et al.*, 1998). Though plants and microorganisms can degrade petroleum hydrocarbons independently of one another (refer to Sections 3.1.2 and 3.1.3), the literature suggests that it is the interaction between plants and microorganisms (i.e., the *rhizosphere effect*) which is the primary mechanism responsible for petrochemical degradation in phytoremediation efforts.

##### 3.1.1 The Rhizosphere Effect

The *rhizosphere* is the region of soil closest to the roots of plants and is, therefore, under the direct influence of the root system. Plants provide *root exudates* of carbon, energy, nutrients, enzymes and sometimes oxygen to microbial populations in the rhizosphere (Cunningham *et al.*, 1996; Campbell, 1985; Vance, 1996). Root exudates of sugars, alcohols, and acids can amount to 10 to 20% of plant photosynthesis annually (Schnoor *et al.*, 1995) and provide sufficient carbon and energy to support large numbers of microbes (e.g., approximately  $10^8 - 10^9$  vegetative microbes per gram of soil in the rhizosphere; Erickson *et al.*, 1995). Due to these exudates, microbial populations and activities are 5 to 100 times greater in the rhizosphere than in bulk soil (i.e., soil not in contact with plant roots) (Figure 3.1) (Atlas and Bartha, 1998; Gunther *et al.*, 1996; Anderson *et al.*, 1993; Paul and Clark, 1989). This plant-induced enhancement of the microbial population is referred to as the *rhizosphere effect* (Atlas and Bartha, 1998) and is believed to result in enhanced degradation of organic contaminants in the rhizosphere.

Several studies serve as examples of the rhizosphere effect in the phytoremediation of petroleum hydrocarbons. Gunther *et al.* (1996) found higher microbial numbers and activity coupled with increased degradation in hydrocarbon-contaminated soil planted to ryegrass compared to unplanted soil. The authors suggested that plant roots stimulated the microbes, which enhanced the degradation of the hydrocarbon mixture. Epuri and Sorensen (1997) reported marginally higher mineralization of [ $^{14}\text{C}$ ]benzo[*a*]pyrene, as well as higher microbial numbers, in soil planted to tall fescue compared to unplanted soil.





**Figure 3.1. Number of microorganisms at increasing distance from the root surface (adapted from Paul and Clark, 1989)**

Jordahl *et al.* (1997) reported that populations of microorganisms capable of degrading benzene, toluene, and xylene were five times more abundant in the rhizosphere of poplar trees (*Populus deltoides x nigra* DN-34, Imperial Carolina) than in bulk soil. Nichols *et al.* (1997) found greater numbers of organic chemical degraders in rhizosphere compared to bulk soil and in contaminated soils compared to uncontaminated soils. Plants creating the rhizosphere in this experiment included alfalfa and alpine bluegrass, while the contaminants included hexadecane, (2,2-dimethylpropyl)benzene, benzoic acid, pyrene, *cis*-decahydronaphthalene, and phenanthrene. Likewise, Radwan *et al.* (1998) identified that the roots of several plants from the Kuwaiti desert (*Senecio glaucus*, *Cyperus conglomeratus*, *Launaea mucronata*, *Picris babylonica* and *Salsola imbricata*) and crop plants (*Vicia faba* and *Lupinus albus*) were densely associated with hydrocarbon-utilizing bacteria (*Cellulomonas flavigena*, *Rhodococcus erythropolis* and *Arthrobacter* species). The rhizosphere soils of all plants contained greater numbers of these hydrocarbon-utilizing bacteria than bulk soils and this rhizosphere effect was more pronounced for plants growing in oil-contaminated soil compared to clean soil. As a result, the authors suggested that phytoremediation may be a feasible approach for cleaning oil-polluted soils.

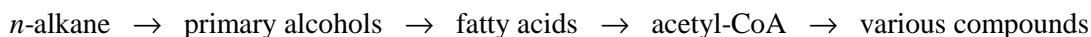
It should also be noted, however, that a few experiments suggest that the degradation of petroleum hydrocarbons from soil may not be enhanced by the rhizosphere effect. Ferro *et al.* (1994) reported that crested wheatgrass [*Agropyron desertorum* (Fisher ex Link) Schultes] had no effect on either the rate or extent of mineralization of [ $^{14}\text{C}$ ]phenanthrene when planted and unplanted systems were compared. For this experiment, the authors speculated that rapid mineralization of the [ $^{14}\text{C}$ ]phenanthrene by microbes prior to the establishment of the plant

root systems – and, therefore, prior to the presence of a rhizosphere effect in the soil – may have resulted in the lack of significant difference between mineralization in planted and unplanted systems. More recently, Ferro *et al.* (1997) reported that alfalfa (*Medicago sativa* Mesa, var. Cimarron VR) planted in artificial loamy sands had no effect on either the rate or extent of mineralization of [<sup>14</sup>C]benzene compared to unplanted soils. It should be noted, however, that in this experiment there was minimal replication (i.e.,  $n \leq 3$  for each of three trials) and the amount of [<sup>14</sup>C]benzene applied to the soils was small (i.e., 40 or 662 µg benzene per kg soil).

### 3.1.2 A Closer Look at the Role of Plants in Degradation

#### Direct Degradation

Evidence regarding the direct degradation of petroleum hydrocarbons by plants is somewhat dated and limited in quantity. Durmishidze (1977) summarized various studies, primarily from the USSR, on degradation pathways of hydrocarbons in plants. Corn seedlings, tea, and poplar shoots were reported to metabolize methane into various acids. The assimilation of radiolabelled methane, ethane, propane, butane, and pentane was recorded for bean and corn seedlings, as well as tea, grape, walnut and quince. The ability to assimilate *n*-alkanes and liberate <sup>14</sup>CO<sub>2</sub> was identified in leaves and roots of both whole and cut plants. The general pathway of conversion for alkanes in plants was generalized as:



Durmishidze (1977) also reported that benzene, toluene, and xylene were metabolized by cereal grasses in only two to three days; within the green mass of corn in four to five days; and by root crops in five to six days. Phenol was reported as the primary conversion product of benzene in plant tissues, with the subsequent production of various acids. The primary cleavage products of toluene were given as glycol, as well as glyoxalic, fumaric, succinic, and malic acid. Benzo[*a*]pyrene was reportedly metabolized by 14-day-old corn and bean plants, alfalfa, ryegrass, chick pea, cucumbers, squash, orchard grass, and vetch, with the amount of degradation ranging from 2 to 18% of the benzo[*a*]pyrene taken up by the plant and varying with plant type.

The results of several other more recent studies also indicate that PAHs can be degraded directly by plants. Edwards (1988) documented the metabolism of [<sup>14</sup>C]anthracene and [<sup>14</sup>C]benz[*a*]anthracene in bush bean grown in a nutrient solution containing the two PAHs. Within the plant, parent compounds were transformed into both polar and non-polar *metabolites*. Interestingly, substantial quantities of the polar metabolites moved into the nutrient solution as root exudates. By maintaining sterile conditions the author ruled out microbial transformation as the pathway for the production of these metabolites. Edwards *et al.* (1982) also reported that soybean was capable of degrading <sup>14</sup>C-anthracene. Evidence of the degradation was given by measuring the <sup>14</sup>CO<sub>2</sub> given off from plants placed in <sup>14</sup>C-anthracene-contaminated soil. It was also measured by analyzing extracts from plants that had their roots placed in a solution containing <sup>14</sup>C-anthracene. Dorr (1970; as cited in Edwards,

1983) reported that after 20 days of increasing concentration of benzo[a]pyrene within rye plants, concentrations began to decline – possibly due to the transformation or degradation of the benzo[a]pyrene within the plants.

### **Indirect Degradation**

There is a considerable body of information available regarding the indirect roles played by plants in the degradation of petroleum hydrocarbons. These include: (i) the supply of root exudates that cause the rhizosphere effect and enhance *cometabolic* degradation, (ii) the release of root-associated enzymes capable of transforming organic pollutants, and (iii) the physical and chemical effects of plants and their root systems on soil conditions (Gunther *et al.* 1996).

### **Root Exudates**

As described above, root exudates are the link between plants and microbes that leads to the rhizosphere effect. The type and amount of root exudate are dependent on plant species and the stage of plant development. For example, Hegde and Fletcher (1996) found that the release of total phenolics by the roots of red mulberry (*Morus rubra* L.) increased continuously over the life of the plant with a massive release at the end of the season accompanying leaf *senescence*. The type of root exudate is also likely to be site and time specific (Siciliano and Germida, 1998b). Site and time factors include variables such as soil type, nutrient levels, pH, water availability, temperature, oxygen status, light intensity, and atmospheric carbon dioxide concentration – all of which significantly affect the type and quantity of root exudates (Siciliano and Germida, 1998b).

The type of root exudate can influence the type of interaction between plants and soil microorganisms. For instance, interactions can be “specific” or “non-specific” depending on the exudate. Specific interactions occur when the plant exudes a specific compound (or compounds) in response to the presence of a contaminant (Siciliano and Germida, 1998b). Non-specific interactions occur when typical or “normal” plant exudates are chemically similar to the organic contaminant, resulting in increased microbial activity and increased degradation of the contaminants (Siciliano and Germida, 1998b). For example, the roots of red mulberry typically exude rhizosphere phenolics that help create a suitable environment for the biodegradation of PCBs, and perhaps PAHs, by selectively promoting the growth of certain microbes, such as the PCB-degrading bacteria *Alcaligenes eutrophus* H850, *Corynebacterium* sp. MB1, and/or *Pseudomonas putida* LB400 (Hegde and Fletcher, 1996; Donnelly *et al.*, 1994).

Results of experiments using rhizosphere soil collected from alfalfa roots and non-rhizosphere (bulk) soil suggest that the continued presence of plant roots and their exudates may be required for degradation of PAHs in soil (Wetzel *et al.*, 1997). Investigations on the bioenergetics of microbial degradation of toluene, phenol, 1,1,1-trichloroethane and trichloroethylene in sandy soil planted to alfalfa also suggest that this is the case (Erickson *et al.*, 1995). Conversely, reduced mineralization of naphthalene was found in soils planted with

bell rhodesgrass (*Chloris gayana*) versus unplanted soils, possibly because root exudates may have placed naphthalene-degrading microorganisms at a competitive disadvantage (Watkins *et al.*, 1994).

### ***Cometabolism***

*Cometabolism* is the process by which a compound that cannot support microbial growth on its own can be modified or degraded when another growth-supporting substrate is present (Cunningham and Berti, 1993). Organic molecules, including plant exudates, can provide energy to support populations of microbes that co-metabolize petroleum hydrocarbons. For example, Ferro *et al.* (1997) hypothesized that plant exudates may have served as co-metabolites during the biodegradation of [<sup>14</sup>C]pyrene in the rhizosphere of crested wheatgrass.

Petroleum hydrocarbons can also serve as cometabolites, particularly for the larger, more persistent (i.e., recalcitrant) hydrocarbons such as PAHs with four or more benzene rings. Indeed, the presence of oil and grease co-substrates significantly enhanced the degradation of fluoranthene, pyrene, indo[1,2,3-*c,d*]pyrene, benz[*a*]anthracene, benzo[*k*]fluoranthene, chrysene, and benzo[*g,h,i*]perylene, all of which have four or five benzene rings (Keck *et al.*, 1989). Benzo[*a*]pyrene is another large (five-ring) PAH that is typically recalcitrant in soil, yet it was almost completely degraded (95% degradation) by soil microbes when suitable co-substrates were present in a crude oil mixture (Kanaly *et al.*, 1997). The recalcitrant nature of PAHs with four or more benzene rings is thought to be due to the inability of microorganisms to use these compounds directly for energy and growth, which emphasizes the importance of cometabolism in their degradation (Kanaly *et al.*, 1997; Keck *et al.*, 1989; Sims and Overcash, 1983).

### ***Plant Enzymes Involved in Phytoremediation***

Another indirect role that plants play in the degradation of petroleum hydrocarbons involves the release of enzymes from roots. These enzymes are capable of transforming organic contaminants by catalyzing chemical reactions in soil. Schnoor *et al.* (1995) identified plant enzymes as the causative agents in the transformation of contaminants mixed with sediment and soil. Isolated enzyme systems included dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase. These findings suggest that plant enzymes may have significant spatial effects extending beyond the plant itself and temporal effects continuing after the plant has died (Cunningham *et al.*, 1996).

### ***Effect of Plants on Physical/Chemical Soil Condition***

Plants and their roots can indirectly influence degradation by altering the physical and chemical condition of the soil. Soil exploration by roots helps bring plants, microbes, nutrients and contaminants into contact with each other (Cunningham *et al.*, 1996). Plants also provide organic matter to the soil, either after they die or as living plants through the loss of root cap cells and the excretion of mucigel, a gelatinous substance that is a lubricant for root penetration through the soil (Cunningham *et al.*, 1996). Organic matter can reduce the *bioavailability* (i.e., the extent to which a contaminant is available to interact with living

organisms) of some petroleum hydrocarbons, particularly those that are *lipophilic* (soluble in lipids) and bind to organic matter. The influence of soil organic matter on phytoremediation efforts is discussed in more detail in Section 4.1.

### **3.1.3 A Closer Look at the Role of Microorganisms in Degradation**

Currently, microorganisms are used to destroy or immobilize organic contaminants in the absence of plants in a process referred to as *bioremediation* (see Section 6.3 for further details). In this section, we focus on issues concerning the role of microorganisms in the degradation of petroleum hydrocarbons in the presence of plants – a mechanism of phytoremediation. These issues include the types of microorganisms involved in phytoremediation, reasons for microbial degradation, differences in degradation by various microorganisms, characteristics of microbial communities involved in degradation, and the role microorganisms play in reducing *phytotoxicity* to plants.

#### **Types of Microorganisms**

A variety of microorganisms are reportedly involved in the degradation of petroleum hydrocarbons (Table 3.1). In general, the bacteria *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Mycobacterium*, and *Nocardia* are reported as the most active bacterial species in the degradation of hydrocarbons in soil (Bossert and Bartha, 1984). *Pseudomonas*, *Arthrobacter*, and *Achromobacter* often occur in greater numbers within rhizosphere soil than bulk soil (Walton *et al.*, 1994b). Soil fungi also play a role in the degradation of petroleum hydrocarbons. For example, Sutherland (1992) reported that a diversity of fungi, including *Aspergillus ochraceus*, *Cunninghamella elegans*, *Phanerochaete chrysosporium*, *Saccharomyces cerevisiae*, and *Syncephalastrum racemosum*, can oxidize various PAHs (e.g., anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, fluoranthene, fluorene, naphthalene, phenanthrene, pyrene) as well as methyl-, nitro-, and fluoro-substituted PAHs.

Radwan *et al.* (1995) investigated the microorganisms associated with various plants grown in soil polluted with 10% crude oil by weight. The plants used in the investigation included various Kuwaiti desert plants together with corn, tomato and termis. Rhizosphere samples of all plants were rich in oil-utilizing microorganisms. Filamentous actinomycetes (possibly *Streptomyces*) were present in all samples and the predominant bacterial genus (>95%) in the rhizosphere of all plants was *Arthrobacter*. On the other hand, *Rhodococcus*, *Pseudomonas*, and *Bacillus* predominated in the contaminated bulk soil – with *Arthrobacter* making up less than 5% of the total culturable bacteria. The predominant fungi in the rhizosphere samples belonged to *Penicillium* and *Fusarium*; in the contaminated bulk soil, however, *Trichoderma* predominated. Eight strains of *Arthrobacter*, two of *Penicillium*, and two of *Fusarium*, isolated from the rhizosphere of various plants growing in the oil-contaminated soil, also grew well in cultures when a variety of individual odd- and even-chain *n*-alkanes (C<sub>10</sub> to C<sub>40</sub>), benzene, naphthalene, and phenanthrene were supplied as the sole sources of carbon and energy. It was further demonstrated that the four predominant *Arthrobacter* strains could

quickly consume the *n*-alkanes dodecane (C<sub>12</sub>), hexadecane (C<sub>16</sub>), and decosane (C<sub>22</sub>) as well as naphthalene and phenanthrene from their growth medium.

**Table 3.1. Genera of hydrocarbon-degrading microorganisms isolated from soil<sup>1</sup>.**

Bacteria		Fungi	
<i>Acidovorax</i> (phenanthrene, anthracene)	<i>Alcaligenes</i> (phenanthrene, fluorene, fluoranthene)	<i>Cunninghamella</i> (benzo[ <i>a</i> ]pyrene)	<i>Fusarium</i> ( <i>n</i> -alkanes (C <sub>10</sub> to C <sub>40</sub> ), benzene, naphthalene, phenanthrene)
<i>Arthrobacter</i> ( <i>n</i> -alkanes (C <sub>10</sub> to C <sub>40</sub> ), benzene, naphthalene, phenanthrene)	<i>Mycobacterium</i> (2-methylnaphthalene, phenanthrene, pyrene, benzo[ <i>a</i> ]pyrene, carbazole)	<i>Penicillium</i> ( <i>n</i> -alkanes (C <sub>10</sub> to C <sub>40</sub> ), benzene, naphthalene, phenanthrene)	<i>Phanerochaete</i> (benzo[ <i>a</i> ]pyrene)
<i>Pseudomonas</i> (phenanthrene, fluoranthene, fluorene, benzo[ <i>a</i> ]pyrene)	<i>Rhodococcus</i> (pyrene and benzo[ <i>a</i> ]pyrene)		
<i>Sphingomonas</i> (phenanthrene, fluoranthene, anthracene)	<i>Xanthomonas</i> (carbazole)		
<i>Achromobacter</i>	<i>Micrococcus</i>	<i>Acremonium</i>	<i>Monilia</i>
<i>Acinetobacter</i>	<i>Norcardia</i>	<i>Aspergillus</i>	<i>Mortierella</i>
<i>Bacillus</i>	<i>Proteus</i>	<i>Aureobasidium</i>	<i>Paecilomyces</i>
<i>Brevibacterium</i>	<i>Sarcina</i>	<i>Beauveria</i>	<i>Phoma</i>
<i>Chromobacterium</i>	<i>Serratia</i>	<i>Botrytis</i>	<i>Rhodotorula</i>
<i>Corynebacterium</i>	<i>Spirillum</i>	<i>Candida</i>	<i>Saccharomyces</i>
<i>Cytophaga</i>	<i>Streptomyces</i>	<i>Chrysosporium</i>	<i>Scolecobasidium</i>
<i>Erwinia</i>	<i>Vibrio</i>	<i>Cladosporium</i>	<i>Sporobolomyces</i>
<i>Flavobacterium</i>		<i>Cochliobolus</i>	<i>Sprotrichum</i>
		<i>Cylindrocarpon</i>	<i>Spicaria</i>
		<i>Debaryomyces</i>	<i>Syncephalastrum</i>
		<i>Geotrichum</i>	<i>Tolypocladium</i>
		<i>Gliocladium</i>	<i>Torulopsis</i>
		<i>Graphium</i>	<i>Trichoderma</i>
		<i>Humicola</i>	<i>Verticillium</i>

<sup>1</sup> Specific information on the type of hydrocarbon degraded is provided when available.

