Phytotechnologies: Plant-Based Systems for the Remediation of Oil Impacted Soils

Richard E. Farrell and James J. Germida
Department of Soil Science, University of Saskatchewan, Saskatoon, SK, Canada S7N 5A8

INTRODUCTION

It is currently estimated that in western Canada alone, there are more than 90,000 active and abandoned oil/gas drilling sites and 10,000 brownfield sites that are, or will be, in need of some degree of remediation. To accomplish this, today's environmental manager can choose from a variety of approaches ranging from intensive engineering techniques to monitored natural attenuation–a "hands-off" approach that relies entirely on natural processes to remediate sites with no human intervention. Phytotechnologies, which involve the use of plants and their associated microorganisms to degrade or contain soil contaminants, are steadily emerging as a "green" biotechnology suitable for the treatment of oil-impacted soil and groundwater. They are essentially a form of ecological engineering–capitalizing on naturally occurring synergistic relationships among plants, microorganisms, and the environment that have evolved over millions of years. As phytotechnologies employs human initiative to enhance natural, plant-assisted degradation processes, they represent a technology that is intermediate between engineering and natural attenuation.

Phytotechnolies take advantage of the fact that plants have extensive rooting systems which explore large volumes of soil; support larger, more active bacterial populations in the rhizosphere (the region immediately surrounding the root) than in the surrounding bulk soil [17] and release cometabolites [35] or degradation-accelerating enzymes [34]. Advantages associated with phytotechnologies include a high level of public acceptance, low costs relative to conventional engineering techniques, and the ability to address multiple contaminants simultaneously. Moreover, phytotechnologies are particularly attractive for sensitive and remote locations. Though generally considered a long-term remediation option, limited to soils where the contamination is shallow and occurs at low to medium concentrations, phytoremediation has been used effectively to remediate a variety of organic contaminants (including petroleum hydrocarbons) in soil and groundwater [2, 5, 34, 49, 51].

MECHANISMS FOR THE PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS

Both plants and microorganisms are involved—directly and indirectly—in the degradation or transformation of PHCs into products that are generally less toxic and less persistent in the environment than the parent compounds [27]. The primary mechanisms for plant-mediated remediation of soils contaminated with PHCs are: phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation. A brief description of these key degradation processes follows.

Phytostabilization involves the use of plants to contain, or immobilize, contaminants in the soil or groundwater; thus, reducing the bioavailability of the contaminant. The mechanisms involved may include absorption and accumulation by roots, adsorption onto root surfaces, binding with soil organic matter, and incorporation into humic materials in the rhizosphere.

Several studies have reported the bioaccumulation of PHCs within plants and on the surfaces of plant roots [21, 22, 25, 26, 29], the amounts of PHCs bioaccumulated are generally quite small. For example, Banks et al. [5] investigated the uptake of [^{14}C]benzo[a]pyrene from an artificially contaminated soil by tall fescue, and reported no significant accumulation of ^{14}C in the above-ground biomass during the six-month greenhouse study. Accumulation of ^{14}C in the roots also was minimal [0.12%].

In general, plant uptake of PHCs appears to be species-specific, and several researchers have reported the complete absence of plant uptake and accumulation of PHCs from contaminated soil. For example, Goodin and Webber [32] found no evidence for the uptake of ^{14}C-labeled anthracene or benzo[a]pyrene by soybean, ryegrass or cabbage from soil to which the ^{14}C-labelled contaminants were added. Biederbeck et al. [1993] found no evidence to indicate the uptake of PHCs from soil amended with oily waste from a heavy oil upgrader. Likewise, Chaineau et al. [14], Qiu et al. [49], and Rogers et al. [53] reported no evidence of contaminant uptake in the tissues of plants grown in soils contaminated with a variety of PHCs [including PAHs and fuel-oil].

Bioaccumulation of hydrocarbons in plants appears to be related to the lipid content of the plant tissues–such that the greater the lipid content of the plant tissue, the greater the hydrocarbon accumulation [24, 55, 59, 60]. Attempts to model the uptake and accumulation of PHCs in plants are generally based on chemical characteristics of the hydrocarbons–in particular, the affinity of the compound for lipids. Based on the octanol-water partition coefficient, K_{ow}, one general model indicates chemicals that are highly water soluble (log K_{ow} <0.5) are not sufficiently sorbed to roots or actively transported through plant membranes [54]. At the other end of the spectrum, hydrophobic chemicals (log K_{ow} >3.0) are unlikely to be translocated within a plant because they are strongly bound to, and may not pass beyond, the surface of the root where the proportion of lipids is high [58]. The chemicals most likely to be taken up by plants are those that are moderately hydrophobic (log K_{ow} = 0.5–3.0). Chemicals in this group include most BTEX compounds, chlorinated solvents, and short-chain aliphatic compounds [54, 58].

Lipophilic organic contaminants (including some PHCs) also can bind with soil organic matter. Thus, the addition of plant-derived organic matter [in the form of exudates, mucigels, lysates, or residues] to the soil may reduce the bioavailability of a contaminant and, hence, its biodegradability [18]. Furthermore, PHCs may be incorporated into humic materials in the rhizosphere. That is, the plants may supply enzymes that bind a contaminant into soil organic matter [humus] or may increase the organic matter content of the soil, thus allowing for increased humification [18].

Finally, plants (especially trees) also act as organic pumps—transpiring water which, in turn, keeps the contaminant in the vadose zone and helps prevent its migration off-site. That is, because plants transpire considerable amounts of water, they create a mass flow of water and water-soluble chemicals (such as BTEX) towards the root zone [54]. In this manner, plants help prevent both parent compounds and any water-soluble degradation intermediates from spreading or leaching to the groundwater. For example, poplar trees have benn used to form a 'hydraulic barrier' to restrict groundwater movement and prevent off-site migration of pollutants at a site contaminated with gasoline and diesel fuel.

Phytodegradation (sometimes referred to as phytotransformation) involves the breakdown of contaminants either internally, through metabolic processes, or externally, through the release of plant-produced enzymes into the soil. To date, however, few studies have have demonstrated that the ability of plants to metabolize PHCs or release enzymes capable of catalyzing the transformation of these contaminants in soil [21, 25, 26, 54].

Phytovolatilization refers to the uptake and transpiration of a contaminant by a plant. In this way, the parent contaminant, or a metabolite of the contaminant (if phytodegradation is also involved), is released into the atmosphere. For example, transpiration provides a route for the transfer of volatile PHCs such as BTEX compounds from the soil to the atmosphere via the plant [30]. Watkins et al. [63] reported that the volatilization of [^{14}C]naphthalene was enhanced in a sandy loam soil planted with bell rhodesgrass compared to unvegetated soil. The authors suggested that the naphthalene was taken up by the roots of the grass, translocated within the plant and volatilized through the stems and leaves. They also noted that although this mechanism of removal would reduce the amount of naphthalene available for biodegradation, it may have implications for air quality monitoring and regulatory compliance.

Rhizodegradation (also referred to as rhizosphere biodegradation, enhanced rhizosphere biodegradation, and plant-mediated biodegradation) involves the breakdown of contaminants in the soil as a result of microbial activity that is enhanced in the presence of the rhizosphere. Unlike the situation with plants, however, it is well established that microorganisms play a primary, direct role in degrading various PHCs [6, 9, 11, 13, 33, 36, 50, 57, 62]. Moreover, though plants and microorganisms can degrade PHCs independenty, it is the interaction between the plant and the microbial community in the rhizosphere that is considered to be the primary mechanism by which PHCs are degraded in soils. Consequently, enhanced rhizosphere biodegradation is examined more closely in the following section.

THE RHIZOSPHERE EFFECT

The rhizosphere is the zone of soil under the direct influence of roots and is generally considered to extend 1–5 mm from the root surface. Plant roots excrete a variety of organic compounds (i.e., root exudates) into the rhizosphere. Due to the presence of these exudates, microbial populations and activities are 5 to 100 times greater in the rhizosphere than in the bulk soil [3, 34, 48]. This plant-induced enhancement of microbial populations and activity in the root zone is referred to as the rhizosphere effect [3].