<sup>2</sup> Sources: Barnsley (1975); Bossert and Bartha (1984); Bumpus *et al.* (1985); Cerniglia and Gibson (1979); Grosser *et al.* (1995); Heitkamp and Cerniglia (1989); Radwan *et al.* (1995); Shuttleworth and Cerniglia (1990); Sutherland (1992).

## Microbial Degradation of Organic Contaminants

Microbial degradation of organic contaminants normally occurs as a result of microorganisms using the contaminant for their own growth and reproduction (Committee on *In Situ* Bioremediation *et al.*, 1993). Organic contaminants not only provide the microorganisms with a source of carbon, they also provide electrons that the organisms use to obtain energy (Committee on *In Situ* Bioremediation *et al.*, 1993). Basic microbial metabolism of contaminants involves aerobic *respiration* (respiration in the presence of oxygen). Variations in metabolism include *anaerobic* respiration, cometabolism, fermentation, reductive dehalogenation, and the use of inorganic compounds as electron donors (Committee on *In Situ* Bioremediation *et al.*, 1993). Interestingly, bacteria are capable of quickly distributing genetic information to each other, thus allowing them to adapt quickly to environmental changes, such as exposure to new contaminants (Bollag *et al.*, 1994).

In general, the metabolic processes of microorganisms act on a wider range of compounds, carry out more difficult degradation reactions, and transform contaminants into more simple molecules than those of plants (Cunningham and Berti, 1993). However, not all microorganisms degrade organic contaminants in the same manner. The pathway of aerobic degradation of PAHs by *prokaryotic* microorganisms, such as bacteria, involves a dioxygenase enzyme, the incorporation of two atoms of molecular oxygen into the contaminant, and the production of less toxic compounds such as acids, alcohols, carbon dioxide and water (Gibson and Subramanian, 1984; Eweis *et al.*, 1998; Pothuluri and Cerniglia, 1994). In contrast, degradation by *eukaryotic* fungi initially involves the incorporation of only one atom of oxygen into the PAH, which is similar to the degradation mechanism found in mammals (Sutherland, 1992; Cerniglia *et al.*, 1986; Cerniglia and Gibson, 1979; Pothuluri and Cerniglia, 1994). Although most fungal transformations result in compounds that are less toxic than the parent PAHs, some of the minor metabolites produced during fungal degradation of PAHs result in compounds that are more toxic than the parent compounds (Sutherland, 1992).

There are several points of interest regarding microbial communities involved in the phytoremediation of organic contaminants. For example, the composition and size of the microbial community in the rhizosphere depends on plant species, plant age, and soil type (Campbell, 1985; Atlas and Bartha, 1998; Bossert and Bartha, 1984). The microbial community also may vary with exposure history; i.e., soil microbial communities may experience selective enrichment of contaminant-tolerant species when exposed to a contaminant for a prolonged period of time (Anderson *et al.*, 1993). On the other hand, some species of bacteria can degrade a wide variety of rarely-occurring compounds without having to first adapt to contaminated conditions (Siciliano and Germida, 1998b). *Catabolic* pathways in pseudomonads, for example, allow these bacteria to degrade a variety of aromatic contaminants (e.g., toluene, *m*-xylene, and naphthalene) without having to synthesize a large number of different enzymes (Houghton and Shanley, 1994).

Evidence suggests that the degradation of certain contaminants may take place only if a specific *consortium* of microbes occurs at the contaminated site (Anderson *et al.*, 1993). Lappin *et al.* (1985) isolated five species of bacteria from the rhizosphere of wheat that could grow on and degrade the herbicide mecoprop [2-(2-methyl 4-chlorophenoxy)propionic acid], but only as long as two or more species occurred together. Individually, none of the species could degrade mecoprop. The requirement of a microbial consortium is not a characteristic exclusive to contaminant degradation, as the degradation of many naturally-occurring organic molecules often involves different microorganisms working in concert or succession to break down the parent molecule and its metabolites (Bollag *et al.*, 1994).

Finally, White and Alexander (1996) found a consortium of microbes that could utilize phenanthrene sorbed to soil without first desorbing it. This has important implications for bioavailability, since it appears that some bacteria may not require certain contaminants to be in the aqueous phase before degradation can occur.

### **Role of Microorganisms in Reducing Phytotoxicity to Plants**

Another role played by microbes involves their ability to reduce the *phytotoxicity* of contaminants to the point where plants can grow in adverse soil conditions, thereby stimulating the degradation of other, non-phytotoxic contaminants (Siciliano and Germida, 1998b). In fact, Walton *et al.* (1994a) have hypothesized that the defenses of plants to contaminants may be supplemented through the external degradation of contaminants by microorganisms in the rhizosphere. That is to say, plants and microbes have co-evolved a mutually-beneficial strategy for dealing with phytotoxicity, where microorganisms benefit from the plant exudates while the plants benefit from the ability of microorganisms to break down toxic chemicals.

Evidence in support of this hypothesis can be found in several studies. Rasolomanana and Balandreau (1987) found improved growth of rice in soil to which oil residues had been applied. The authors hypothesized that the increased growth resulted from the removal of the oil residues by various bacterial species of the genus *Bacillus*, which used plant exudates to cometabolize the oil residues in the rhizosphere. Radwan *et al.* (1995) found the plant *Senecio glaucus* growing along the polluted border of an oil lake in the Kuwaiti desert. The plant roots and adhering sand particles were white and clean, while the surface of the transitional zone between the root and shoot was black and polluted. The authors suggested that microbes detoxified contaminants in the rhizosphere, which allowed the plants to survive in the oil-contaminated soils.

### **3.2 Containment**

Containment involves using plants to reduce or eliminate the bioavailability of contaminants to other biota. Contaminants are not necessarily degraded when they are contained. Direct mechanisms of containment by plants include the accumulation of petroleum hydrocarbons within the plants and *adsorption* of the contaminants on the root surface (Figure 1.1). Another direct mechanism involves the use of plants as organic pumps to isolate the contaminant



within the root zone, thus preventing the contaminant from spreading. These direct mechanisms of containment are discussed in greater detail in Sections 3.2.1 and 3.2.2, respectively.

Plants act indirectly to contain contaminants by supplying enzymes that bind contaminants into soil organic matter (or *humus*) in a process called *humification* and by increasing soil organic matter content, which allows for humification (Cunningham *et al.*, 1996). For example, preliminary studies by Walton *et al.* (1994a) suggest that  $^{14}\text{C}$  originating from radiolabeled fluoranthene, phenanthrene and naphthalene may be incorporated by sweet clover (*Melilotus alba*) and its associated microorganisms into humic and fulvic acids found in the rhizosphere.

### **3.2.1 Accumulation of Petroleum Hydrocarbons by Plants**

Various studies have documented the accumulation of petroleum hydrocarbons in plants as well as the adsorption of these compounds onto the surface of plant roots. Researchers have identified that the *lipid* content of the plant may influence the degree of accumulation of petroleum hydrocarbons. Attempts have been made to model the uptake and accumulation of petroleum hydrocarbons in plants based on the chemical characteristics of the various hydrocarbons, particularly the compound's affinity for lipids. These issues are addressed in greater detail below.

#### **Examples of Accumulation**

Several studies illustrate that plants take up petroleum hydrocarbons via their roots and may accumulate them to a small degree in their roots and shoots. Durmishidze (1977) reported that rice seedlings take up [ $^{14}\text{C}$ ]methane through their roots and that bean and corn seedlings take up radiolabelled methane, ethane, propane, butane, and pentane through their roots and leaves. It also was reported by Durmishidze that benzene, toluene, and xylene entered plants with irrigation water, becoming incorporated into the metabolic processes of the plant. In experiments involving the fate of [ $^{14}\text{C}$ ]benzene in soils planted with alfalfa, Ferro *et al.* (1997) found that 2% to 8% of the  $^{14}\text{C}$  was recovered in the root fraction, which included small portions of rhizosphere soil attached to unwashed roots, and that less than 2% of the recovered  $^{14}\text{C}$  occurred in the shoots of plants.

Edwards *et al.* (1982) documented the uptake of [ $^{14}\text{C}$ ]anthracene in soybean via roots exposed to [ $^{14}\text{C}$ ]anthracene-contaminated soil or solution culture and via leaves exposed to [ $^{14}\text{C}$ ]anthracene in the air surrounding the plant. Once the anthracene was taken up by the plant, either through its leaves or roots, it was *translocated* to other parts of the plant. Edwards (1988) also investigated the uptake and translocation of [ $^{14}\text{C}$ ]anthracene and [ $^{14}\text{C}$ ]benz[*a*]anthracene in bush bean, using plants grown in a nutrient solution to which PAHs were added individually. Results indicated that 54% of the total  $^{14}\text{C}$  dose was taken up into the roots of anthracene-treated plants after 30 days, while 60% was taken up into the roots of benz[*a*]anthracene-treated plants. For both PAHs,  $^{14}\text{C}$  was present in much smaller amounts in the stems (3.4% for anthracene-treated plants; 0.4% for benzo[*a*]anthracene-treated plants)

and leaves (3.1% for anthracene-treated plants; 0.4% for benzo[*a*]anthracene-treated plants). The amount of <sup>14</sup>C recovered in the stems and leaves of anthracene-treated plants was greater than the amount recovered in the benz[*a*]anthracene-treated plants. This indicates that anthracene (the smaller, more water-soluble of the two PAHs), together with its metabolites, is more readily translocated from the roots and assimilated within the plant.

Duxbury *et al.* (1997) identified the uptake of intact and photomodified [<sup>14</sup>C]anthracene, phenanthrene and benzo[*a*]pyrene by the aquatic plant duckweed (*Lemna gibba*) under light (i.e., simulated solar radiation) and dark conditions. Uptake was generally lower for both the intact and photomodified forms of the chemicals under simulated solar radiation compared to dark conditions.

Wild and Jones (1992) determined that when carrots were grown in sewage sludge contaminated with PAHs, the total PAH content of the carrot root peels plateaued at 200 µg kg<sup>-1</sup> dry weight given total soil PAH levels ≥500 µg kg<sup>-1</sup>. They also found that the lower molecular weight PAH compounds – such as naphthalene, acenaphthene, and fluorene – were relatively enriched in the peel, most likely due to their greater water solubility and bioavailability. Transfer from the root peel to the core of the root appeared to be minimal.

In contrast, the results of several other studies suggest that not all plants take up petroleum hydrocarbons from contaminated soil. For example, Goodin and Webber (1995) found no evidence for the uptake of intact [<sup>14</sup>C]anthracene or [<sup>14</sup>C]benzo[*a*]pyrene by soybean, ryegrass (*Lolium multiflorum* Lam.) or cabbage (*Brassica oleracea* var. capitata L.) from municipal sludge-treated soil to which the radio-labelled contaminants were added. Similarly, Biederbeck *et al.* (1993) found no uptake by oat or wheat of petroleum hydrocarbons from soil contaminated with an oily waste sludge. Chaineau *et al.* (1997) did not identify any hydrocarbon components in the tissues of maize exposed to fuel oil in soil. Results of a study by Qui *et al.* (1997) showed no accumulation of PAHs in the shoots or roots of common buffalograss, prairie buffalograss, zoysiagrass, or kleingrass exposed to contaminated soil. Rogers *et al.* (1996) found no contaminants in the tissues of plants exposed to a mixture of organic chemicals (MOC). The MOC contained equal molar amounts of benzoic acid, hexadecane, phenanthrene, pyrene, 2,2-dimethyl 4, npropyl-benzene, and either cycloheptane or cis-decahydronaphthalene (cis-decalin). The plants used were red clover, white clover, alfalfa, birdsfoot trefoil, tulesy sage, alpine bluegrass, bering hairgrass, reed canarygrass, and quackgrass.

### **Influence of Lipid Content**

Research has indicated that the lipid content of the plant influences the degree of PAH-accumulation. For example, Edwards (1983) reported higher concentrations of PAHs and, specifically, benzo[*a*]pyrene in oil extracted from plants than from plant tissues in general. Schwab *et al.* (1998) found that the roots of alfalfa adsorbed naphthalene to a greater degree than fescue (*Festuca arundinacea* Schreber), i.e., alfalfa roots had approximately twice the affinity for naphthalene than did the roots of fescue. This difference in affinity was related to

the greater lipid content of alfalfa (10 g lipid kg<sup>-1</sup> dry root) compared to fescue (4.5 g lipid kg<sup>-1</sup> dry root). Simonich and Hites (1994a & b) reported that white pine (*Pinus strobus*) and sugar maple (*Acer saccharum*) accumulated PAHs in their above ground tissues. They also found that plant tissue types with higher lipid contents (e.g., the needles of white pine) generally contained higher concentrations of PAHs than tissue types with lower lipid contents (e.g., the leaves and seeds of sugar maples).

### **Modelling Accumulation**

Models using the octanol-water partition coefficient have been developed and tested to varying degrees for the uptake of organic chemicals by plants (see Paterson *et al.*, 1994; Trapp *et al.*, 1990; Ryan *et al.*, 1988; and Briggs *et al.*, 1982). The octanol-water partition coefficient ( $K_{ow}$ , often expressed as  $\log K_{ow}$ ) is a measure of a chemical's affinity for water versus lipids or fats (Mackay, 1991). In general, chemicals that are highly water soluble (i.e., *hydrophilic* compounds with a  $\log K_{ow} < 0.5$ ) are not sufficiently sorbed to roots or actively transported through plant membranes (Schnoor *et al.*, 1995). Hydrophobic chemicals ( $\log K_{ow} > 3.0$ ) are not easily transported within the plant because they are strongly bound to and may not pass beyond the root's surface due to the high proportion of lipids present at the surface (Siciliano and Germida, 1998b). Two exceptions are the uptake of polychlorinated dibenzo-*p*-dioxins and dibenzofurans ( $\log K_{ow} > 6$ ) by the roots of zucchini (*Curcubita pepo* L. convar. *gircomontiina*) and pumpkin (*Curcubita pepo* L. cv. *Gelber Zentner*) (Hulster *et al.*, 1994). Moderately *hydrophobic* organic chemicals ( $\log K_{ow} = 0.5$  to 3.0) are effectively taken up by plants; these chemicals include most BTEX, chlorinated solvents, and short-chain aliphatic chemicals (Schnoor *et al.*, 1995; Siciliano and Germida, 1998b).

In addition to the octanol-water partition coefficient, the size and molecular weight of an organic contaminant may play a role in the ability of a plant to take up the contaminant. Anderson *et al.* (1993) reported that plant root uptake usually favors small, low molecular weight polar compounds, whereas large, high molecular weight compounds tend to be excluded from the root. Environmental conditions and plant characteristics also may affect uptake by roots (Anderson *et al.*, 1993).

#### **3.2.2 Plants as Organic Pumps**

Plants *transpire* considerable amounts of water and, thus, can reverse the downward migration of water-soluble chemicals (Schnoor *et al.*, 1995). When Aprill and Sims (1990) were evaluating the use of prairie grasses for treatment of PAH-contaminated soil, they found that the cumulative volume of *leachate* collected after 219 days was significantly higher from unplanted than planted *microcosms*. In fact, there was no leachate from the vegetated systems once the grasses had established sufficient root and shoot mass. In this manner, the plants act as organic pumps, preventing both parent compounds and degradation intermediates dissolved in water from spreading or leaching beyond the rooting zone. This upward pumping action can be expected to be most prevalent in semi-arid to arid regions where *evapotranspiration* exceeds precipitation (Davis *et al.*, 1994).

### 3.3 Transfer of Petroleum Hydrocarbons to the Atmosphere

Soil may be phytoremediated by using plants to transfer volatile petroleum hydrocarbons from the soil to the atmosphere (see Figure 1.1). In the phytoremediation literature, this process is also known as *phytovolatilization* (Flathman and Lanza, 1998). Wiltse *et al.* (1998) observed leaf burn in alfalfa plants growing in crude oil-contaminated soil. The authors suggested that an unidentified compound from the contaminated soil was being translocated through the plant and then transpired. The leaf burn gradually disappeared as the experiment progressed, indicating that the contaminants responsible for this effect had dissipated. Watkins *et al.* (1994) found that the *volatilization* of [<sup>14</sup>C]naphthalene was enhanced in sandy loam soil planted to Bell rhodesgrass compared to unplanted soil. The results of the study suggest that naphthalene was taken up by the roots of the grass, translocated within the plant, and transpired through the stems and leaves. The authors noted that this mechanism of removal would reduce the amount of naphthalene available in soil, but may have implications regarding subsequent contamination of the atmosphere and, consequently, regulatory compliance with air quality guidelines.

## 4. INFLUENCE OF ENVIRONMENTAL FACTORS ON PHYTOREMEDIATION

A variety of environmental factors affect or alter the mechanisms of phytoremediation. Soil type and organic matter content can limit the bioavailability of petroleum contaminants. Water content in soil and wetlands affects plant/microbial growth and the availability of oxygen required for aerobic respiration. Temperature affects the rates at which various processes take place. Nutrient availability can influence the rate and extent of degradation in oil-contaminated soil. Finally, sunlight can transform parent compounds into other compounds, which may have different toxicities and bioavailability than the original compounds. These various environmental factors cause *weathering* – the loss of certain fractions of the contaminant mixture – with the end result being that only the more resistant compounds remain in the soil.

### 4.1 Soil Structure, Texture, and Organic Matter Content

Soil type is defined according to various characteristics including structure, texture, and organic matter content. In terms of the influence of *soil structure*, Alexander *et al.* (1997) identified that phenanthrene may be trapped within and sorbed to the surfaces of *nanopores* (soil pores with diameters < 100 nm) that are inaccessible to organisms (i.e., not bioavailable).

*Soil texture* can also affect phytoremediation efforts by influencing the bioavailability of the contaminant. For example, clay is capable of binding molecules more readily than silt or sand (Brady and Weil, 1996). As a result, the bioavailability of contaminants may be lower in soils with high clay contents. In support of this concept, Carmichael and Pfaender (1997) found that soils with larger particles (e.g., sand) typically had greater mineralization of PAHs than soils with smaller particles (e.g., silt and clay), possibly due to the greater bioavailability of the contaminants in the sandy soils. Similarly, Edwards *et al.* (1982) found that the amount of <sup>14</sup>C-anthracene taken up by soybean in soil was considerably lower than the amount taken up by plants in nutrient solution. The authors stated that they had anticipated this result since PAHs are known to adsorb to soil constituents and, in doing so, are no longer available for uptake from the soil.

Soil organic matter binds lipophilic compounds, thereby reducing their bioavailability (Cunningham *et al.*, 1996). A high organic carbon content (>5%) in soil usually leads to strong adsorption and, therefore, low availability, while a moderate organic carbon content (1 to 5%) may lead to limited availability (Otten *et al.*, 1997).

Soil type may influence the quality or quantity of root exudates, which may influence phytoremediation efforts. More specifically, research by Bachmann and Kinzel (1992) indicates an interrelationship between soil type and levels of amino acids, sugars, and certain enzyme activities in the rhizosphere. On the other hand, Siciliano and Germida (1997) found that the effectiveness of phytoremediation to reduce concentrations of 2-chlorobenzoic acid in three soils from Saskatchewan was not influenced by soil type.