Living plants have extensive root systems that help to bring microbes, nutrients, and contaminants into contact with each other [18]. Thus, the presence of plants in contaminated soils greatly increases the volume of soil in which active microbial degradation can be stimulated. Several studies serve as examples of the rhizosphere effect in the phytoremediation of PHCs. Gunther et al. [34] found higher microbial numbers and activity coupled with increased degradation in hydrocarbon-contaminated soil planted to ryegrass (*Lolium perenne* L.) compared to unvegetated soil. The authors suggested that plant roots stimulated microbes, which enhanced the degradation of the hydrocarbons. Jordahl et al. [41] 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 the bulk soil. Nichols et al. [47] found higher numbers of organic chemical degraders in rhizosphere and contaminated soils compared to bulk and uncontaminated soils, respectively. Plants creating the rhizosphere in this experiment included alfalfa (*Medicago sativa* L.) and alpine bluegrass (*Poa alpina* L.), while the contaminants included hexadecane, (2,2-dimethylpropyl) benzene, cis-decahydronaphthalene, benzoic acid, phenanthrene, and pyrene. More recently, Miya and Firestone [46] reported that increased degradation of phenanthrene in the rhizosphere of slender oat (*Avena barbata*) was associated with increased numbers of phenanthrene-degraders in the rhizosphere soil compared to bulk soil.

Role of the Plant in Rhizodegradation. Plants play a key role in the rhizodegradation of PHCs–namely, without the plant there is no rhizosphere and, hence, no rhizosphere effect. In addition to supplying root exudates and nutrients that increase the size and activity of the microbial community, plants also play a role in the rhizodegradation of PHCs by releasing organic compounds into the soil that affect the bioavailability of the contaminant, producing contaminant analogs that stimulate the growth of hydrocarbon-degrading species, and releasing exudates that serve as primary substrates for the cometabolic degradation of PHCs.

Perhaps the most important role played by plants in rhizosphere biodegradation is production and release of root exudates that induce the rhizosphere effect. In addition, root exudates may potentially interact with PHCs (PAHs in particular) to alter their bioavailability [23, 43]. However, whether root exudates are produced in sufficient quantity to significantly affect the bioavailability of PHCs in soils remains to be seen. Root exudates may also contain 'contaminant analogs'; i.e., compounds that may act to stimulate the biodegradation of contaminant hydrocarbons. For example, Atlas and Cerniglia [4] attributed the rapid biodegradation of pristane and phytane in soils contaminated as a result of the Exxon Valdez oil spill to microbes that had evolved to consume naturally occurring terpenes produced by the surrounding pine trees. Root exudates also can provide energy to support populations of microbes that co-metabolize specific PHCs. For example, Kanaly et al. [42] found that benzo[a]pyrene was almost completely degraded (95% degradation) by microbes in soil containing suitable co-substrates present in a crude oil mixture.

Role of the Microorganism in Rhizodegradation. Microbial degradation of an organic contaminant normally occurs because the degrading microorganisms are able to use the contaminant for their own growth and reproduction [15]. Organic contaminants not only provide the microorganisms with a source of carbon, one of the basic building blocks of new cell components, they also provide electrons that the organisms use to obtain energy. Basic microbial metabolism of contaminants involves aerobic respiration (i.e., respiration in the presence of oxygen). Variations in metabolism include anaerobic respiration, secondary utilization and cometabolism, using inorganic compounds as electron donors, fermentation, and reductive dehalogenation [61]. In general, microorganisms act on a wider range of substrates, carry out more difficult degradative steps, and generally take the contaminant to a molecularly simpler end point than plants [19].