## 4.2 Water and Oxygen Availability

Water and oxygen are important to the general health of plants and microbes (Eweis *et al.*, 1998). Water is not only a major component of living organisms, it also serves as a transport medium to carry nutrients to biota and carry wastes away. If the moisture content of the soil is low, there will be a loss of microbial activity and dehydration of plants. Too much moisture results in limited gas exchange and the creation of anoxic zones where degradation is dominated by anaerobic microorganisms.

Interestingly, oxygen may be provided to the rhizosphere as a plant exudate. The extent of oxygen-transfer from the root depends on the type of plant (Vance 1996). Herbaceous wetland plants typically have a high capacity to transport oxygen from their leaves to their roots and then into the rhizosphere. Conversely, non-wetland and woody plants have a poor capacity for the downward transport of oxygen. Downward oxygen transport in wetland plants allows the plants to survive in saturated conditions under which most terrestrial plants would die. It also allows wetland plants to support an enhanced bacterial population in the rhizosphere, which may help degrade organic contaminants.

## 4.3 Temperature

Temperature affects the rates at which the various mechanisms of phytoremediation take place. In general, the rate of microbial degradation or transformation doubles for every 10 °C increase in temperature (Eweis *et al.*, 1998; Wright *et al.*, 1997). In an experiment involving oil bioremediation in salt marsh mesocosms, degradation of applied hydrocarbons averaged 72% during summer compared to 56% during winter, even though the winter exposure was 42 days longer (Wright *et al.*, 1997). The seasonal difference was thought to be the result of a 10 °C difference in temperature between the warm summer and cool winter periods. Likewise, the biodegradation of kerosene in a contaminated sandy loam soil reached its maximum rate during the months of July and August – when the temperature was  $\geq 20$  °C (Dibble and Bartha, 1979). Simonich and Hites (1994a & b) reported that concentrations of PAHs in plants were higher during spring and autumn when ambient temperatures were relatively low compared to summer. Conversely, during the summer, when ambient temperatures were higher, lower concentrations of PAHs were found in the plants.

## 4.4 Nutrients

Adequate soil nutrients are required to support the growth of plants and their associated microorganisms. This may be especially true during phytoremediation efforts, when the plant/microbe community is already under stress from the contaminant. Xu and Johnson (1997) have shown that petroleum hydrocarbons can significantly reduce the availability of plant nutrients in soil. Low nutrient availability results from the fact that petroleum hydrocarbons have high carbon contents, but are poor suppliers of nitrogen and phosphorus. As soil microorganisms degrade the hydrocarbons, they use up or immobilize available nutrients (i.e., nitrogen and phosphorus) creating nutrient deficiencies in contaminated soil.

Biederbeck *et al.* (1993) found that, following initial applications of an oily waste sludge to sandy soil, the soil had very low nitrate levels due to immobilization of nitrogen by rapidly growing populations of oil degrading bacteria as well as suppression of nitrogen-fixing bacteria. Two years following oil application, however, sludge treated plots contained more nitrate than untreated controls, presumably due to the gradual remineralization of the previously immobilized nitrogen. The trend was similar for phosphorus, which was initially low following incorporation due to immobilization by an expanding microbial biomass, but became more available one year later.

Petroleum hydrocarbons also may limit the accessibility of nutrients to plants and microorganisms by reducing the availability of water in which the nutrients are dissolved (Schwendinger, 1968). Nutrient deficiencies in soil caused by petroleum hydrocarbons may be offset by the application of fertilizer or *green manure* to the soil (see Section 5.5).

#### **4.5 Solar Radiation**

Photomodifications of PAHs by ultraviolet light can occur in contaminated water or on the surface of soil – increasing the polarity, water solubility, and toxicity of the compounds prior to uptake by the plant (McConkey *et al.*, 1997; Ren *et al.*, 1994; Huang *et al.*, 1993). PAHs that can be modified in this manner include anthracene, phenanthrene, benzo[*a*]pyrene, fluoranthene, pyrene, and naphthalene. Enhanced toxic effects (such as reduced growth) also can result from penetration of ultraviolet radiation into plant tissue, followed by photomodifications and photosensitizations of PAHs accumulated within these tissues (Duxbury *et al.*, 1997).

#### **4.6 Weathering**

Weathering processes include volatilization, evapotranspiration, photomodification, hydrolysis, leaching and biotransformation of the contaminant. These processes selectively reduce the concentration of easily-degradable contaminants, with the more recalcitrant compounds remaining in the soil. The contaminants left behind are typically non-volatile or semi-volatile compounds that preferentially partition to soil organic matter or clay particles, which limits their bioavailability and the degree to which they can be degraded (Bossert and Bartha, 1984; Cunningham and Ow, 1996; Bollag, 1992; Cunningham *et al.*, 1996). Carmichael and Pfaender (1997) noted that contaminant bioavailability was a major factor limiting the degradation of weathered (>60 years) PAHs.

## 5. SPECIAL CONSIDERATIONS ASSOCIATED WITH PHYTOREMEDIATION

Special considerations associated with the phytoremediation of petroleum hydrocarbons include establishment of appropriate plant and microbial communities on the contaminated site; effects of various hydrocarbon concentrations on plants and microbes; biotransformation and bioaccumulation of the contaminants together with disposal of contaminated biomass; implications regarding mixtures of inorganic and organic compounds at contaminated sites; and techniques to enhance phytoremediation. The following section provides information on these various considerations.

### 5.1 Establishment of Appropriate Plants and Microorganisms

Successful phytoremediation of petroleum hydrocarbons will require the establishment of appropriate plants and microorganisms at the contaminated site. Factors to consider include (i) the influence of contaminants on germination of plants or survival of transplanted vegetation, (ii) the effectiveness of inoculating contaminated soils with microorganisms and (iii) the use of native versus non-native plants and microorganisms to phytoremediate the site.

#### 5.1.1 Seed Germination and Transplanting

An important factor in establishing plants in an oil-contaminated site involves getting seeds to germinate. Seed germination is enhanced when soil is moist (but not too wet), the temperature is appropriate, and the soil is not toxic to the seeds (Cunningham *et al.*, 1996). A good way of knowing whether the plant(s) being considered for phytoremediation will germinate successfully is to carry out germination tests in the contaminated soil prior to planting (Cunningham *et al.*, 1996). For example, Epuri and Sorensen (1997) tested the germination of four grasses [tall fescue, perennial rye grass, reliant hard fescue (*Festuca longifolia* Thuill.) and nassau Kentucky bluegrass (*Poa pratensis* L.)] exposed to approximately 220 mg of PAHs and 26 mg of PCBs per kilogram of soil (0.022% w/w PAHs and 0.0026% w/w PCBs). Of the four species, tall fescue had the highest germination rate (individual rates not reported).

According to the results of germination tests by Dibble and Bartha (1979), germination of wheat and soybean in kerosene-contaminated soil (0.34% w/w) was delayed compared to germination in uncontaminated soil. However, the overall percentage of germination after 10 days was similar in both the contaminated and uncontaminated soil. The authors suggested that the slower rate of germination in the soil contaminated with kerosene may have been a result of decreased oxygen availability and, consequently, increased competition for oxygen between the germinating seeds and microorganisms.

Wiltse *et al.* (1998) found that alfalfa seed germinates in soils contaminated with up to 50 g crude oil per kg (5% w/w). Udo and Fayemi (1975) determined that at levels of 10.6% crude oil, maize did not germinate and that ungerminated kernels removed from the soil were swollen and shiny indicating oil absorption. Schwendinger (1968) reported that germination



of oats in a loamy sand decreased from 90% to 25% as the volume of crude oil applied to 410 g of soil increased from 0 to 25 ml. Chaîneau *et al.* (1997) identified the concentration of fuel oil that prohibited 50% of seeds from germinating (i.e., lethal concentration or LC<sub>50</sub>) following 8 days of exposure; the LC<sub>50</sub> values varied from 0.3 to 4% (oil/soil, w/w) for lettuce (*Lactuca sativa* L.), barley, clover (*Trifolium repens* L.), and maize (*Zea mays* L.) and from 4 to 9% for bean (*Phaseolus vulgaris* L.), wheat, and sunflower.

Rogers *et al.* (1996) conducted germination tests on nine species of legumes and grasses using a mixture of organic chemicals (MOC) and test temperatures of 10 °C and 25 °C (Table 5.1). The MOC contained equal molar amounts of benzoic acid, hexadecane, phenanthrene, pyrene, 2,2-dimethyl 4,npropyl-benzene, and either cis-decahydronaphthalene (cis-decalin) (at 25 °C only) or cycloheptane (at 10 °C only) and was applied to the soil at rates ranging from 0 to 8000 mg per kg (0 to 0.8% w/w). Germination rates generally exceeded 50% when the MOC concentration was less than or equal to 2000 mg per kg soil and were less than 50% when the MOC concentration was greater than 2000 mg per kg soil. Germination rates at 10 °C were greater than those at 25 °C, particularly at higher MOC concentrations. Red clover, alfalfa, and alpine bluegrass generally exhibited higher germination rates for most MOC concentrations at both temperatures.

Although more labor-intensive than planting seeds, transplanting of seedlings or mature plants also may be used to establish plants on a site. Lin and Mendelssohn (1998) were able to transplant mature *Spartina patens* into marsh soil with a crude oil content as high as 100 mg crude oil per gram of soil (0.01% w/w) and *S. alterniflora* into soils contaminated with oil concentrations as high as 250 mg crude oil per g soil (0.025% w/w). Likewise, Longpre *et al.*, (1999) found that transplants of the wetland plant *Scirpus pungens* (three-square bulrush) can survive, grow, and produce new shoots in sediments contaminated with oil concentrations as high as 72.9 g oil per kg soil (7.29% w/w). It should be noted, however, that the biomass of *S. pungens* was significantly less in heavily contaminated compared to lightly or moderately contaminated sediments.

### **5.1.2 Inoculation of Microorganisms**

There are conflicting reports on the effectiveness of inoculating contaminated sites with microorganisms. Cunningham *et al.* (1996) state that it is a common experience for soil and plant inoculants to be out-competed by native microflora. They go on to say that this is even true in some symbiotic relationships (e.g. soybean and their *Bradyrhizobia* symbiont). Heitkamp and Cerniglia (1989) found that competition with indigenous microorganisms did not adversely affect the degradation of pyrene by a *Mycobacterium* species inoculated into sediments, unless organic nutrients (glucose and peptone) were added, in which case there was an overgrowth of indigenous bacterial species.

On the other hand, Radwan *et al.* (1997) found that the population of indigenous, oil-degrading *Arthrobacter* species (strain KCC201) in sand treated with weathered crude oil (20% w/w) decreased dramatically following inoculation with non-indigenous, oil-degrading

*Arthrobacter* and *Candida* species. Decreases in the number of indigenous *Arthrobacter* were thought to be due to competition with the inoculated species and the authors suggested that seeding with microbial “cocktails” irrespective of their origin may be useless or harmful if inoculated species out-competed indigenous, oil-degrading species.

**Table 5.1. Germination rate (%) of plant species exposed to a mixture of organic chemicals (MOC)<sup>1</sup> at 10 and 25 °C**

Temp. (°C)	Plant Species	MOC Concentration (mg per kg soil)				
		0	1000	2000	4000	8000
10	Red clover ( <i>Trifolium pratense</i> L.)	75	98	64	64	16
	Alfalfa ( <i>Medicago sativa</i> L.)	100	96	74	76	39
	Alpine bluegrass ( <i>Poa alpina</i> L.)	84	96	98	98	81
	Birdsfoot trefoil ( <i>Lotus corniculatus</i> )	59	77	62	30	17
	White clover ( <i>Trifolium repens</i> L.)	52	92	66	50	32
	Tilesy sage ( <i>Artemisia tilesii</i> )	23	19	11	18	18
	Bering hairgrass ( <i>Deschampsia beringensis</i> Hulten)	42	97	57	87	42
	Reed canarygrass ( <i>Phalaris arundinacea</i> L.)	70	45	61	94	63
	Quackgrass ( <i>Elytrigia repens</i> L.)	nd	87	46	82	34
	25	Red clover	100	94	63	36
Alfalfa		90	69	51	34	0
Alpine bluegrass		95	84	83	12	0
Birdsfoot trefoil		63	51	35	20	0
White clover		36	41	65	56	0
Tilesy sage		9	6	11	7	0
Bering hairgrass		48	41	20	10	0
Reed canarygrass		98	91	85	44	0
Quackgrass		72	78	86	78	0

<sup>1</sup>The MOC was applied to a silt loam soil: pH = 5.4; OM = 3.5%; Texture = 13% sand, 72% silt, 15% clay; P = 53 mg kg<sup>-1</sup>; K = 192 mg kg<sup>-1</sup>; Ca = 608 mg kg<sup>-1</sup>; Mg = 47 mg kg<sup>-1</sup>. (Adapted from Rogers *et al.*, 1996).

Other studies have found that inoculation enhances degradation of organic contaminants. Madsen and Kristensen (1997) determined that soil inoculation with phenanthrene-degrading bacteria (a possible *Pseudomonas fluorescens*, an *Arthrobacter* species, and an unidentified species of gram-positive, rod-shaped bacterium) enhanced the degradation of

[<sup>14</sup>C]phenanthrene – particularly in soils that had no known history of hydrocarbon contamination (specifically, coal tar contamination). It should be noted that a fairly large quantity of inoculum (at least 10<sup>6</sup> cells per gram of soil) was required to establish the introduced bacteria. Grosser *et al.* (1995) determined that the isolation, propagation, and reintroduction of 10<sup>7</sup> colony forming units of *Mycobacterium* species per gram of soil resulted in enhanced mineralization of phenanthrene, anthracene, and pyrene above that found with just indigenous soil microbes. Schwendinger (1968) identified drastically reduced adaptation times and greater total CO<sub>2</sub> production over a 7-week period after seeding *Cellulomonas* (a cellulose-decomposing bacteria) into sandy loam soil with relatively high levels of oil (100 mL kg<sup>-1</sup> soil). Siciliano and Germida (1997) found that inoculating two pseudomonad bacterial species onto meadow brome (*Bromus biebersteinii*) increased degradation of 2-chlorobenzoic acid (a PCB-degradation compound) in soil.

### **5.1.3 Using Native Versus Non-Native Plants and Microorganisms**

Situations may arise where plants and microorganisms most appropriate for phytoremediation may not be native to the contaminated site. A decision must then be made as to whether non-native plants or microorganisms should be introduced. The introduction of non-native biota into any ecosystem should not be taken lightly. Indeed, research indicates that 4 to 19% of non-native organisms introduced into natural and agricultural ecosystems in the United States have had severe adverse effects on both the environment and economy (OTA, 1993).

Genetically modified (engineered) organisms (GMOs) are considered a type of non-native organism; consequently, similar issues arise when considering the release of GMOs into the environment (OTA, 1993). Preliminary studies are already being conducted concerning the engineering of organisms that could be used in phytoremediation. Kochetkov *et al.* (1997), for example, have genetically modified two rhizosphere bacteria (*Pseudomonas putida* and *P. aureofaciens*) by supplying them with naphthalene-degradation *plasmids*. Unfortunately, it is difficult to identify beforehand the exact ecological consequences of introducing non-native and genetically engineered organisms into the environment.

The ecological risks associated with the use of non-native and genetically modified species in phytoremediation can be avoided by using native species whenever possible. Whatever the plant chosen for phytoremediation, the species must be well adapted to the soil and climate of the region – making soil characteristics, length of growing season, average temperature, and annual rainfall important considerations in phytoremediation planning (Cunningham *et al.*, 1996; Newman *et al.*, 1998). Another advantage of using native plants is the fact that they are pre-adapted to the climatic and soil conditions at the site.

## **5.2 Concentrations of Petroleum Hydrocarbons**

Within certain concentration ranges, plants and microbes can tolerate petroleum hydrocarbons, thus laying the foundation for the phytoremediation of contaminated sites. Phytoremediation of petroleum hydrocarbons may be ineffective, however, if concentrations

of the contaminants are either too high (causing toxicity) or too low (resulting in poor bioavailability).

### **5.2.1 Effects of Low and High Concentrations**

Initial low concentrations of contaminants may limit the extent to which phytoremediation can further reduce contaminant levels. That is to say, if microbial uptake and metabolism of organic compounds ceases when the contaminant concentration reaches a certain level, the microorganisms will not be physiologically capable of reducing contaminant concentrations any further (The Committee on *In Situ* Bioremediation *et al.*, 1993). Low concentrations may also cause microbes capable of degrading the contaminant to switch to alternative substrates or even result in the death of the microbes due to lack of sustenance (Committee on *In Situ* Bioremediation *et al.*, 1993; Hrudey and Pollard, 1993). Similarly, readily biodegradable contaminants in groundwater may remain undegraded, or degrade only very slowly, if their bioavailability is limited by low concentrations (Committee on *In Situ* Bioremediation *et al.*, 1993).

Concentrations of contaminants that are too high, on the other hand, can cause toxic effects and may even kill exposed microorganisms and plants – again limiting the effectiveness of phytoremediation. High concentrations of contaminants may prevent or slow the metabolic activity of microorganisms; this, in turn, would prevent the growth of new microbial biomass needed to increase or maintain degradation (Hrudey and Pollard, 1993; Committee on *In Situ* Bioremediation *et al.*, 1993). As well, a study by Baldwin (1992) indicates that the presence of petroleum hydrocarbons can reduce the number of bacterial species in a community to only one or two, even though the total number of individuals of these two selected species increases with increasing amounts of crude oil.

Walker *et al.* (1978) found little to no recovery of mosses, lichens and most dicotyledonous plants (i.e., herbs) in the plant communities of Prudhoue Bay, Alaska, one year following exposure to crude oil (applied at a rate of 12 L per m<sup>2</sup>). Similarly, various plants (red clover, alfalfa, birdsfoot trefoil, white clover, alpine bluegrass, tiley sage, bering hairgrass, reed canarygrass, and quackgrass,) did not germinate or were killed shortly after germination given exposure to concentrations of an organic chemical mixture of 2000 mg per kg soil or more (Rogers *et al.* 1996).

### **5.2.2 Concentrations Tolerated by Plants**

Different plants can tolerate different levels of petroleum hydrocarbons. For example, Schwendinger (1968) found that oat grown in oil-contaminated soils did not exhibit severe symptoms of damage at contaminant concentrations up to about 3% oil by weight. Schwendinger also found that fairly sensitive crops, such as tomato, kale and leaf lettuce, could tolerate a considerable quantity of crude oil in the soil – though the amount of crude oil tolerated was species dependent. Radwan *et al.* (1995) reported that some annual plants in Kuwait (such as *Senecio glaucus*) grow well in areas considered to be weakly to moderately contaminated (i.e., < 10% by weight of oil sediments). Alfalfa has been shown to grow

actively for up to one year in the presence of water saturated with toluene (e.g., 500 ppm) (Davis *et al.*, 1994). Rogers *et al.* (1996) found that alfalfa, red clover, white clover, birdsfoot trefoil, alpine bluegrass, Bering hairgrass, reed canary grass, and quackgrass grew well in soil contaminated with less than or equal to 2000 mg per kg (0.2% w/w) of a mixture of organic chemicals (see Section 5.1.1). Walker *et al.* (1978) determined that sedges (*Carex* species) and willows (*Salix* species) were the Arctic plants that recovered from a crude oil application of 12 L per m<sup>2</sup>.

Exposure of plants to tolerable concentrations of petroleum hydrocarbons can, however, affect their health. *Chlorosis* of leaves, plant dehydration, stunted growth, and death of the growing point were the effects of exposing maize seedlings to increasing concentrations of crude oil (0 to 10.6% w/w) (Udo and Fayemi, 1975). Hydrocarbon contamination of 5, 25, or 55 g hydrocarbon per kg soil (i.e., 0.5%, 2.5% or 5.5% w/w, respectively) reduced plant growth but increased microbial activity associated with barley and field pea (Xu and Johnson, 1995). Barley was more sensitive than field pea to hydrocarbon content as indicated by reduced root growth with increasing hydrocarbon concentration, a trend that was not seen with the field pea.

Wiltse *et al.* (1998) found that the overall agronomic performance of alfalfa was reduced in soil contaminated with 20,000 mg crude oil per kg soil (2% w/w). Total forage yield in contaminated soils averaged only 32% of the yield obtained in uncontaminated soil, while root weight in contaminated soil averaged only 47% of that in uncontaminated soil. As well, alfalfa plants in contaminated soil were shorter and slower to mature compared to those in uncontaminated soil. Interestingly, forage yield, plant height and maturity of plants in the contaminated soil improved in the later stages of the study – when contaminant concentrations may have been reduced.

Whether the effect of the contaminant is beneficial or adverse depends, to a certain degree, on the concentration of the contaminant. For example, Carr (1919) found that soybean can tolerate fairly large amounts (4% w/w) of crude oil mixed with the soil, and that the growth of soybean in a sandy peat soil was improved with the addition of a small amount (0.75% w/w) of crude oil. Likewise, Rogers *et al.* (1996) found the growth of white clover, tulesy sage, Bering hairgrass, and alpine bluegrass was enhanced by exposure to a low concentration (1000 mg per kg) of a mixture of organic chemicals (see Section 5.1.1). However, their growth was severely limited by exposure to higher concentrations of the same mixture.

### **5.3 Biotransformation, Bioaccumulation, and Disposal**

Although *biotransformation* may ultimately lead to the mineralization of toxic contaminants, intermediate metabolites formed along the way may be more toxic than the original compound. The degradation of PAHs by fungi, for example, incorporates only one atom of oxygen, which can result in the production of carcinogenic epoxides (Sutherland, 1992). Therefore, under soil conditions favoring fungal activity, early PAH metabolic products may

actually increase the toxicity of the contamination. As degradation proceeds, however, the majority of fungal transformations would detoxify the PAH compounds (Reilley *et al.*, 1996).

Toxic intermediate metabolites may build up in the soil as a result of either slow reactions by key bacteria or the production of “dead-end” products (Committee on *In Situ* Bioremediation *et al.*, 1993). Vinyl chloride, a cancer-causing agent, may build up during trichloroethylene biodegradation because bacteria can convert trichloroethylene to vinyl chloride relatively quickly, but the subsequent degradation of vinyl chloride occurs slowly. Dead-end products may form during cometabolism; i.e., the incidental metabolism of a contaminant may create a product that cannot be further transformed by bacterial enzymes. In the cometabolism of chlorinated phenols, for example, dead-end products such as the toxic chlorocatechols sometimes accumulate in the soil.

*Bioaccumulation*, the uptake and accumulation of a chemical by biota, may cause direct toxicity to the organism that accumulates the chemical or to a consumer of that organism (Mackay, 1991). The accumulation of petroleum hydrocarbons in plants was discussed generally in Section 3.2.1. The results of these studies indicated that, although some plants may take up petroleum hydrocarbons from contaminated soil, typically only small amounts are recovered in the plant. In review, experiments on the fate of [<sup>14</sup>C]benzene in soils planted with alfalfa found that less than 2% of the recovered <sup>14</sup>C was associated with the plant shoots and between 2% to 8% of the <sup>14</sup>C was recovered in the roots (root tissue plus rhizosphere soil attached to unwashed roots) (Ferro *et al.*, 1997). The findings of a study by Edwards (1988) support the relatively small recovery of <sup>14</sup>C from stems and leaves, but indicate that relatively large concentrations of the <sup>14</sup>C can be associated with the roots. Specifically, Edwards (1988) investigated the uptake and translocation of [<sup>14</sup>C]anthracene and [<sup>14</sup>C]benz[*a*]anthracene from nutrient solutions into bush bean. Results indicated that, after 30 days, anthracene-treated plants had taken up 54% of the total <sup>14</sup>C dose into their roots, 3.4% into their stems, and 3.1% into their leaves. Similarly, benz[*a*]anthracene-treated plants had taken up 60% of the total <sup>14</sup>C into their roots, 0.4% into their stems and 0.4% into their leaves. Keep in mind that the root uptake in this study may be augmented by the fact that the roots were in nutrient solution, where contaminants would be relatively more bioavailable compared to contaminants in soil.

*Biomagnification* occurs when lipophilic organic chemicals, which concentrate in fatty tissues, increase in concentration at higher levels of the food chain (Mackay, 1991). In the past, lipophilic organic chemicals, such as the PCBs, dioxins and furans, found at low concentrations in water and sediments of the Great Lakes of North America have biomagnified in the aquatic food chain – resulting in substantial health risks to higher trophic level organisms (e.g., lake trout) (Gelowitz, 1995). Although the studies summarized above indicate that the uptake and above-ground accumulation of petroleum hydrocarbons by plants is small, the daily consumption by herbivores of large quantities of plant material containing low-level contaminants may still result in biomagnification within the grazing food chain, that is, unless the higher trophic level organisms themselves can efficiently degrade the contaminants (Cunningham *et al.*, 1996). Moreover, even if the plants completely degrade PAHs, the constant uptake of chronic amounts (i.e., greater than background levels) could

potentially keep the contaminant concentration in the plants comparatively high; thus, providing a constant source of exposure for consumers of the plant material (Edwards *et al.*, 1982). Clearly, more research is needed to assess the potential for biomagnification of petroleum hydrocarbons in the grazing food chain.

The disposal of contaminated plant matter is a common practice with plants that accumulate metals, since harvesting the plants effectively removes the contaminants from the site. However, no information concerning the disposal of plant tissues containing accumulated petroleum hydrocarbons was found in the literature. This may reflect the fact that the issue is relatively new and, therefore, little to no research has been carried out to date. As well, there may be little interest in the topic since, as stated above, the few studies that do exist on accumulation of petroleum hydrocarbons indicate that there is only minor uptake and accumulation of hydrocarbons in the stems and leaves of plants.

#### **5.4 Mixtures of Contaminants**

Soils from oil-contaminated sites may also be contaminated with metals, salts, and/or pesticides, thus complicating phytoremediation efforts. Cunningham *et al.*, (1996) found that sodium salts as well as a variety of heavy metals (e.g., chromium, lead, mercury, zinc, nickel, copper, and cadmium) are commonly encountered on sites contaminated with organics. However, relatively little information exists on the effect of mixtures of these contaminants on phytoremediation efforts. In general, metal concentrations may inhibit microbial metabolism (Hrudey and Pollard, 1993), while high salinity levels can disrupt protein structures, denature enzymes and dehydrate cells (Atlas and Bartha, 1998).

Mixtures of organic contaminants also may cause difficulties in phytoremediation. For example, certain petroleum hydrocarbons can inhibit the growth of bacteria that degrade other petroleum contaminants. More specifically, naphthalene competitively inhibits the growth of the phenanthrene-degrading bacteria *Acidovorax delafieldii* strain TNA921 and *Sphingomonas paucimobilis* strain TNE12 (Shuttleworth and Cerniglia, 1996). As well, in a phenomenon known as *diauxy*, microbes may selectively degrade organic compounds that are easiest to digest or provide the most energy (Committee on *In Situ* Bioremediation *et al.*, 1993). Thus, if the target contaminant (e.g., petroleum hydrocarbons) occurs in combination with an adequate supply of a more preferred substrate, diauxy could result in limited microbial degradation of the target compound(s). Mixtures of organic compounds can, however, promote microbial degradation of petroleum hydrocarbons, particularly if one or more components of the mixture is a cometabolite of others. Likewise, biota primarily degrading one type of organic compound may also degrade a second compound present at concentrations too low to independently support bacterial growth (Committee on *In Situ* Bioremediation *et al.*, 1993).

## 5.5 Techniques Used to Enhance Phytoremediation

Several techniques, primarily agronomic, can be used to enhance the effectiveness of phytoremediation efforts. These techniques include the application of fertilizers and/or green manures, surfactants, and tillage to the contaminated soil.

Even if contaminated soil is not initially nutrient limited, available nutrients in soil can be quickly used up or immobilized when microbial populations degrade petroleum hydrocarbons, which typically serve as rich sources of carbon, but are poor nutrient suppliers (Xu and Johnson, 1997). Fertilizer application may, therefore, enhance the degradation of petroleum hydrocarbons by reducing competition among plants and microbes for limited nutrients in oil-contaminated soil. Steffensen and Alexander (1995) found that competition between two bacterial species (*Pseudomonas putida* and *P. aeruginosa*) for phosphorus limited the amount of organic compounds degraded by the bacteria; however, both the rate and extent of degradation increased when adequate quantities of phosphorus were provided. Cutright (1995) found that increasing the amount of nitrogen and phosphorus in soil under aerobic conditions increased the degradation of a PAH mixture by *Cunninghamella echinulata* var. *elegans*, a soil fungus. Likewise, Brown *et al.* (1998) identified more rapid losses of 2- and 3-ring PAHs from soil contaminated with weathered petroleum compounds when the soil was amended with a sludge compost high in nitrogen compared to an unamended control and a soil treatment of wood products low in nitrogen.

While conducting experiments on the effects of crude oil on tomato, kale and leaf lettuce, Schwendinger (1968) recognized that plants in oil-polluted soil exhibited stress symptoms comparable to those of extreme nutrient deficiency. Schwendinger went on to suggest that the damage of oil pollution to plants could be minimized by heavy fertilization, which would move the necessary nutrients into the plant despite the reduced capacity of the roots to take up nutrients in the oil-contaminated soil water. In a recent study, Lin and Mendelsohn (1998) found that fertilizer not only enhanced the establishment and growth of *Spartina alterniflora* and *S. patens* transplanted into crude-oil contaminated soil, but also enhanced the reduction of oil content in the soil compared to unfertilized soil with transplants. Mulhern *et al.* (1987) identified that herbaceous plants grew better on scrubber sludge from a coal-fired power plant when the sludge was treated with both cattle manure and commercial fertilizer. Amadi *et al.* (1993) reported that addition of poultry manure to soils contaminated with crude oil had a positive effect on the growth of maize (*Zea mays* LTZ-SR-Y) compared to contaminated soil without manure supplements. Green manure crops – typically, nitrogen fixing, legumes incorporated into soil to improve soil fertility – could also be used to provide soil nitrogen at contaminated sites (Biederbeck *et al.*, 1996), and, in so doing, may enhance phytoremediation efforts.

Tillage has been applied in bioremediation efforts where microorganisms, in the absence of plants, were used to remediate hydrocarbon-contaminated sites (Loehr and Webster, 1996; Genouw *et al.*, 1994; Bollag *et al.*, 1994). Tillage may also play an important role in



phytoremediation by aerating the soil and mixing fertilizer applications into contaminated soil prior to planting.

The use of surfactants in laboratory experiments suggests that their application to contaminated soil may enhance degradation of PAHs. Specifically, Madsen and Kristensen (1997) found that the application of non-ionic surfactants such as alcohol ethoxylate and glycoside enhanced the degradation of [<sup>14</sup>C]phenanthrene, [<sup>14</sup>C]pyrene, as well as unlabelled pyrene, benzo[*b,j,k*]fluoranthene, and benzo[*a*]pyrene. The authors also suggested that the ease with which the surfactants themselves are degraded will have an impact on their effectiveness; i.e., surfactants that are rapidly degraded may be less effective at increasing the availability of PAHs in soil compared to surfactants that are mineralized more slowly.

## 6. ALTERNATIVES TO PHYTOREMEDIATION

Phytoremediation is not the only means of reclaiming a site contaminated with petroleum hydrocarbons. *Natural attenuation*, *engineering* techniques and *bioremediation* are three alternatives for which more detailed information is provided below.

### 6.1 Natural Attenuation

Natural attenuation is a “hands-off” remediation approach that relies entirely on natural processes with no human intervention. The natural processes include physical/chemical mechanisms such as dilution, *dispersion* and adsorption of the contaminant (USEPA, 1996a). Biological processes, such as the unassisted growth of plants and microbial communities that break down contaminants, can be involved as well.

The rupture of an oil pipeline near Bemidji, Minnesota serves as an example of natural attenuation (Committee on *In Situ* Bioremediation *et al.*, 1993). The pipeline burst in August 1979, spilling approximately 100,000 gallons of crude oil into the surrounding soil and groundwater. In 1983 researchers from the U.S. Geological Survey began monitoring the site carefully to determine the fate of the crude oil. Although components of the crude oil initially migrated a short distance, native microorganisms capable of degrading the oil prevented widespread contamination of the groundwater. It is important to keep in mind, however, that only a fraction of sites offer naturally occurring conditions where microorganisms alone will degrade contaminants quickly enough to prevent contaminants from spreading (Committee on *In Situ* Bioremediation *et al.*, 1993).

Berry and Burton (1997) provide a Canadian example of natural attenuation. Heavy clay soil was treated with 5000 mg diesel per kg soil (0.5% w/w) and monitored for natural attenuation over a 74-day period. Natural attenuation resulted in the near-complete disappearance of the diesel fuel from the extractable fraction after 30 days.

### 6.2 Engineering

Engineering techniques are primarily physical, chemical, and mechanical in nature. They can involve *ex situ* methods such as excavation, transportation, and incineration of the contaminated soil. *In situ* processes, such as pump-and-treat, soil vapour extraction, sparging, and chemical washes, also may be involved (Otten *et al.*, 1997; Cunningham *et al.*, 1995).

#### 6.2.1 Ex Situ Engineering

*Ex situ* remediation begins with excavation of the contaminated soil. Following excavation, the contaminated soil is transported to another location where it is either landfilled or incinerated.

### 6.2.2 *In Situ Engineering*

The focus of *in situ* techniques is to remove the contaminant from the soil and/or groundwater without excavating the soil or transporting extracted groundwater off-site (Otten *et al.*, 1997; Pierzynski *et al.*, 1994). Some *in situ* engineering techniques focus specifically on soil reclamation (e.g., soil vapour extraction, solidification, chemical treatment, thermal treatment), while others are used to reclaim groundwater (e.g., pump-and-treat). Several engineering techniques (e.g., vacuum extraction and sparging) remediate soils and groundwater simultaneously. The various *in situ* engineering techniques are described briefly below.

*In situ* engineering methods for soil reclamation include soil vapour extraction, chemical treatment, solidification, and thermal treatment (Pierzynski *et al.*, 1994). Soil vapour extraction is a technique that uses filters or drains to withdraw soil vapour from the unsaturated zone of the soil (Otten *et al.*, 1997). The aim of this technique, also known as stripping, is to enhance volatilization of volatile contaminants. Chemical treatment involves applying a chemical agent on the soil or through an injection system to the subsurface environment (Pierzynski *et al.*, 1994). The chemical agent can neutralize, immobilize or chemically-alter the organic contaminant so that it is no longer a threat to the environment. Solidification involves physically or chemically binding the contaminated soil into a solid mass, often involving the use of cement or plastic (Pierzynski *et al.*, 1994). In thermal treatment, heat is used to remove contaminants by increasing volatilization (Pierzynski *et al.*, 1994). Significant reclamation can occur by this means when the contaminants are composed of highly volatile organic chemicals.

Pump-and-treat is an *in situ* method that works to reclaim groundwater (Otten *et al.*, 1997; Pierzynski *et al.*, 1994). With this method, water is pumped out of extraction wells, treated and then re-injected into injection wells, facilitating the movement of mostly water-soluble contaminants toward the extraction wells. Water pumped into the injection wells may contain added nutrients and/or other substances that increase degradation or recovery of the contaminant. Contaminated water pumped to the surface can be remediated using a variety of physical (adsorption, separation, flotation, air and steam stripping, and thermal treatment), chemical (ion exchange and oxidation/reduction) or biological techniques (land treatment, activated sludge, aerated surface impoundments, and biodegradation) (Pierzynski *et al.*, 1994).

Methods that facilitate the simultaneous remediation of both soil and groundwater include sparging and vacuum extraction. Sparging involves the injection of compressed air into the soil via sparging wells (compressed air filters) installed beneath the groundwater table (Otten *et al.*, 1997). It can be used to strip volatile contaminants from the soil, as well as to stimulate biological degradation or transformation of the contaminants. When sparging is used to treat the saturated zone, the unsaturated zone is treated at the same time. Vacuum extraction involves the use of a vacuum system to pump contaminated vapour and/or liquid out of the soil and groundwater (Malot, 1989). The extraction process is designed to recover both free and adsorbed hydrocarbons and has proven to be effective in a variety of *hydrogeologic*

settings, including clays, silts, sands, gravel, alluvium, glacial till, and fractured rock (Malot, 1989). At most sites, treatment of the extracted vapors and liquids is required.

### **6.3 Bioremediation**

Bioremediation uses microorganisms, such as bacteria, protozoa, and fungi, to degrade contaminants into less toxic or non-toxic compounds (USEPA, 1996b; Pierzynski *et al.*, 1994; Committee on *In Situ* Bioremediation *et al.*, 1993; Bollag *et al.*, 1994). The three basic components of any bioremediation process include (i) microorganisms, (ii) a potentially biodegradable contaminant, and (iii) a *bioreactor* in which the process can take place. Proper temperature, oxygen, and nutrient levels may need to be provided in the bioreactor. The microbes in the bioreactor use carbon in the organic contaminants as a source of energy, and in doing so, degrade the contaminant.

Bioremediation can be applied both *ex situ* and *in situ*. With *ex situ* bioremediation, the contaminated soil is excavated or the groundwater is extracted. With *in situ* remediation, there is no excavation or extraction. As a result, the contaminated soil or groundwater serves as the bioreactor. The proper microorganisms may occur at the site naturally or may need to be introduced from other locations. Both *ex situ* and *in situ* bioremediation methods are described in greater detail in the following sections.

#### **6.3.1 Ex Situ Bioremediation**

The primary methods used in *ex situ* bioremediation are slurry-phase and solid-phase treatment (USEPA, 1996b; Pierzynski *et al.*, 1994). In slurry-phase treatment, contaminated soil is combined with water and other additives in a bioreactor. The resultant slurry is then mixed continuously to keep the microorganisms in contact with the contaminants. Upon completion of the treatment, water is removed from the solids, which are either disposed of or treated further if still contaminated.