There are several points of interest with regards to the microbial communities involved in the phytoremediation of organic contaminants. First, soil microorganisms may experience selective enrichment of contaminant-tolerant species when exposed to a contaminant for prolonged periods of time. Such selective enrichment may, in turn, result in enhanced degradation of the contaminant. Second, some species of bacteria appear to be able to degrade a wide variety of rarely-occurring compounds without having to first adapt to contaminated conditions [58]. For example, catabolic pathways in pseudomonads allow these bacteria to degrade a variety of aromatic contaminants, such as toluene, m-xylene, and naphthalene, without having to synthesize a large number of different enzymes [37]. Third, evidence suggests that the degradation of certain contaminants occurs only when a specific consortium of microbes is present together at a contaminated site [58]. Fourth, it appears that some bacteria may not require certain contaminants to be in the aqueous phase before degradation can occur. Finally, the composition and size of the microbial community in the rhizosphere is dependent on the species and age of the plant, as well as on soil type [3, 9, 12]. It also may depend on the exposure history of the plant roots to contaminants. 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 and thereby stimulate the degradation of other non-phytotoxic contaminants [58]. Radwan et al. [50] found that the plant *Senecio glaucus* grew along the polluted border of an oil lake in the Kuwaiti desert. Interestingly, 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 for the survival of the plants in the oil-contaminated soils.

SPECIAL CONSIDERATIONS WITH PHYTOREMEDIATION

Several considerations must be met when implementing a phytoremediation system to reclaim/restore contaminated soils. The three primary considerations are (i) type and concentration of contaminant, (ii) establishment of appropriate plants and microorganisms, and (iii) influence of environmental factors on phytoremediation potential.

Type and Concentration of Petroleum Hydrocarbons. The inherent degradability of a hydrocarbon depends on its chemical nature; thus, certain PHCs are easier to phytoremediate than are others. In general, BTEX compounds are relatively easy to remediate because they are rapidly degraded in the presence of oxygen; are relatively soluble, thus making them bioavailable; and can serve as the primary electron donor for a wide variety of soil bacteria [15]. In general, weathering processes involving volatilization, photomodification, hydrolysis, leaching, and biotransformation, selectively reduce the concentration of easily-degradable components of a contaminant (e.g., BETX compounds); thus making older, highly weathered sites more difficult to phytoremediate than younger, less weathered sites [9, 18]. Likewise, large lipophilic molecules (such as the four and five-ring PAHs) are more difficult to remediate than smaller molecules. The difficulty in degrading these compounds is a reflection of their limited bioavailability (a consequence of their strong adsorption to soil organic matter and clay) as well as their limited ability to cross cellular membranes, which prevents their entry into microbes and plants [16]. Nevertheless, cometabolism by microorganisms has been shown to result in the degradation of some fairly large PAHs, such as benzo[a]pyrene [42].

The phytoremediation of PHCs may be ineffective if concentrations of the contaminants are either too high or too low. Concentrations of contaminants that are too high will result in a toxic response, which may include the death of the plant or soil microorganisms. Contaminants generally exhibit a treatable concentration range, above which the contaminant prevents or slows metabolic activity. This, in turn, prevents the growth of new microbial biomass needed to stimulate rapid removal of the contaminant [15, 38]. For example, Rogers et al. [53] found that the growth of white clover, tilesy sage, Bering hairgrass, and alpine bluegrass was enhanced by exposure to 0.1% (w/w) of a mixture of organic chemicals, but was severely limited by exposure to higher concentrations of the same mixture. Conversely, low concentrations of contaminants may limit the extent to which phytoremediation can further reduce the contaminant level. That is, degrader-microorganisms may be physiologically incapable of reducing contaminant concentrations to very low levels because the uptake and metabolism of the contaminant stops at low concentrations–even when environmental conditions are optimal. Low contaminant concentrations also may cause microbes capable of degrading the contaminant to switch to alternative substrates or may result in the death of the microbes due to lack of sustenance [15, 38].

Establishment of Appropriate Plant and Microorganisms. The successful implementation of any phytoremediation system will require the establishment of appropriate plants (and/or microorganisms) at the contaminated site. Factors to consider include: (i) selection of an appropriate plant species, including the use of native versus non-native plants; (ii) the influence of contaminants on seed germination; and (iii) the effectiveness of inoculating contaminated soils with microorganisms.

Plant selection is considered to be site-specific. Nevertheless, many plants have a wide habitat and/or geographic distribution. Thus, it may be possible to select suitable 'candidate' plants from the growing list of phytoremediator plants and–based on environmental and phytological characteristics–'apply' that plant (or a close relative) to a different location. To assist in the selection of appropriate phytoremediator plants, Farrell et al. [28] constructed a database of plants that play a role in the phytoremediation of PHCs. The PhytoPet© database is essentially an inventory of plants with a demonstrated ability to phytoremediate or, at the very least, tolerate soils contaminated with PHCs. Used in conjunction with other tools (e.g., the Phytoremediation Decision Tree [40]), the PhytoPet© database can be used with site-specific information to evaluate whether phytoremediation is appropriate for a particular site.