With solid-phase treatment, soils are remediated in above ground treatment areas equipped with collection systems to prevent contaminants from escaping (USEPA, 1996b; Pierzynski *et al.*, 1994). *Landfarming*, *soil biopiles*, and *composting* are three types of solid-phase treatment. Landfarming involves spreading the contaminated soil thinly over land or a pad with a leachate-collection system. In some landfarming cases, reduction of contaminant concentrations may actually be due more to volatilization, leaching or dilution through mixing with uncontaminated soil than from actual degradation from microorganisms. Genouw *et al.* (1994) demonstrated that landfarming can be used effectively to clean up oil sludge applied to soil, but only if appropriate technical measures (e.g., nutrient and organic amendment, inoculation, and tillage) are employed and sufficient time (at least 15 years) is allowed for bioremediation to take place. Loehr and Webster (1996) found similar results with land-treatment of creosote-contaminated soils.

With soil biopiles, contaminated soil is piled in heaps several meters high over an air distribution system (USEPA, 1996b). A vacuum pump is used to pull air through the biopile.

As a result, volatile contaminants are easily controlled since they are usually part of the air stream pulled through the pile.

In composting, biodegradable contaminants are mixed with straw, hay, or corn cobs to make it easier to achieve optimum levels of air and water (USEPA, 1996b; Bollag *et al.*, 1994). The compost can be (i) formed into piles and aerated with blowers or vacuum pumps, (ii) placed in a treatment vessel where it is mixed and aerated, or (iii) placed in long piles known as windrows and periodically mixed using tractors or similar equipment. Compost piles typically have elevated temperatures due to microbial activity, which sets them apart from biopiles.

### **6.3.2 *In Situ* Bioremediation**

*In situ* bioremediation is similar to phytoremediation in its use of microorganisms on-site to degrade contaminants. However, *in situ* bioremediation does not involve the use of plants and generally employs more invasive engineering techniques than phytoremediation. For example, the oxygen required by aerobic microorganisms during *in situ* bioremediation may be provided by pumping air into the soil above the water table – in a process known as *bioventing* – or by delivering the oxygen in liquid form as hydrogen peroxide (USEPA, 1996b).

*In situ* bioremediation has been successful in remediating groundwater as well as surface soils and subsoils contaminated with petroleum hydrocarbons (Pierzynski *et al.*, 1994; USEPA, 1996b). *In situ* groundwater bioremediation systems involve extraction wells to collect the contaminated groundwater, an above-ground water treatment system to “condition” the extracted water by adding nutrients and oxygen, and injection wells to return the conditioned groundwater to the subsurface. Contaminants in the conditioned, subsurface water are then degraded using microorganisms. This system is very similar to the pump-and-treat system described in Section 6.2.2, with the exception that degradation occurs within the ground rather than in above-ground treatment facilities.

## 7. COMPARISON OF PHYTOREMEDIATION TO ALTERNATIVE REMEDIATION STRATEGIES

A comparative summary of various characteristics of phytoremediation, natural attenuation, engineering techniques and bioremediation is provided in Table 7.1 (located at the end of this section). The summary characteristics include whether the method is *in situ* or *ex situ*, a general description of the method, whether human intervention is involved, the direct and indirect benefits of the method, as well as the limitations and costs associated with each method. As descriptions of the methods and the degree of human intervention have been provided in detail in previous sections, the current section deals exclusively with the benefits, limitations, and costs of phytoremediation and its alternatives.

### 7.1 Benefits, Limitations, and Costs of Phytoremediation

#### 7.1.1 Direct Benefits of Phytoremediation

Phytoremediation is an *in situ*, solar driven technique, which limits environmental disturbance and reduces costs (Shimp *et al.*, 1993). Moreover, it is particularly well-suited to the treatment of large areas of surface contamination, when other methods may not be cost-effective (Schnoor, 1999).

In general, both the public and government regulators look favorably upon phytoremediation because it involves exploiting the natural ability of the environment to restore itself (Cunningham *et al.*, 1996). Indeed, there was a high level of public support for the use of plants in phytoremediation at a series of public focus group meetings to gauge public perceptions and awareness of environmental applications of biotechnology in Canada (McIntyre and Lewis, 1997). Phytoremediation also is considered to be more aesthetically pleasing than other remediation techniques (Shimp *et al.*, 1993; Cunningham *et al.*, 1996).

Plant samples can be harvested and used as indicators of the extent of remediation or, conversely, contamination (Shimp *et al.*, 1993). Similarly, a field of plants may serve as a direct, visual bioassay (Cunningham *et al.*, 1996). There is also the potential to grow various phytoremediator species together on the same site in an attempt to simultaneously remediate various contaminants, including salts, metals, pesticides, and petroleum hydrocarbons. Plants help contain the region of contamination by removing water from soil, thereby keeping the contaminants from spreading or confining them within or near the root-system (Shimp *et al.*, 1993). Some wetland plants can transport oxygen to the rhizosphere under conditions that may otherwise limit the amount of oxygen available to soil microorganisms, as is the case in soils and sediments saturated with water or contaminated with oil (Shimp *et al.*, 1993; Schnoor *et al.*, 1995). Microbial communities in the rhizosphere may be able to biodegrade a wide variety of organic contaminants (Shimp *et al.*, 1993). Finally, phytoremediation may be applied with relative ease using existing agricultural practices at contaminated sites (McIntyre and Lewis, 1997).

### **7.1.2 Indirect Benefits of Phytoremediation**

An indirect benefit of phytoremediation is improvement of soil quality by improving soil structure (aggregates and peds), increasing porosity/aggregation and, therefore, water infiltration, providing nutrients (nitrogen-fixing legumes), accelerating nutrient cycling, and increasing soil organic carbon (Schnoor *et al.*, 1995; Cunningham *et al.*, 1996). The use of plants in a remediation effort stabilizes the soil, thus preventing erosion and direct human exposure (i.e., by preventing the consumption of contaminated soil by children and the inhalation of soil particles carried in the wind) (Schnoor *et al.*, 1995; McIntyre and Lewis, 1997). Phytoremediation also helps eliminate secondary air- or water-borne wastes. For example, some plants accumulate PAHs from the atmosphere (Simonich and Hites, 1994a, 1994b; Edwards, 1983). Likewise, phytoremediation has the potential to help reduce greenhouse gas emissions because it does not require the use of pumps or motors that give off greenhouse gases and plants used in phytoremediation may serve as sinks for the greenhouse gas carbon dioxide (Tsao, 1999a). Trees used in phytoremediation may reduce noise levels from industrial sites (Tsao, 1999a). Likewise, phytoremediation itself is less noisy than other reclamation alternatives.

Another indirect benefit is that the growth of certain hardy plants in a contaminated soil can allow for the growth of other, less hardy plants. An experiment outlined by Cunningham *et al.* (1996) indicated that a tolerant grass species (*Vetiveria zizanioides*) thrived in a clay soil contaminated with up to 3% total petroleum hydrocarbons. The same soil was initially extremely phytotoxic to a variety of crop plants tested. However, after a 1-year period when the soil was cropped to only *V. zizanioides*, several crop species could be grown together with *V. zizanioides* – even though there was no detectable change in the quantity of contaminants.

### **7.1.3 Limitations of Phytoremediation**

Petroleum hydrocarbon contamination must occur at shallow depths for phytoremediation to be effective. Though some plants, such as trees, may have root systems that can extend to a depth of 60 m, most plants do not produce roots to anywhere near this depth and root density generally decreases with depth (Cunningham *et al.*, 1996). Consequently, as depth increases beyond one or two metres, relatively immobile contaminants – those that cannot migrate to the plant roots during water uptake – are increasingly unlikely to be affected by phytoremediation (Cunningham *et al.*, 1996).

Phytoremediation is slower than *ex situ* methods, typically requiring several seasons for site clean-up (McIntyre and Lewis, 1997). The time required to achieve clean-up standards using phytoremediation may be particularly long for hydrophobic pollutants that are tightly bound to soil particles (Schnoor *et al.*, 1995). Because it is slow, phytoremediation is not an appropriate solution where the target contaminant presents an immediate danger to human health or the environment (McIntyre and Lewis, 1997).

If contaminants are tightly bound to soil particles or organic matter, they may not be available to plants or microbes for degradation (Otten *et al.*, 1997; Cunningham *et al.*, 1996).

Environmental conditions, such as soil texture, pH, salinity, oxygen availability, temperature and level of non-hydrocarbon contaminants (e.g., metals) must all be within the limits tolerated by plants (McIntyre and Lewis, 1997; Cunningham *et al.*, 1996; Hrudey and Pollard, 1993). In addition, plants will not grow if concentrations of the target contaminant are too high. Thus, phytoremediation of the target contaminant will not proceed unless the soil is pretreated to reduce phytotoxicity or a resistant plant species is selected (Cunningham *et al.*, 1996).

Although petroleum hydrocarbons may not accumulate in plant tissue to a great extent (e.g., Ferro *et al.*, 1997), the potential for biomagnification in the food chain may still exist if consumer organisms at higher trophic levels can not biodegrade, detoxify or eliminate the contaminants they acquire from eating plants (Cunningham *et al.*, 1996). As well, biodegradation of the target contaminant may lead to the formation of toxic intermediates, such as those created during the initial biotransformation of petroleum hydrocarbons by fungi (Sutherland, 1992).

The effectiveness of phytoremediation also will depend on the chemical nature of the contaminants themselves. For example, there is the potential for water-soluble contaminants to leach away before phytoremediation can reclaim the area (Cunningham *et al.*, 1996; Pierzynski *et al.*, 1994). Similarly, evaporation of volatile petroleum hydrocarbons into the air from the soil directly or through the plant is simply a transfer of the contaminant from one environmental medium to another. As a result, there may be air quality issues resulting from this transfer.

#### **7.1.4 Costs of Phytoremediation**

Cunningham *et al.* (1996) estimated the general cost of phytoremediation at \$17 to \$100 per m<sup>3</sup> (based on a cost of \$2,500 to \$15,000 per hectare to a depth of 15 cm). More recently, BP Amoco estimated a cost of approximately \$3.00US per m<sup>3</sup> per year to phytoremediate a site contaminated with oil, gas, phenol, volatile organic compounds and semi-volatile compounds using deep-rooted plants and trees (Tsao 1999b).

Where site circumstances permit, phytoremediation may use the same equipment and materials common to agricultural practices and costs can be equated to those associated with crop production (McIntyre and Lewis, 1997). The cost of applying cropping systems in phytoremediation has been estimated at \$0.02US to \$1.00US per m<sup>3</sup> per year (or \$200US to \$10,000US per hectare with roots extending down to one meter), which is several orders of magnitude less than the costs associated with physical/chemical remediation technologies (Cunningham *et al.*, 1995).

A number of other factors also contribute to the generally low cost of phytoremediation. First, because the process is *in situ* and driven by solar energy, there are few energy inputs required – greatly reducing the cost of phytoremediation. As well, because phytoremediation is slower than *ex situ* methods, costs are spread out over longer time periods – resulting in relatively low annual costs. Indeed, maintaining a site under cultivation for 10 years costs substantially



less than conventional methods of site clean-up (McIntyre and Lewis, 1997). It is interesting to note that in phytoremediation, agronomic costs, including planting, tillage, fertilization, and harvesting, can be insignificant in comparison with associated administrative costs, such as site management, regulatory reporting, and analysis of data (Cunningham *et al.*, 1996).

## **7.2 Benefits, Limitations and Costs of Alternatives**

As with phytoremediation, the various alternative remediation methods have their benefits, limitations, and costs (summarized in Table 7.1).

### **7.2.1 Natural Attenuation**

The primary benefits of natural attenuation are (i) minimal disturbance of the site; i.e., the site is simply left to be remediated by natural processes and (ii) since it involves no human intervention, the operational costs are low to non-existent. The costs associated with natural attenuation are typically related to monitoring to make sure the process is working.

The main limitation of natural attenuation is that it is slower than any other remediation method. In addition, the most appropriate plants and microorganisms may not be present and/or natural environmental conditions may not be optimal to facilitate natural remediation of the contamination. Health risks from the contaminated site may therefore exist for a period of time that is not acceptable from either a social or business standpoint.

### **7.2.2 Engineering**

#### **Benefits and Limitations of *Ex Situ* Engineering Methods**

One of the major advantages of *ex situ* engineering is that it involves proven, dependable technologies (Malot, 1989; Cunningham and Berti, 1993) that are viewed favorably by government regulators. In general, it can be completed in a relatively short time, with definite start- and end-points, and it leaves behind a “clean” site (Cunningham and Berti, 1993).

One of the major limitations of *ex situ* engineering is that it involves further disturbance of the site by way of excavation (Cunningham and Berti, 1993; Malot, 1989). Landfilling of excavated material simply transfers the contaminants to a second location (Cunningham and Berti, 1993; Malot, 1989) and incineration produces fly ash that must be disposed of properly (Boeve, 1989). As a result, questions of residual liability remain after the initial site has been remediated (Cunningham and Berti, 1993). In addition, the public may have concerns about air pollution problems associated with incineration or having a contaminated landfill in their neighborhood (Pierzynski *et al.*, 1994). *Ex situ* methods also may be impractical due to physical conditions at the site, such as the locations of buildings, roadways or utilities, and proximity to residents (Malot, 1989).

#### **Benefits and Limitations of *In Situ* Engineering Methods**

The chief benefit of *in situ* engineering is that it generally involves less surface disturbance than *ex situ* methods and thereby reduces the potential for human exposure to contaminants

(Pierzynski *et al.*, 1994). *In situ* methods also require a minimal amount of facilities (Pierzynski *et al.*, 1994). Cleanup of contaminated soil and groundwater with vacuum extraction is *in situ*, rapid and low cost (Malot, 1989). As well, vacuum extraction systems are not limited by depth to groundwater (Malot, 1989); i.e., applications have been successful at sites with groundwater as deep as 300 feet or as shallow as 3 inches.

The effectiveness of *in situ* extraction processes, however, can be limited by heterogeneities in the soil with depth, the presence of silts and clays and uncertainties in flow conditions within the unsaturated zone (Malot, 1989). In addition, contaminants removed from soil and groundwater via extraction may still require treatment. The use of chemical treatments for *in situ* treatment often creates unwanted by-products, leaves residues, and can be more toxic or hazardous than the target contaminant (Boeve, 1989).

Some limitations of pump-and-treat systems involve the fact that they are very slow, taking decades to restore aquifers, and do not treat soils directly (Malot, 1989). Pump-and-treat systems are typically effective only if the contaminants are water soluble and contained in highly permeable, homogenous soils. The efficiency of pump-and-treat systems used to clean up most types of hydrocarbon contamination is hampered by the fact that many contaminants are not water-soluble and, hence, are difficult to extract. Furthermore, surfactants or other compounds added to increase the water-solubility of hydrocarbons may, themselves, be difficult to remove afterward (Malot, 1989).

### **Costs of Engineering**

Costs associated with the use of engineering techniques to remediate contaminated soil can range from \$10 to over \$1,000 per m<sup>3</sup>. The cost of remediation will depend on the type of engineering technique employed and the type of contaminant (Cunningham *et al.*, 1995; Cunningham *et al.*, 1996). On average, *in situ* remediation costs for volatile or water-soluble contaminants range from \$10 to \$100US per m<sup>3</sup>. Landfilling or low-temperature thermal treatment can cost anywhere from \$60 to \$300US per m<sup>3</sup>, while special landfilling or high-temperature thermal treatments can cost from \$200 to 700US per m<sup>3</sup>. Bollag *et al.* (1994) quote incineration or secured landfill costs of \$260 to \$1064 per m<sup>3</sup> (i.e., \$200 to \$800 per cubic yard).

Engineering costs will also vary depending on whether the method is *in situ* or *ex situ* (Cunningham *et al.*, 1996). *In situ* pump-and-treat or vapor stripping are comparatively inexpensive with total project costs of approximately \$15 per m<sup>3</sup> or \$10 per ton<sup>1</sup> treated. *Ex situ* treatment projects, although faster, are more costly, ranging from \$60 to over \$1,180 per m<sup>3</sup> (or \$40 per ton to more than \$800 per ton). Investment costs of *in situ* remediation are usually lower than those for *ex situ* treatment, as the installation of devices for air and water

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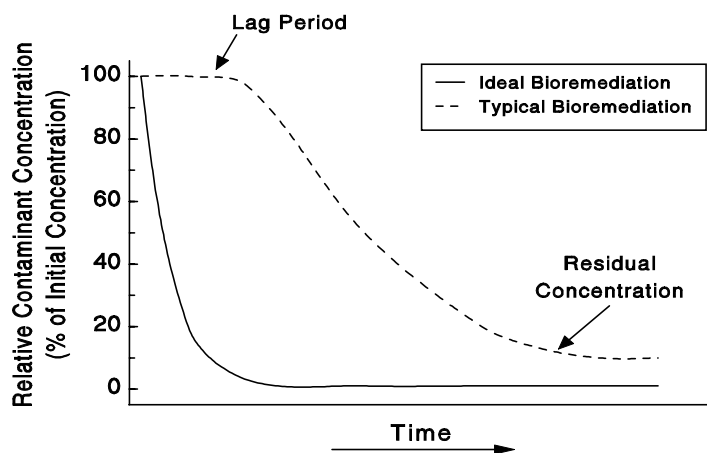
<sup>1</sup> Assumes a soil bulk density of 1500 kg m<sup>-3</sup> with 1 ton = 1016 kg. The same values for bulk density and the ton-to-kg conversion were used for all conversions from \$ per ton to \$ per m<sup>3</sup>.

extraction/injection is less expensive than the excavation and treatment of soil (Otten *et al.*, 1997). Costs also are dependent on soil properties, site conditions, the total volume to be remediated, proximity to incineration unit, landfill charges, and stabilizing agents (Cunningham *et al.*, 1996; Cunningham *et al.*, 1995). Contaminant levels that must be attained to satisfy regulatory guidelines also impact the ultimate cost of site remediation.

### 7.2.3 Bioremediation

#### Benefits and Limitations of Bioremediation

One of the advantages of bioremediation is that it has a proven track record; i.e., it has been used successfully to clean up spills of oils and other hydrocarbons for more than 20 years (Pierzynski *et al.*, 1994). However, bioremediation may not occur if the contaminant is toxic to microorganisms. As well, typical bioremediation (i) involves a lag time before degradation begins, (ii) may have rates that are slower than expected, and (iii) may have final contaminant concentrations that reach a limit or residual concentration, which is greater than the desired concentration, i.e. a regulatory standard (Figure 7.1) (Reynolds *et al.*, 1999b). Likewise, bioremediation may contribute little to the reclamation process if the rate of biodegradation is slower than other processes, such as leaching or volatilization (Pierzynski *et al.*, 1994). As a result, bioremediation can require intensive monitoring of soils, leachate, and air to ensure that the process is proceeding as designed (Pierzynski *et al.*, 1994).



**Figure 7.1** Ideal versus typical microbial biodegradation curves for contaminated soil. The initial lag time, slower treatment rates, and residual concentration of contaminants in soil are frequently observed in practice (adapted from Reynolds *et al.*, 1999b).

As with all *ex situ* methods, the main limitation of *ex situ* bioremediation is that it requires excavation of the contaminated soil/groundwater. Moreover, treatment of the contaminated

soil before and/or after the actual bioremediation step may be required (USEPA, 1996b). In general, solid-phase systems may require larger amounts of space and more time to complete, particularly compared to slurry-phase processes (USEPA, 1996b).

One of the primary benefits of *in situ* bioremediation is that it does not require excavation, but instead involves minimal site disruption with relatively less dust and a subsequently smaller release of contaminants to the atmosphere than *ex situ* methods (USEPA, 1996b; Pierzynski *et al.*, 1994; Bollag *et al.*, 1994). Likewise, transportation of contaminated soil to another location is not required (Pierzynski *et al.*, 1994). Operational requirements may be lower than on-site incineration, solidification, or soil washing, resulting in potentially fewer mechanical problems and lower costs (Bollag *et al.*, 1994). As well, it is possible to treat a large volume of soil at once and it is effective at sites with permeable (sandy or uncompacted) soil (USEPA, 1996b; Malot, 1989).

The limitations of *in situ* bioremediation are that it may be slower than *ex situ* techniques, it may be difficult to manage, and it may not work well in clay soils or in highly layered subsurface environments where oxygen cannot be evenly distributed throughout the treatment area (USEPA, 1996b). Difficulties with flow control in the unsaturated zone also are frequently encountered (Malot, 1989). For example, differences in underground soil layering and density may cause reinjected conditioned groundwater to follow certain preferred flow paths, with the result that conditioned water may not reach some areas of contamination (USEPA, 1996b). Clogging of nutrient injection systems with biogrowth also has the potential to limit the effectiveness of bioremediation systems (Malot, 1989). As is the case with all *in situ* remediation techniques, intermediate by-products may be more mobile and hazardous than the original components (Malot, 1989).

### **Costs of Bioremediation**

Costs associated with bioremediation generally range from about \$98 to \$260 per m<sup>3</sup> (Bollag *et al.*, 1994). The cost of *ex situ* bioremediation of petroleum hydrocarbons in a fully contained facility ranges from \$133 to \$400 per m<sup>3</sup> (or \$20,000 to \$60,000 per hectare to a depth of 15 cm) (Cunningham *et al.*, 1996). *In situ* bioremediation techniques are cheaper at \$50 to \$133 per m<sup>3</sup> (or \$7500 to \$20,000 per hectare – assuming a 15 cm depth) (Cunningham *et al.*, 1996). However, these costs can quickly escalate depending on the level of monitoring, analytical costs, and degree of security and safety that are required (Cunningham *et al.*, 1996).

**Table 7.1. Comparison of Phytoremediation to Alternative Remediation Methods Including Natural Attenuation, Engineering, and Bioremediation**

Characteristics	Phytoremediation	Natural Attenuation	Engineering	Bioremediation
1. <i>In Situ</i> or <i>Ex Situ</i>	<i>in situ</i>	<i>in situ</i>	<i>ex situ</i> or <i>in situ</i>	<i>ex situ</i> or <i>in situ</i>
2. General Description	Use plants and microbes to degrade, contain, or transfer <sup>1</sup> contaminants	Use plants and microbes to degrade, contain, or transfer <sup>1</sup> contaminants	<i>Ex situ</i> = excavation, landfilling, incineration. <i>In situ</i> = soil vapour extraction; chemical or thermal treatment, solidification; pump-and-treat; vacuum extraction; sparging	Use microbes to degrade or contain contaminants; <i>ex situ</i> involves excavation coupled with solid-phase or slurry-phase treatment
3. Human Intervention	Yes; agronomic – tillage, fertilizer, inoculation, planting	No	Yes; extensive	Yes; extensive – provide proper temperature, oxygen, and nutrients to optimize microbial activity
4. Direct Benefits	<i>In situ</i> ; solar driven; well-suited to large areas of surface contamination; good esthetics; favorable public perception; plants as indicators of contamination; microbes degrade a variety of contaminants; plants transfer oxygen to rhizosphere; plants help contain contaminants; relatively easy to apply	<i>In situ</i> ; no disturbance	Dependable; leaves clean site; has definite starting and end points; <i>ex situ</i> especially faster than other remediation methods; proven to be effective; vacuum extraction is not limited by depth to groundwater	Limited disturbance with <i>in situ</i> ; proven to be effective
5. Indirect Benefits	Improves soil quality; prevents erosion; plants help eliminate secondary air- and water-borne wastes, including greenhouse gases; trees can reduce noise from industrial sites; hardy plants can help other less hardy plants grow on contaminated sites	Hardy plants can help other less hardy plants grow on contaminated sites; once established plants help prevent erosion and help eliminate secondary air- and water-borne wastes		
6. Limitations	Contamination must typically be shallow; plants may not grow if contamination high; slower than <i>ex situ</i> methods; contaminants may not be bioavailable; environmental conditions have to be right; leaching or volatilization may occur before phytoremediation	Slower than any other remediation method, therefore longer period of higher risks to human and ecosystem health; plants, microbes, or environmental conditions most beneficial to remediation may not be naturally present	Highly disruptive, especially excavation; landfill only transfers contaminants to a second site; disposal issues of fly ash with incineration; pump-and-treat does not treat soils directly and is very slow	Highly disruptive with <i>ex situ</i> excavation; <i>in situ</i> requires extensive collection systems; treatment longer than engineering but not as long as attenuation; may not work if contaminant toxic to microbes; requires intensive monitoring
7. Cost	\$17 to \$100/m <sup>3</sup> ; \$3US/m <sup>3</sup> each year; cropping system = \$0.02 – 1.00US/m <sup>3</sup> per year	No operational costs; may have costs associated with monitoring	Generally, from \$10 to over \$1,000/m <sup>3</sup> ; \$10-100US/m <sup>3</sup> for volatile or water-soluble contaminants <i>in situ</i> ; \$60-300US/m <sup>3</sup> for landfilling or low-temp. thermal; \$200-700US/m <sup>3</sup> for special landfill or high-temp. thermal; incineration or secured landfill costs of \$260-1064 per m <sup>3</sup> ; <i>in situ</i> typically cheaper than <i>ex situ</i>	\$50 to \$133/m <sup>3</sup> for <i>in situ</i> ; \$133 to \$400/m <sup>3</sup> for <i>ex situ</i>

<sup>1</sup> Refers to the transfer of contaminants from soil to the atmosphere by transpiration associated with plants.

Sources: Boeve, 1989; Cunningham *et al.*, 1996; Cunningham *et al.*, 1995; Cunningham and Berti, 1993; Cunningham and Ow, 1996; Hrudey and Pollard, 1993; Malot, 1989; McIntyre and Lewis, 1997; Otten *et al.*, 1997; Pierzynski *et al.*, 1994; Schnoor *et al.*, 1995; Shimp *et al.*, 1993; Tsao, 1999b; USEPA, 1996a; USEPA, 1996b.

## 8. SUMMARY AND CONCLUSION

### 8.1 When is Phytoremediation Most Effective?

Research on phytoremediation indicates that it has the potential to serve as an effective method for the clean-up of petroleum hydrocarbons from contaminated soils. Successful trials have involved a variety of plants, but the majority of research has focused on grass and legume species (see Table 2.1; *Aprill and Sims, 1990; Qiu et al., 1997; Gunther et al., 1996; and Reilley et al., 1996*). In fact, a number of legumes have been found to grow naturally in oil-contaminated areas (*Gudin and Syrratt, 1975*).

Plants and microorganisms can participate both directly and indirectly in the remediation of petroleum-contaminated soils and groundwater through three main mechanisms: (i) degradation, (ii) containment and (iii) transfer of the contaminant from the soil to the atmosphere (*Cunningham et al., 1996; Siciliano and Germida, 1998b; Sims and Overcash, 1983*). Both plants and microorganisms accomplish degradation, either independently or through joint interactions, such as the rhizosphere effect. Indeed, data found in the literature indicate that the primary loss mechanism for petroleum hydrocarbons is the degradation of these compounds by microorganisms in the rhizosphere of plants. Limited information suggests that plants also may degrade petroleum hydrocarbons directly (*Durmishidze, 1977; Edwards et al., 1982; Edwards, 1988*). However, more information is available regarding the indirect roles played by plants in degradation. These roles include supplying root exudates (e.g., sugars, alcohols, and acids) for microbial use, releasing root-associated enzymes that degrade contaminants in the soil, and altering the soil to promote phytoremediation (*Gunther et al., 1996; Erickson et al., 1995; Siciliano and Germida, 1998b; Schnoor et al., 1995; Cunningham et al., 1996*).

Studies regarding containment and transfer of petroleum hydrocarbons focus on the direct roles played by plants. Plants prevent the spread of petroleum hydrocarbons in soil and groundwater by taking up these contaminants (to a small degree), adsorbing them onto their roots, or keeping them near the root zone via water uptake (*Ferro et al., 1997; Schnoor et al., 1995; Aprill and Sims, 1990; Schwab et al., 1998*). Plants also are capable of transferring volatile petroleum hydrocarbons (e.g., naphthalene) from the soil to the atmosphere via transpiration (*Watkins et al., 1994*). Though this mechanism removes contaminants from the soil, it simply moves them into the atmosphere, which can serve as an alternative source of exposure; therefore, health risks associated with the contaminant may still arise.

Research suggests that certain petroleum hydrocarbons are easier to phytoremediate than others. In general, BTEX compounds are relatively easy to remediate because they are (i) rapidly degraded in the presence of oxygen; (ii) relatively soluble, thus making them bioavailable; and (iii) can serve as the primary electron donor for many bacteria widely distributed in nature (*Committee on In Situ Bioremediation et al., 1993; also refer to Appendix A*). Large and lipophilic compounds such as the four and five-ring PAHs are more difficult, though not impossible, to remediate. The remediation of these compounds is more

difficult because of their limited bioavailability, which is a result of their adsorption to soil organic matter and clay, as well as their limited ability to pass through cellular membranes of plants and microbes (Cookson, 1995). Nevertheless, cometabolism by microorganisms has been shown to result in the degradation of some large PAHs, such as benzo[*a*]pyrene (Kanaly *et al.*, 1997). In general, weathering processes, involving volatilization, evapotranspiration, photomodification, hydrolysis, leaching and biotransformation, selectively reduce the concentration of easily-degradable contaminants, leaving behind more recalcitrant compounds (Bossert and Bartha, 1984; Cunningham *et al.*, 1996).

The effectiveness of phytoremediation is site-specific in that it can be greatly affected by environmental conditions, as well as the concentrations and types of petroleum hydrocarbons found on-site. Phytoremediation is facilitated by adequate quantities of nutrients, water and oxygen and is enhanced at warmer temperatures (Wright *et al.* 1997). Phytoremediation may be restricted in soils with abundant organic matter or clay – soil components that tend to reduce the bioavailability of petroleum hydrocarbons, particularly the large and lipophilic PAHs (Otten *et al.*, 1997; Cunningham *et al.* 1996). Phytoremediation may also be inhibited if contaminant concentrations are too high causing toxicity or are too low resulting in reduced bioavailability (Atlas and Bartha 1998; Committee on *In Situ* Bioremediation *et al.* 1993; Hrudey and Pollard 1993; Rogers *et al.* 1996).

The three major alternatives to phytoremediation are natural attenuation, engineering, and bioremediation. Limited information suggests that phytoremediation is slightly less expensive than bioremediation and can be orders of magnitude less expensive than some of the more costly engineering techniques. Phytoremediation is faster than natural attenuation, but typically slower than engineering and bioremediation. Phytoremediation is most likely to have the greatest effectiveness with shallow contamination, which is co-incidental with the greatest root density. Because phytoremediation is an *in situ* technique, it is typically less disruptive to the site than *ex situ* engineering and bioremediation that involve excavation efforts.

Several significant research gaps appear in the literature that are worth noting. First, very few studies have been conducted on phytoremediation of petroleum hydrocarbons in Canada. The cold climate and short growing season make it particularly important to conduct phytoremediation research on plants adapted to the Canadian climate. One applicable study involves the use of constructed wetlands for the treatment of dissolved phase hydrocarbons in Rocky Mountain House, AB (Moore *et al.*, 1999). Another is a preliminary study regarding the use of jack pine (*Pinus banksiana*) for phytoremediation of diesel-contaminated soils in Northern Saskatchewan (McLean *et al.*, 1999). Longpre *et al.* (1999) are presently conducting research on the response of *Scirpus pungens* – a wetland plant common to the shores of the St. Lawrence River – to crude oil contaminated sediments. Although not a phytoremediation project specifically, Biederbeck *et al.* (1993) investigated the response of five crops (barley cv. Leduc; oat cv. Calibre; pea cv. Trapper; spring rye cv. Gazelle; wheat cv. Katepwa) to oily waste sludge applied to sandy soils near Maidstone, SK. Of course, studies conducted outside

of Canada on cold tolerant plants or plants with distributions extending into Canada may still be applicable to Canadian phytoremediation efforts.

A second data gap is related to the fact that there is little evidence detailing the mechanisms involved in the phytoremediation of petroleum hydrocarbons. For example, studies often state that concentrations of petroleum hydrocarbons are reduced in soil to a greater extent when plants are present than in their absence (e.g., Aprill and Sims, 1990; Qiu *et al.* 1997; Reynolds and Wolf 1999). Although these results indicate that phytoremediation is effective in reducing the contaminant load in soil, they, unfortunately, do not identify the specific mechanism(s) that cause the reduction. Two other data gaps involve the biomagnification of petrochemicals in the food chain and the use of a variety of plants to remediate mixtures of contaminants (e.g., petrochemicals and metals) at a given site.

## **8.2 Conclusion**

In conclusion, phytoremediation is emerging as a technique to be used in the reclamation of oil-contaminated sites. When considering phytoremediation as a reclamation strategy, environmental managers must consider the climate and soil type in the area they are reclaiming as these things will influence the effectiveness of phytoremediation and the types of plants to be used (Jackson, 1999). Managers also must know the types of petroleum hydrocarbons at the site, together with any metals, salts, and/or pesticides associated with the target contaminants. Following site and contaminant characterization, the manager should screen for appropriate plants and microorganisms (Jackson, 1999), identifying whether there are native species that could be used in the phytoremediation process. Preliminary screenings indicate that there are several plant species native and introduced to the Prairie and Boreal Plains Ecozones that may be used for phytoremediation efforts in these areas (see Appendix B). Further assessment of these plants in field situation would be useful.

Finally, it is important to keep in mind that a variety of remediation approaches may be required to accomplish all reclamation goals at a contaminated site (Hrudey and Pollard, 1993). The type of approach or approaches chosen will most likely be site-specific and depend on the desired speed of reclamation as well as the number of dollars dedicated to the reclamation effort.



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**Table 9.1 PTAC phytoremediation project technical steering committee members**

Name	Affiliation
Sandra Blenkinsopp	Environment Canada
Terry McIntyre	Environment Canada
Helen Jacobs	Amoco
Gary Webster	Canadian Association of Petroleum Producers
Stuart Lunn	Imperial Oil
Vasile Klaassen	PanCanadian Petroleum, Ltd.
Andrew Higgins	Quest – An Alliance Corporation
Gordon Dinwoodie	Alberta Environmental Protection
Phil Langille	Talisman Energy Inc.
Lin Callow	Gulf Canada Resources Ltd.
Todd Han	Saskatchewan Energy & Mines

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## 11. GLOSSARY

### **Adsorption**

Retention of a molecule or molecules on a surface.

### **Aerobic**

In the presence of oxygen.

### **Aerobic respiration**

A respiration pathway in which molecular oxygen is the final electron acceptor.

### **Anaerobic**

In the absence of oxygen.

### **Bioaccumulation**

Uptake of a chemical by an organism. Bioaccumulation may cause toxicity to the organism that has taken up the chemical or to a predator/consumer that ingests the affected organism. In phytoremediation, bioaccumulation may also be referred to as *phytoextraction*.

### **Bioavailability**

The extent to which a contaminant is available to living things (i.e., biota). Lipophilic compounds (i.e., those attracted to lipids) tend to bind to soil organic matter or soil particles (particularly clay) and are, therefore, typically less available to biota than water-soluble compounds. A consortium or mixture of microbes has been identified that can degrade the PAH phenanthrene adsorbed to soil without first desorbing it (White and Alexander, 1996). This has important implications for bioavailability, since it appears that bacteria may not require certain contaminants to be in an aqueous phase before degradation occurs.

### **Biomagnification**

Increasing concentrations of a chemical at increasing levels of the food chain due to the consecutive consumption of contaminated, lower trophic-level biota by higher trophic level biota. For example, consumption of grass contaminated with cadmium or methyl mercury will increase concentrations of these contaminants in cattle that feed on the grass.

### **Biopiles**

Contaminated soil piled in heaps several meters high over an air distribution system.

### **Bioreactor**

A controlled environment used to grow microorganisms.

### **Bioremediation**

The use of microorganisms to reclaim soil and water contaminated by hazardous substances.

### **Biotransformation**

The modification of a molecule by a living organism.

### **Bioventing**

Pumping air into the soil above the water table to provide oxygen to aerobic bacteria.

**Catabolic**

Break down of complex organic molecules by living things for energy.

**Catalyze**

The acceleration of a chemical reaction, typically produced by a *catalyst*, which is any substance that accelerates a chemical reaction but itself remains unaltered in form and amount (e.g., enzymes).

**Chlorosis**

The yellowing of leaves or other plant components due to bleaching of chlorophyll.

**Cometabolism (or cometabolic)**

The process by which a compound that cannot support the growth of microorganisms can be modified or degraded when another growth-supporting substrate is present. The cometabolized compound is not used for energy or incorporated into the biomass of the organism that modified it.

**Composting**

Decomposition of organic matter in a pile by microorganisms. Contaminated soil to be composed is often amended with a bulking material such as straw or hay. Compost piles differ from biopiles in that compost piles have elevated temperatures due to microbial activity.

**Consortium**

An interacting group of different microbes that generally result in combined metabolic activities.

**Containment**

In phytoremediation, containment involves using plants to reduce or eliminate the availability of contaminants to other biota. Contaminants are not necessarily degraded when they are contained.

**Degradation**

The breakdown of a compound into different compounds.

**Diauxy**

When a microorganism selectively degrades the organic compound in a mixture of compounds that is easiest to digest or provides the most energy.

**Dispersion**

Breaking up and spreading in various directions.

**Eukaryotic**

Organisms that have a membrane-bound nucleus containing chromosomes composed of DNA; examples are algae, fungi, protozoa, plants and animals.

**Evapotranspiration**

The combined loss of water from a given area, during a specific time-period, by evaporation from the soil surface and transpiration by plants.

**Ex situ**

Involving excavation or extraction of contaminated soil or water. May involve transport of contaminated material away from the contaminated site, but not necessarily.

**Extracellular**

External to a cell.

**Exudates**

In phytoremediation, substances excreted from the roots of plants.

**Fibrous root**

Plant roots with many secondary and tertiary branches spreading out from the primary branch.

**Green manure**

A crop that is incorporated into the soil to improve soil quality.

**Humification**

The process of transforming carbon from organic residues to humus through biochemical and physical processes.

**Humus**

Organic compounds in the soil other than undecayed plant and animal tissues, their 'partial decomposition' products, and the soil biomass; humus is synonymous with the term soil organic matter.