In general, establishing plants at a phytoremediation site involves getting seeds to germinate in the contaminated soil. Thus, Cunningham et al. [18] suggest that prior to planting at a given site, tests be conducted to determine whether the plant(s) being considered for phytoremediation will germinate successfully in the contaminated soil. To date, our research group has conducted germination tests for a variety of plants and petroleum-contaminated soils. For example Robson et al. [52] assessed 39 cold-tolerant plants native, or exotic and naturalized, to western Canada for their ability to survive in crude oil-contaminated soil. Four naturalized grasses (*Agropyron pectiniforme*, *Bromus inermis*, *Phleum pratense*, and *Poa pratensis*), three naturalized legumes (*Medicago sativa*, *Melilotus officinalis*, and *Trifolium repens*), two native forbs (*Artemisia frigida* and *Potentilla pensylvanica*), one native grass (*Bromus ciliatus*) and two native legumes (*Glycyrrhiza lepidota* and *Psoralea esculenta*) exhibited phytoremediation potential based on survival. The effect of increasing crude oil concentrations on total and root biomass, and relative growth rate of those species with the highest rates of survival also were determined. The addition of 0.5%, 1% and 5% (crude oil wt/fresh soil wt) crude oil to soil significantly decreased total biomass by at least 22% of the control and relative growth rate of all species except P. esculenta. Root biomass significantly decreased by at least 22%with crude oil addition in all species except P. esculenta and A. frigida. Total biomass production in contaminated soil had a significant negative correlation with the relative growth rate in uncontaminated soil.

The utility and effectiveness of inoculating oil-impacted sites with contaminant-degrading microorganisms is the subject of much debate. For example, Cunningham et al. [18] state that it is a common experience for soil and plant inoculants to be out-competed by native microflora. However, this is true even for some symbiotic relationships (e.g., soybean/*Bradyrhizobium*). Heitkamp and Cerniglia [36] found that competition with indigenous microorganisms did not adversely affect the degradation of pyrene by a *Mycobacterium* species inoculated into sediments; that is, unless organic nutrients (glucose and peptone) were added to the sediments, in which case there was an overgrowth of indigenous bacterial species. Likewise, Grosser et al. [33] determined that the isolation, propagation, and reintroduction of *Mycobacterium* species (at 10^7 cfu g^{-1} soil) resulted in enhanced mineralization of phenanthrene, anthracene, and pyrene; i.e., relative to mineralization by the indigenous microbial population. In our own research group, Hynes et al. [39] have reported that inoculation of white mustard (*Sinapis albus*) with *Sphingomonas macrogoltabidus* significantly enhanced the degradation of phenanthrene in both sterile and nonsterile soil.

TECHNIQUES USED TO ENHANCE PHYTOREMEDIATION

On-site phytoremediation of PHCs can be enhanced by employing a combination of common agronomic practices [e.g., fertility testing and fertilizer application, tillage, and irrigation]. Even if a soil contaminated with PHCs is not initially nutrient limited, available soil nutrient reserves can be quickly depleted as the microbial community begins to degrade the contaminant[s]. Fertilizer applications, therefore, may enhance the degradation of PHCs in soil by reducing competition for limited nutrients. Cutright [21] found that increasing the amount of nitrogen and phosphorus in soil under aerobic conditions increased the degradation of PAHs by the soil fungus *Cunninghamella echinulata* var. elegans. Loss of 2- and 3-ring PAHs from soil contaminated with weathered petroleum compounds also was more rapid when the soil was amended with a sludge compost high in nitrogen compared to no amendment or a low nitrogen amendment [10].