**Hydrogeologic**

Involving geology and hydrology (i.e., the study of the movement of water).

**Hydrolysis**

Breaking a chemical bond by adding the elements of water (i.e., hydrogen and oxygen).

**Hydrophilic**

Molecules and surfaces that have a strong affinity for water molecules. These molecules tend to be polar in chemical structure.

**Hydrophobic**

Molecules and surfaces that have little to no affinity for water, and typically have more affinity for other hydrophobic substances than for water. These molecules tend to be bi-polar or non-polar in chemical structure, such as lipids.

**Hydrophobicity**

The relative measure of the affinity of a molecule for water. A molecule that has a high hydrophobicity has a small affinity for water (and is known as a hydrophobic molecule), while a molecule that has a low hydrophobicity has an affinity for water (and is known as a hydrophilic molecule).

**Inoculate**

To introduce microorganisms into an environment that typically supports their growth.

**In situ**

Treatment at the site of contamination without excavation.

**Landfarming**

Spreading contaminated soil thinly over land or a pad with a leachate-collection system.

**Leachate**

Liquids that have moved downward through the soil and that contain substances in solution or suspension.

**Legumes (or leguminous)**

A plant whose roots serve as hosts for nitrogen-fixing bacteria, which are in a symbiotic relationship with the plant.

**Lipid**

Fats or fat-like substance that are not soluble in water but soluble in non-polar solvents.

**Lipophilic**

Molecules that are preferentially soluble in lipids or non-polar solvents.

**Metabolites**

The chemical products of changes to a parent molecule, including chemicals participating in *metabolism*, which is the total of all chemical reactions by which energy is provided for vital processes and new cell substances are assimilated.

**Microcosms**

An artificial environment that is a scaled-down version of a larger, natural system.

**Mineralization**

The ultimate degradation and recycling of an organic molecule into inorganic materials, such as carbon dioxide and water. In phytoremediation, the mineralization or metabolism of contaminants within plant tissue is also referred to as *phytodegradation*.

**Nanopores**

Soil pores with diameters < 100 nanometres.

**Natural attenuation**

A reclamation approach that relies on natural processes to remediate sites with no human intervention. The natural processes include physical/chemical mechanisms such as dilution, *dispersion* and adsorption of the contaminant. Biological processes, such as the unassisted growth of plants and microbial communities that break down contaminants, may be involved as well.

**Octanol-water partition coefficient (Kow, often expressed as log Kow)**

A measure of a chemical's affinity for water versus lipids or fats. A higher Kow indicates a greater affinity for lipids than water.

**Organic matter**

In soil, the organic fraction exclusive of undecayed plant and animal remains; also see humus.

**Photolytic loss**

Chemical loss through photolysis, which occurs when the energy from sunlight ruptures chemical bonds in a molecule that absorbs the light.

**Phytotoxicity**

Toxicity in plants.

**Phytovolatilization**

In phytoremediation, refers to the movement of a contaminant out of the soil, into, through and out of a plant, and then into the atmosphere. Also referred to in this report as *transfer* of the contaminants.

**Plasmid**

Small, circular molecules of DNA in bacteria. They contain a limited amount of specific information (e.g., resistance to antibiotics) that supplements the essential genetic information contained in the primary bacterial chromosome.

**Prokaryotic**

Organisms, typically unicellular, that do not have a membrane-bound nucleus; e.g., bacteria.

**Rhizosphere**

The surface of plant roots and the region of soil directly surrounding the roots where microbial populations are affected by the presence of the roots.

**Rhizosphere Effect**

The direct effect of plant roots and their exudates on microorganisms, including the fact that microbial populations are usually larger within the rhizosphere than in the root-free soil.

**Root exudates**

The compounds that come out of the plant roots and go into the rhizosphere.

**Senescence**

Aging; deterioration with aging; e.g., when perennial plant tissues dry up in preparation for winter dormancy.

**Soil structure**

The combination or arrangement of primary soil particles into secondary units or *peds*, with secondary units being classified on the basis of size, shape, and grade.

**Soil texture**

The relative proportions of sand, silt and clay in soil.

**Transfer**

In phytoremediation, refers to the movement of a contaminant out of the soil, into, through and out of a plant, and then into the atmosphere. Also referred to as *phytovolatilization* of contaminants.

**Transformation**

Change or modification.



**Translocated (or Translocation)**

Moved to a different place; movement of material in solution within an organism (e.g., phloem of a plant).

**Transpire (or Transpiration)**

The loss of water vapor from plants primarily through pores (stomata) in the leaves.

**Volatilization**

Transfer of a chemical into the atmosphere as a gas or vapour.

**Weathering**

In phytoremediation, the selective reduction of easily-degradable contaminants in soil due to natural processes such as wind, sun, and rain. In general, the action of external factors such as rain, frost, sun or wind on the Earth's surface.

**Note:** This glossary was constructed using information from the following sources: Abercrombie *et al.* (1970); Alexander *et al.* (1997); Atlas (1995); Atlas and Bartha (1998); Brady and Weil (1996); Flathman and Lanza (1998); Macey (1992); Committee on In Situ Bioremediation *et al.* (1993); Lawrence (1989); Mackay (1991); Pierzynski *et al.* (1994); Purves *et al.* (1998); Soil Science Society of America (SSSA) (1997); White and Alexander (1996); USEPA (1996a); USEPA (1996b).

## Appendix A

### TYPES AND BEHAVIOR OF PETROLEUM HYDROCARBONS

Petroleum hydrocarbons are naturally occurring chemicals exploited by humans for a wide range of purposes, such as the fueling of vehicles and heating of homes (Committee on *In Situ* Bioremediation *et al.*, 1993). Petroleum hydrocarbons are found in gaseous (natural gas), liquid (crude oil), or solid (tars and asphalts) forms and are basically mixtures of various classes of hydrocarbon compounds (Lyons, 1996). Classes of compounds include alkanes, aromatics, and polycyclic aromatic hydrocarbons (PAHs) (Committee on *In Situ* Bioremediation *et al.*, 1993; Mackay, 1991). Non-hydrocarbon components of petroleum hydrocarbons include sulphur, nitrogen, and oxygen, as well as small amounts of metals such as cadmium, vanadium, mercury, nickel, sodium, or potassium (Baxendell, 1983).

Alkanes are composed of carbon and hydrogen in unbranched (i.e., normal) or branched chains and cyclic structures (Lide, 1990; Mackay, 1991). Methane, ethane, propane, butane, pentane and hexane are several examples of normal alkanes. They differ in the number of carbons present in each molecule (Table A.1).

**Table A.1** Examples of the carbon content and chemical formulas of alkanes (adapted from McMurry, 1986)

Number of Carbons ( <i>n</i> )	Name	Formula ( $C_nH_{2n+2}$ )
1	Methane	CH <sub>4</sub>
2	Ethane	C <sub>2</sub> H <sub>6</sub>
3	Propane	C <sub>3</sub> H <sub>8</sub>
4	Butane	C <sub>4</sub> H <sub>10</sub>
5	Pentane	C <sub>5</sub> H <sub>12</sub>
6	Hexane	C <sub>6</sub> H <sub>14</sub>
7	Heptane	C <sub>7</sub> H <sub>16</sub>
8	Octane	C <sub>8</sub> H <sub>18</sub>
9	Nonane	C <sub>9</sub> H <sub>20</sub>
10	Decane	C <sub>10</sub> H <sub>22</sub>

Aromatic hydrocarbons are a class of compounds composed of a single benzene ring, which consists of six carbon molecules bound together in a ring with three double bonds and one hydrogen atom per carbon (McMurry, 1986). Different compounds result from the substitution of one or more of the hydrogen atoms with various functional groups. For example, toluene is composed of a benzene ring with a methyl group (i.e., CH<sub>3</sub>), ethylbenzene has a benzene ring with an ethyl group (CH<sub>2</sub>CH<sub>3</sub>), and xylene has a benzene ring with two methyl groups. Collectively, benzene, toluene, ethylbenzene, and xylene are known as BTEX

compounds, and are important constituents in gasoline (Committee on *In Situ* Bioremediation *et al.*, 1993).

The fusion of two or more benzene rings results in the creation of polycyclic aromatic hydrocarbons (Wetzel *et al.*, 1997). These chemicals are generally found in minute concentrations almost everywhere in the environment and are generally formed when a fuel is burned (Wetzel *et al.*, 1997; Mackay, 1991).

### **Petroleum Hydrocarbons at Well Sites and Pipeline Ruptures**

Natural gas and crude oil are petroleum hydrocarbons associated with well sites and pipeline ruptures. Natural gas is composed primarily of methane, but may also contain ethane, propane and butane (Olah and Molnar, 1995). Crude oil is a mixture of saturated, straight-chain alkanes, with smaller amounts of slightly branched alkanes, cycloalkanes, and aromatics (Olah and Molnar, 1995). The exact proportions of these chemical classes do, however, vary from one crude oil to another (Lyons, 1996). When gases are “flared” or burned off in flare pits at well sites, the burning can produce PAHs, resulting in an increase in the concentration of PAHs in the pit relative to those originally found in the crude oil. A similar increase in PAHs may be found when the general area of the well site is burned, for whatever reason. Metals, together with nitrogen and sulfur, may also be present in the crude oil to varying degrees at different locations (Lyons, 1996).

### **Behavior of Petroleum Hydrocarbons**

The behavior of petroleum hydrocarbons depends on several physical/chemical properties including molecular structure, composition, weight, solubility, vapor pressure, and affinity for lipids or oils (Paterson *et al.*, 1994; Mackay, 1991). In general, alkanes are not very soluble in water (e.g. hexane solubility = 10 mg/L) and solubility decreases by a factor of about 3 or 4 for every carbon added (Mackay, 1991). The branched and cyclic alkanes tend to be more soluble in water because of their smaller molecular weight. The aromatic BTEX compounds also are relatively soluble in water (e.g. benzene solubility = 1780 mg/L; Mackay, 1991).

The number of rings and their arrangement in the various PAHs results in a wide range of physical and chemical characteristics (Table A.2). PAHs with three or fewer rings, such as naphthalene (2 rings), are relatively volatile and water soluble compared to PAHs with more than three rings, which have negligible volatility, very low aqueous solubility (0.26 mg/L or less), and a relatively high affinity for lipids as indicated by their larger octanol-water partition coefficients (Sims and Overcash, 1983). Leaching of larger PAHs is generally limited by the low water solubility and strong partitioning of the compounds to clay surfaces and soil organic matter (Wetzel *et al.*, 1997). Most PAHs are considered to be persistent organic pollutants, with persistence increasing with ring number (Henner *et al.*, 1997).

**Table A.2 Physical and Chemical Properties of Several Polycyclic Aromatic Hydrocarbons (adapted from Sims and Overcash, 1983)**

PAH	Number of Rings	Aqueous Solubility (mg/L)	Vapour Pressure (torr at 20°C)	Log of Octanol-Water Partition Coefficient (log $K_{ow}$ )	Melting Point (°C)/Boiling Point (°C)
Naphthalene	2	30	$4.92 \times 10^{-2}$	3.37	80/218
Acenaphthene	3	3.47	$2.0 \times 10^{-2}$	4.33	96/279
Acenaphthylene	3	3.93	$2.9 \times 10^{-2}$	4.07	92/265
Anthracene	3	0.07	$1.96 \times 10^{-4}$	4.45	216/340
Phenanthrene	3	1.29	$6.8 \times 10^{-4}$	4.46	101/340
Fluorene	3	1.98	$1.3 \times 10^{-2}$	4.18	116/293
Fluoranthene	4	0.26	$6.0 \times 10^{-6}$	5.33	111/- -
Benz[a]anthracene	4	0.014	$5.0 \times 10^{-9}$	5.61	158/400
Chrysene	4	0.002	$6.3 \times 10^{-7}$	5.61	255/448
Pyrene	4	0.14	$6.85 \times 10^{-7}$	5.32	149/360
Benz[a]pyrene	5	0.0038	$5.0 \times 10^{-7}$	6.04	179/496
Benzo[b]-fluoranthene	4	0.0012	$5.0 \times 10^{-7}$	6.57	167/- -
Benzo[k]-fluoranthene	4	0.00055	$5.0 \times 10^{-7}$	6.84	217/480
Dibenzo[a,h]-anthracene	4	0.0005	$1.0 \times 10^{-10}$	5.97	262/- -
Benzo[ghi]perylene	6	0.00026	$1.0 \times 10^{-10}$	7.23	222/- -
Indeno[1,2,3-cd]-pyrene	6	0.062	$1.0 \times 10^{-10}$	7.66	163/- -

Concern over PAHs stems from evidence of mutagenic effects in bacterial and animal cells and carcinogenic effects in animals (Table A.3) (Wetzel *et al.*, 1997; Henner *et al.*, 1997). Benzo[a]pyrene is typically considered one of the most toxic PAHs. Light aromatics, alkanes (nC9 to nC14), and alkyl naphthalene have been found to be more acutely toxic to plants than heavier hydrocarbon compounds above nC15 (Chaîneau *et al.*, 1997), potentially because the lighter fractions are more bioavailable.

**Table A.3 Carcinogenicity levels of some PAHs (adapted from Henner *et al.*, 1997)**

<b>PAH</b>	<b>Carcinogenicity</b>
Naphthalene	No effect
Anthracene	No effect
Pyrene	No effect
Chrysene	Very weakly active
Benz[ <i>a</i> ]anthracene	Weakly active
Dibenz[ <i>a,h</i> ]anthracene	Active
Benzo[ <i>a</i> ]pyrene	Very active

## Appendix B

### POTENTIAL APPLICATION OF PHYTOREMEDIATION IN THE PRAIRIE AND BOREAL PLAIN ECOZONES OF WESTERN CANADA

Oil production in Western Canada currently takes place in both the Prairie and Boreal Plain Ecozones (Acton *et al.*, 1998). The Prairie Ecozone encompasses an extensive geographic area, extending from the Rocky Mountains in the west to the deciduous forests in the east; from the boreal forests in the north to the Gulf of Mexico in the south. Grasslands cover a vast area of the Prairie Ecozone. In Western Canada, the Boreal Plain Ecozone is north of the Prairie Ecozone, but south of the Churchill River Upland and Athabasca Plain Ecozones. It extends east-west from Newfoundland to the Rocky Mountains. The majority of the Boreal Plain is covered by boreal forest.

One of the major challenges in applying phytoremediation in the Prairie and Boreal Plain Ecozones is identifying plants that are both tolerant of the relatively cold, harsh climate and effective in phytoremediation. The following information includes a brief description of each ecozone, along with a preliminary screening of native and introduced plants with the potential to phytoremediate or tolerate oil-contaminated sites in the two ecozones. **Note:** the preliminary plant screening was based solely on the information found in the database (PhytoPet) associated with this report.

#### The Prairie and Boreal Plain Ecozones

In Canada, the Prairie Ecozone includes the Aspen Parkland, Moist Mixed Grassland and the Mixed Grassland Ecoregions (Acton *et al.*, 1998). In general, the climate is semiarid to humid continental and typically involves long, cold winters and short, very warm summers. Soils are highly variable, reflecting the climate, natural vegetation, and landform of the area. As stated above, a majority of the area is covered by grasses, although aspen groves are abundant in the Aspen Parkland.

The Boreal Plain Ecozone is composed of the Mid-Boreal Upland and Lowland Ecoregions together with the Boreal Transition Ecoregion (Acton *et al.*, 1998). The climate is characterized by long, cold, snowy winters and short, warm, moist summers. The soils are largely Luvisols (loamy- and clayey- textured sediments), Brunisols (sandy deposits), with organic soils found in low-lying areas. As stated above, the boreal forest covers most of this Ecozone.

#### The Plant Screening

Based on studies summarized in the database, there are several native and introduced plants with the potential to phytoremediate or tolerate petroleum hydrocarbons in the Prairie and Boreal Plain Ecozones. Twelve plants native to the Prairie Ecozone (western wheatgrass, big bluestem, side oats grama, blue grama, common buffalograss, Prairie buffalograss, Canada

wild rye, red fescue [Alberta only], switchgrass, poplar trees, little bluestem, and Indiangrass) have a demonstrated potential for use in the phytoremediation of oil-contaminated soil (Table B.1a). Alfalfa, which has been introduced extensively into the Prairie Ecozone as a forage, also has a demonstrated ability to assist in the phytoremediation of various petroleum hydrocarbons (Table B.1b). Indeed, more studies have been conducted on the phytoremediation potential of alfalfa than any other plant recorded in the database. Other plants introduced to the Prairie Ecozone that may be used in phytoremediation include carrot, red fescue [Manitoba/SK], soybean, annual ryegrass and perennial ryegrass. Plants in the Prairie Ecozone that tolerate various petroleum hydrocarbons include the native plants tilesy sage, water sedge, sunflower, reed grass, alpine bluegrass, three-square bulrush and cattail (Table B.1c), as well as the introduced plants crested wheatgrass, oat, canola, quackgrass, soybean, barley, birdsfoot trefoil, black medick, field pea, alsike clover, red clover, white clover, wheat, fababeans, and maize (Table B.1d).

Red fescue and poplar trees are the two native plants in the Boreal Plain Ecozone that have been identified as potential phytoremediators of petroleum hydrocarbons (Table B.2a). The garden vegetable, carrot, is an introduced plant to the Boreal Plain that has been shown to have the potential to accumulate PAHs. The small number of identified phytoremediators for the Boreal Plain may be explained by the fact that research to date has focused on grasses, which make up only a small percentage of the plants native to the Boreal Plain (based on species listed in Acton *et al.*, 1998). It is worth noting that there are several native plants of the Boreal Plain that tolerate petroleum hydrocarbons; these include water sedge, round sedge, rock sedge, tall cotton-grass, reed canarygrass, jack pine, alpine bluegrass, snow willow, and cattail (Table B.2c). As well, oat, black medick, field pea, alsike clover, red clover, white clover, and wheat are tolerant plants introduced into this zone (Table B.2d).

Tall fescue (*Festuca arundinacea*), and hybrid poplar trees (*Populus deltoides x nigra*) are plants in the database that have been identified for their potential in phytoremediation. Although these exact species are not present in the Prairie and Boreal Ecozones, various other species of fescue (e.g., plains rough fescue *F. hallii*; sheep fescue *F. saximantana*), and poplar (cottonwoods *P. deltoides*; trembling aspen *P. tremuloides*; balsam poplar *P. balsamifera*) are native to one or both of the ecozones. Research on the phytoremediation potential of these other species may prove to be beneficial.

In conclusion, results of the preliminary screening indicate that there are several native and introduced plants with the potential to be used for phytoremediation efforts in the Prairie and Boreal Plain Ecozones. Since many of the studies that identified the phytoremediation potential of these plants were conducted in laboratories, it is suggested that research efforts be made to confirm this potential under field conditions prior to using these plants in large-scale phytoremediation projects. Also keep in mind that, although a plant may have been listed in Tables B.1 and B.2 as native or introduced to the two Ecozones, the plant may not be naturally present (either as a native or introduced plant) at the actual site to be remediated since the plant occurrences in the tables are a general summary for the Ecozones, which represent vast and diverse areas.

**Table B.1 Plants in the Prairie Ecozone of Western Canada with the potential to phytoremediate or tolerate petroleum hydrocarbons**

**a) Native plants that phytoremediate petroleum hydrocarbons in the Prairie Ecozone**

Plant Common Name (Genus, Species – Family – Growth Form)	Petroleum Hydrocarbons	Mechanism of Phytoremediation	Plant Occurrence	Salinity Tolerance <sup>2</sup>
Western wheatgrass <sup>1</sup> ( <i>Agropyron smithii</i> - Gramineae - grass)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native	moderately tolerant
Big bluestem <sup>1</sup> ( <i>Andropogon gerardi</i> – Gramineae - grass)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native	-
Side oats grama <sup>1</sup> ( <i>Bouteloua curtipendula</i> – Gramineae)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native – rare	-
Blue grama <sup>1</sup> ( <i>Bouteloua gracilis</i> – Gramineae – grass)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native - common	moderately sensitive
Common buffalograss ( <i>Buchloe dactyloides</i> – Gramineae - grass)	naphthalene, fluorene, phenanthrene	unknown	native – rare	-
Prairie buffalograss ( <i>Buchloe dactyloides</i> var. <i>Prairie</i> – Gramineae - grass)	naphthalene, fluorene, phenanthrene	unknown	native – rare	-
Canada wild rye <sup>1</sup> ( <i>Elymus canadensis</i> – Gramineae - grass)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native	moderately tolerant
Red fescue ( <i>Festuca rubra</i> var. <i>Arctared</i> – Gramineae – grass)	crude oil and diesel	rhizosphere effect (suspected)	native - Rocky Mtns of Alberta	-
Switchgrass <sup>1</sup> ( <i>Panicum virgatum</i> – Gramineae - grass)	alone = anthracene & pyrene; with other plants = chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, & dibenz[ <i>a,h</i> ]anthracene	anthracene & pyrene degradation through rhizosphere effect; mechanism unknown for group of plants	native – very rare	-
Poplar trees ( <i>Populus deltoides</i> x <i>nigra</i> – Salicaceae – deciduous tree)	potential to phytoremediate benzene, toluene, <i>o</i> -xylene	rhizosphere effect: 5 times more BTX degraders in rhizosphere compared to bulk soil; all soil uncontaminated	<i>Populus deltoides</i> = native	-
Little bluestem <sup>1</sup> ( <i>Schizchyrium scoparium</i> or <i>Andropogon scoparium</i> – Gramineae - grass)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native – rare; along Bow River	-
Indiangrass <sup>1</sup> ( <i>Sorghastrum nutans</i> – Gramineae - grass)	chrysene, benzo[ <i>a</i> ]pyrene, benz[ <i>a</i> ]anthracene, dibenz[ <i>a,h</i> ]anthracene	unknown	native - rare	-

<sup>1</sup> Aprill and Sims (1990) evaluated the phytoremediation potential of these plants for the group as a whole, not as individual species. Switchgrass, however, also was investigated as an independent species by Reilley *et al.* (1996). Likewise, little bluestem was investigated as an independent species by Pradhan *et al.* (1998).

<sup>2</sup> Salinity tolerance based on information obtained from the United States Salinity Laboratory website: <http://www.usssl.ars.usda.gov/saltoler.htm>



Table B.1 continued...

**b) Introduced plants that phytoremediate petroleum hydrocarbons in the Prairie Ecozone**

Plant Common Name (Genus, Species – Family – Growth Form)	Petroleum Hydrocarbons	Mechanism of Phytoremediation	Plant Occurrence	Salinity Tolerance
Carrot ( <i>Daucus carota</i> – Umbelliferae – herb) <sup>1</sup>	PAHs in sewage sludge	accumulation	introduced	sensitive
Red fescue ( <i>Festuca rubra</i> var. <i>Arctared</i> – Gramineae – grass)	crude oil and diesel	rhizosphere effect (suspected)	introduced to Manitoba and Saskatchewan	-
Soybean ( <i>Glycine max</i> - Leguminosae – herb/legume)	anthracene	uptake and direct degradation	introduced - rare	-
Annual ryegrass ( <i>Lolium multiflorum</i> – Gramineae – grass)	crude oil and diesel	rhizosphere effect (suspected)	introduced	-
Ryegrass or perennial ryegrass ( <i>Lolium perenne</i> – Gramineae - grass)	hydrocarbon mixture: n- alkane (C10,C14 to C18, C22,C24), pristane, hexadecane, phenanthrene, anthracene, fluoranthene, pyrene	suspected mechanism = degradation/rhizosphere effect: plant roots stimulated microbial degradation of hydrocarbons	introduced - rare	moderately tolerant
Alfalfa ( <i>Medicago sativa</i> L. – Leguminosae – herb/legume)	phytoremediate = benzene, anthracene, pyrene, naphthalene; tolerate = asphalt dust from mine (France), crude oil, hydrocarbon mixture of benzoic acid, hexadecane, 2,2-dimethyl 4,n-propyl- benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	minor benzene containment in tissues; anthracene & pyrene degradation through rhizosphere effect; naphthalene containment by adsorption to roots	introduced - common	moderately sensitive

<sup>1</sup> Wild and Jones (1992) found that carrots accumulated PAHs in their peels to a maximum value of 200 ug total PAHs per kg dry weight in a laboratory setting. As with many other plants listed here, since the evaluation was conducted in the laboratory, small-scale field validation of the results should be conducted prior to application to larger scale remediation efforts.

**Table B.1 continued...**

**c) Native plants that tolerate petroleum hydrocarbons in the Prairie Ecozone<sup>1</sup>**

<b>Plant Common Name (Genus, Species – Family – Growth Form)</b>	<b>Petroleum Hydrocarbons</b>	<b>Plant Occurrence</b>	<b>Salinity Tolerance</b>
Tilesy age ( <i>Artemisia tilesii</i> – Compositae – herb/sub-shrub)	hydrocarbon mixture: benzoic acid, hexadecane, 2,2-dimethyl 4,n-propyl- benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	native - rare	-
Water sedge ( <i>Carex aquatilis</i> – Cyperaceae – grass-like)	crude oil	native	-
Sunflower ( <i>Helianthus annuus</i> – Compositae – herb)	oil and creosote	native	-
Reed grass ( <i>Phragmites australis</i> – Gramineae – grass)	hydrocarbon mixture containing ~50% BTEX	native – wet places in Parkland	-
Alpine bluegrass ( <i>Poa alpina</i> – Gramineae - grass)	very tolerant; hydrocarbon mixture 1: hexadecane, (2,2-dimethylpropyl)benzene, cis- decahydronaphthalene (decalin), benzoic acid, phenanthrene, pyrene; hydrocarbon mixture 2 = benzoic acid, hexadecane, 2,2-dimethyl 4,n-propyl- benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	native – Foothills Fescue grassland	-
Three-square bulrush ( <i>Scirpus pungens</i> – Cyperaceae – grass- like sedge family)	crude oil	native	tolerant of moderate salinity
Cattail ( <i>Typha latifolia</i> – Typhaceae – herb)	hydrocarbon mixture containing ~50% BTEX	native - common	-

<sup>1</sup> Tolerance is defined here as the ability of a plant to grow in hydrocarbon contaminated soil; it does not necessarily mean the plant is healthy.

**Table B.1 continued...**

**d) Introduced plants that tolerate petroleum hydrocarbons in the Prairie Ecozone**

<b>Plant Common Name (Genus, Species – Family – Growth Form)</b>	<b>Petroleum Hydrocarbons</b>	<b>Plant Occurrence</b>	<b>Salinity Tolerance</b>
Crested wheatgrass ( <i>Agropyron desertorum</i> – Gramineae – grass)	phenanthrene	introduced - common	moderately tolerant to tolerant
Oat ( <i>Avena sativa</i> – Gramineae – grass)	oily waste sludge	introduced	moderately sensitive
Canola ( <i>Brassica rapa</i> – Cruciferae – herb) <sup>1</sup>	oil and creosote	introduced - common	-
Quackgrass ( <i>Elytrigia repens</i> or <i>Agropyron repens</i> – Gramineae - grass)	hydrocarbon mixture: benzoic acid, hexadecane, 2.2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	introduced – common	-
Soybean ( <i>Glycine max</i> - Leguminosae – herb/legume)	crude oil	introduced - rare	-
Barley ( <i>Hordeum vulgare</i> – Gramineae - grass)	crude oil	introduced	moderately tolerant
Birdsfoot trefoil ( <i>Lotus corniculatus</i> – Leguminosae- herb/legume)	bituminous shale (Scotland); asphalt dust from mine (France); hydrocarbon mixture: benzoic acid, hexadecane, 2.2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	introduced - rare	moderately tolerant
Black medick ( <i>Medicago lupulina</i> Leguminosae – herb/legume)	mixed product spill (France)	introduced – common in parkland but not prairie	-
Field pea ( <i>Pisum arvense</i> – Leguminosae – herb/legume)	crude oil	introduced	-
Alsike clover ( <i>Trifolium hybridum</i> – Leguminosae – herb/legume)	oil and creosote	introduced - common	moderately sensitive
Red clover ( <i>Trifolium pratense</i> – Leguminosae – herb/legume)	hydrocarbon mixture = benzoic acid, hexadecane, 2.2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	introduced – common in parklands but not prairie	moderately sensitive
White clover ( <i>Trifolium repens</i> – Leguminosae – herb/legume)	hydrocarbon mixture = benzoic acid, hexadecane, 2.2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	introduced - common	moderately sensitive
Wheat ( <i>Triticum aestivum</i> – Gramineae – grass) <sup>2</sup>	oil and creosote	introduced - common	moderately tolerant
Fababean ( <i>Vicia faba</i> – Leguminosae – herb/legume)	oil and creosote	introduced	-
Maize ( <i>Zea mays</i> – Gramineae – grass)	fuel oil	introduced – more common in southern Prairies	moderately sensitive

<sup>1</sup> Although canola and wheat tolerated exposure to oil- and creosote-contaminated soil, they exhibited very poor growth (Bailey and McGill 1999; Biederbeck *et al.*, 1993)

**Table B.2 Plants in the Boreal Plain Ecozone of Western Canada with the potential to phytoremediate or tolerate petroleum hydrocarbons**

**a) Native plants that phytoremediate petroleum hydrocarbons in the Boreal Plain Ecozone**

<b>Plant Common Name (Genus, Species – Family – Growth Form)</b>	<b>Petroleum Hydrocarbons</b>	<b>Mechanism of Phytoremediation</b>	<b>Plant Occurrence</b>	<b>Salinity Tolerance</b>
Red fescue ( <i>Festuca rubra</i> var. Arctared – Gramineae – grass)	crude oil and diesel	rhizosphere effect (suspected)	native –Boreal forest of Alberta	-
Poplar trees ( <i>Populus deltoides</i> x <i>nigra</i> – Salicaceae – deciduous tree)	potential to phytoremediate benzene, toluene, o-xylene	rhizosphere effect: 5 times more BTX degraders in rhizosphere compared to bulk soil; all soil uncontaminated	<i>Populus deltoides</i> = native	-

**b) Introduced plants that phytoremediate petroleum hydrocarbons in the Boreal Plain Ecozone**

<b>Plant Common Name (Genus, Species – Family – Growth Form)</b>	<b>Petroleum Hydrocarbons</b>	<b>Mechanism of Phytoremediation</b>	<b>Plant Occurrence</b>	<b>Salinity Tolerance<sup>1</sup></b>
Carrot ( <i>Daucus carota</i> – Umbelliferae – herb) <sup>2</sup>	PAHs in sewage sludge	accumulation	introduced	sensitive

<sup>1</sup> Salinity tolerance based on information obtained from the United States Salinity Laboratory website: <http://www.ussl.ars.usda.gov/saltoler.htm>

<sup>2</sup> Wild and Jones (1992) found that carrots accumulated PAHs in their peels to a maximum value of 200 ug total PAHs per kg dry weight in a laboratory setting. Since the evaluation was conducted in the laboratory, small-scale field validation of the results should be conducted prior to application to larger scale remediation efforts.

**c) Native plants that tolerate petroleum hydrocarbons in the Boreal Plain Ecozone<sup>1</sup>**

<b>Plant Common Name (Genus, Species – Family – Growth Form)</b>	<b>Petroleum Hydrocarbons</b>	<b>Plant Occurrence</b>	<b>Salinity Tolerance</b>
Water sedge ( <i>Carex aquatilis</i> – Cyperaceae – grass-like)	crude oil	native	-
Round sedge ( <i>Carex rotundata</i> – Cyperaceae – grass-like)	crude oil	native	-
Rock sedge ( <i>Carex rupestris</i> – Cyperaceae – grass-like)	crude oil	native	-
Tall cotton-grass ( <i>Eriophorum angustifolium</i> – Cyperaceae – grass-like)	crude oil	native	-
Reed canarygrass ( <i>Phalaris arundinacea</i> – Gramineae - grass)	hydrocarbon mixture: benzoic acid, hexadecane, 2,2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	native	moderately tolerant
Reed grass ( <i>Phragmites australis</i> – Gramineae – grass)	hydrocarbon mixture containing ~50% BTEX	native – wet places	-
Jack pine ( <i>Pinus banksiana</i> – Pinaceae – tree)	diesel	native	-
Alpine bluegrass ( <i>Poa alpina</i> – Gramineae - grass)	very tolerant; mixture 1: hexadecane, (2,2- dimethylpropyl)benzene, cis- decahydronaphthalene (decalin), benzoic acid, phenanthrene, pyrene; mixture 2 = benzoic acid, hexadecane, 2,2- dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	native – Foothills Fescue grassland	-
Snow willow ( <i>Salix reticulata</i> – Salicaceae – shrub)	crude oil	native – Boreal forest of Manitoba and Saskatchewan	-
Cattail ( <i>Typha latifolia</i> – Typhaceae – herb)	hydrocarbon mixture containing ~50% BTEX	native - common	-

<sup>1</sup> Tolerance is defined here as the ability of a plant to grow in hydrocarbon contaminated soil; it does not necessarily mean the plant is healthy.

Table B.2 continued...

d) Introduced plants that tolerate petroleum hydrocarbons in the Boreal Plain Ecozone<sup>1</sup>

Plant Common Name (Genus, Species – Family – Growth Form)	Petroleum Hydrocarbons	Plant Occurrence	Salinity Tolerance
Oat ( <i>Avena sativa</i> – Gramineae – grass)	oily waste sludge	introduced	moderately sensitive
Black medick ( <i>Medicago lupulina</i> - Leguminosae – herb/legume)	mixed product spill (France)	introduced – common	-
Field pea ( <i>Pisum arvense</i> – Leguminosae – herb/legume)	crude oil	introduced	-
Alsike clover ( <i>Trifolium hybridum</i> – Leguminosae – herb/legume)	oil and creosote	introduced - common	moderately sensitive
Red clover ( <i>Trifolium pratense</i> – Leguminosae – herb/legume)	hydrocarbon mixture = benzoic acid, hexadecane, 2,2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	introduced – common	moderately sensitive
White clover ( <i>Trifolium repens</i> – Leguminosae – herb/legume)	hydrocarbon mixture = benzoic acid, hexadecane, 2,2-dimethyl 4,n-propyl-benzene, phenanthrene, pyrene, and either cycloheptane or cis-decalin	introduced - common	moderately sensitive
Wheat ( <i>Triticum aestivum</i> – Gramineae – grass) <sup>2</sup>	oil and creosote	introduced	moderately tolerant

<sup>1</sup> Tolerance is defined here as the ability of a plant to grow in hydrocarbon contaminated soil; it does not necessarily mean the plant is healthy.

<sup>2</sup> Although wheat tolerated exposure to oil- and creosote-contaminated soil, it exhibited poor growth (Bailey and McGill 1999; Biederbeck *et al.*, 1993).

## Appendix C

### INTERNET RESOURCES ON PHYTOREMEDIATION

The following are several web sites on the Internet relating to phytoremediation in general, as well as phytoremediation of petroleum hydrocarbons.

1. Remediation Technologies Development Forum (RTDF)
  - Phytoremediation of Organics Action Team:  
<http://www.rtdf.org/public/phyto/>
  - Field study protocol for the phytoremediation of petroleum hydrocarbons in soil put together by the Phytoremediation Action Team (1998)  
<http://www.rtdf.org/public/phyto/protocol.htm>
  - Phytoremediation bibliography associated with the site contains 1446 references on phytoremediation of inorganic and organic compounds:  
<http://www.rtdf.org/public/phyto/phytobib/biba-b.html>
  - Total Petroleum Hydrocarbons (TPH) in Soil Subgroup Conference Call Summaries:  
<http://www.rtdf.org/public/phyto/minutes/tph/default.htm>
2. Federal Remediation Technologies - Roundtable Remediation Technologies Screening Matrix and Reference Guide Version 3.0:  
[http://www.frtr.gov/matrix2/top\\_page.html](http://www.frtr.gov/matrix2/top_page.html)  
[http://www.frtr.gov/matrix2/section4/4\\_5.html](http://www.frtr.gov/matrix2/section4/4_5.html)
3. U.S. EPA Citizens Guides to Bioremediation, Phytoremediation and Natural Attenuation:  
<http://www.clu-in.org/>  
<http://clu-in.org/products/citguide/phyto2.htm>  
<http://clu-in.org/products/citguide/biorem.htm>  
<http://clu-in.org/products/citguide/natural.htm>
4. PHYTONET Phytoremediation Electronic Newsgroup Network:  
<http://www.dsa.unipr.it/phytonet/>
5. International Journal of Phytoremediation  
<http://www.aehs.com/phytohome.htm>
6. Phytoremediation: A Growing Field with Some Concerns (has additional web-sites listed):  
[http://www.the-scientist.library.upenn.edu/yr1999/mar/black\\_p1\\_990301.html](http://www.the-scientist.library.upenn.edu/yr1999/mar/black_p1_990301.html)

7. Great Plains/Rocky Mountain Hazardous Substance Research Center – Phytoremediation  
Kansas State University (KSU) leads a fourteen-institution consortium for the Great Plains/Rocky Mountain Hazardous Substance Research Center.  
Site has list of other phytoremediation web sites.  
<http://www.engg.ksu.edu/HSRC/phytoem/home.html>
8. Phytoremediation Bibliography - Prepared by Victor F. Medina, Ph.D. Assistant Professor  
Washington State University:  
<http://hano.tricity.wsu.edu/~vmedina/biblio.html>
9. Ground-water Remediation Technologies Analysis Center  
U.S. Environmental Protection Agency Documents / Software  
Bibliography of Phytoremediation Resources – 845 refs.  
<http://www.gwrtac.org/html/external/phytoem.htm>
10. Environment Canada – Environmental Technology Centre Oil Properties Database  
<http://www.etcentre.org/main/e/db/db.html>
11. U.S. EPA - Phytoremediation Resource Guide (EPA 542-B-99-003)  
<http://www.epa.gov/tio> - go to publications to download copy
12. Interstate Technology and Regulatory Cooperation Work Group Phytoremediation Work  
Team - Phytoremediation Decision Tree (November 1999)  
<http://clu-in.org/techpubs.htm> - hard copies unavailable as of December 1999