While conducting experiments on the effects of crude oil on tomato, kale, and leaf lettuce, Schwendinger [56] recognized that plants in oil-contaminated soil exhibited stress symptoms comparable to those of extreme nutrient deficiency. Schwendinger went on to suggest that the damage to plants from oil pollution 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 more recent study, Lin and Mendelssohn [44] found that fertilizer applications enhanced both the establishment and growth of Spartina alterniflora and S. patens transplanted into crude oil contaminated soil and degradation of the crude oil (as evidenced by a more pronounced decrease in the oil content of the fertilized soil compared to the planted, unfertilized control soil). Amadi et al. [1] reported that the 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–also can be used to provide soil nitrogen at contaminated sites [7] and, in doing so, may enhance phytoremediation efforts.

Various tillage practices are used to increase production in agricultural fields by aerating the soil, providing a more homogeneous distribution of added fertilizers throughout the upper portion of the rooting zone, returning and incorporating readily-decomposable organic matter into the soil, and improving aeration. The beneficial effects of tillage may then lead to enhanced biological activity [and, presumably, biodegradation efficiency] in the soil. Thus, it is to be expected that proper tillage practices may play an important role in maximizing the phytoremediation potential of plant systems in contaminated soils. Indeed, tillage practices are commonly employed in the landfarming of PHCs [8, 31, 45].

SUMMARY

Phytotechnologies, plant-based systems for the treatment of contaminated soils, continue to emerge as acceptable, ‘green’ technologies with potential for the effective and inexpensive cleanup of oil-impacted sites. Initial indications are that plant-based systems are effective at degrading and containing PHCs in soil and groundwater. Phytotechnologies are in situ, solar driven techniques that control erosion, runoff, infiltrattion, and dust emissions; create habitat and promote biodiversity; are energy efficient and require minimal maintenance; and are well-suited to treatment in remote areas, when other methods may not be cost-effective. In general, phytotechnologies are looked upon favorably because they exploit the natural ability of the environment to restore itself. Indirect benefits of phytoremediation include 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. The use of plants to remediate contaminated soils also 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).

Limitations associated with phytotechnologies reflect the state of the science; i.e., not all of the mechanisms involved are fully understood. Successful application of phytotechnologies generally requires more time than engineering techniques, but less time than natural attenuation. Because these technologies are plant-based, they are climate dependent and seasonal. Moreover, as is the case with all biological remediation systems, phytotechnologies are incapable of achieving a 100% reduction in contaminant concentration (i.e., ultimately, they are limited by the bioavailability of the contaminant). [Though this makes them an ideal component of risk-based remediation strategies.] However, a lack of sound economic performance data is perhaps the greatest barrier to the wide-spread adoption of phytotechnologies. Obtaining this type of data for wesetrn Canadian conditions is one component of the phytotechnology research project at the University of Saskatchewan.

REFERENCES

1. Amadi, A., Dickson, A.A. and Maate, G.O. 1993. Remediation of oil polluted soils: 1. Effects of organic and inorganic nutrient supplements on the performance of maize (*Zea may* L.). Water Air Soil Pollut.. 66: 59-76.
2. Aprill, W. and Sims, R.C. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere. 20: 253-265.
3. Atlas, R.M., Bartha, R., 1998. Microbial Ecology: Fundamentals and Applications. Benjamin/Cummings Publishing Company Inc., Don Mills, ON.
4. Atlas, R.M., C.E. Cerniglia., 1995. Bioremediation of petroleum pollutants: Diversity and environmental aspects of hydrocarbon biodegradation. BioSci. 45, 332-339.
5. Banks, M.K., Govindaraju, R.S., Schwab, A.P., and Kulakow, P. 2000. Phytoremediation of Hydrocarbon-Contaminated Soil. S. Fiorenza, C.L. Oubre, and C.H. Ward (eds.). Lewis Publishers, Boca Raton, FL.
6. Barnsley, E.A. 1975. The bacterial degradation of fluoranthene and benzo[*a*]pyrene. Can. J. Microbiol. 21: 1004-1008.
7. Biederbeck, V.O., Bouman, O.T., Campbell, C.A., Bailey, L.D., and Winkleman, G.E. 1996. Nitrogen benefits from four green-manure legumes in dryland cropping systems. Can. J. Plant Sci. 76: 307-315.
8. Bollag, J.-M., Mertz, T. and Otjen, L. 1994. Role of Microorganisms in soil bioremediation. *In* Bioremediation Through Rhizosphere Technology. ACS Symposium Series 563. *Edited by* T.A. Anderson and J.R. Coats. American Chemical

Society, Washington, D.C. pp. 2-10.

9. Bossert, I., Bartha, R., 1984. The fate of petroleum in soil ecosystems. In: Atlas R.M. (Ed.). Petroleum Microbiology. MacMillan, New York, NY. pp. 435-473.
10. Brown, J.L., Syslo, J., Lin, Y.-H., Getty, S., Vemuri, R., and Nadeau, R. 1998. On-site treatment of contaminated soils: an approach to bioremediation of weathered petroleum compounds. J. Soil Contam. 7: 773-800.
11. Bumpus, J.A., Tien, M., Wright, D. and Aust, S.D. 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science. 228: 1434-1436.
12. Campbell, R., 1985. Plant Microbiology. Edward Arnold, Baltimore, MD.
13. Cerniglia, C.E. and Gibson, D.T. 1979. Oxidation of benzo[*a*]pyrene by the filamentous fungus *Cunninghamella elegans*. J. Biol. Chem. 254: 12174-12180.
14. Chaineau, C.H., Morel, J.L., and Oudot, J. 1997. Phytotoxicity and plant uptake of fuel oil hydrocarbons. J. Environ. Qual. 26: 1478-1483.
15. Committee on In Situ Bioremediation, Water Science and Technology Board, Commission on Engineering and Technical Systems, and National Research Council., 1993. *In-Situ* Bioremediation: When Does It Work? National Academy Press, Washington, DC.
16. Cookson, H.T.J., 1995. Bioremediation Engineering: Design and Application. McGraw-Hill Inc., Toronto.
17. Crowley, D. E. 1997. Rhizosphere ecology of xenobiotic-degrading microorganisms. In: *Phytoremediation of Soil and Water Contaminants*, ACS Symposium Series No. 664, pp. 20-36. (Kruger, L., Anderson, T. A. and Coats, J. R., Eds.). American Chemical Society, Washington, DC.
18. Cunningham, S.D., Anderson, T.A., Schwab, A.P. and Hsu, F.C. 1996. Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 56: 55-114.
19. Cunningham, S.D., Berti, W.R., 1993. Remediation of contaminated soils with green plants: An overview. In vitro cell. dev. biol., Plant 29P, 207-212.
20. Cutright, T.J. 1995. Polycyclic aromatic hydrocarbon biodegradation and kinetics using *Cunninghamella echinulata* var. *elegans*. Internat. J. Biodeter. Biodegrad. 14: 397-408.
21. Durmishidze, S.V. 1977 (Transl. 1978). Metabolism of certain air-polluting organic compounds in plants (review). Appl. Biochem. Microbiol. 13: 646-653.
22. Duxbury, C.L., Dixon, D.G. and Greenberg, B.M. 1997. Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed *Lemna gibba*. Environ. Toxicol. Chem. 16: 1739-1748.
23. Eadie, B.J., Morehead, N.R., Landrum, P.F., 1990. Three-phase partitioning of hydrophobic organic compounds in Great Lakes (USA) waters. Chemosphere 20, 161-178.
24. Edwards, N.T. 1983. Polycyclic aromatic hydrocarbons (PAH's) in the terrestrial environment - a review. J. Environ. Qual. 12: 427-441.
25. Edwards, N.T. 1988. Assimilation and metabolism of polycyclic aromatic hydrocarbons by vegetation - an approach to this controversial issue and suggestions

for future research. Polycyclic Aromatic Hydrocarbons: A Decade of Progress - 10th International Symposium, Columus, OH, Battelle Press.

26. Edwards, N.T., Ross-Todd, B.M. and Garver, E.G. 1982. Uptake and metabolism of 14C anthracene by soybean (*Glycine max*). Environ. Exp. Bot. 22: 349-357.
27. Eweis, J.B., Ergas, S.J., Chang, D.P.Y. and Schroeder, E.D. 1998. Bioremediation Principles. McGraw-Hill, Inc., Toronto.
28. Farrell, R.E., Frick, C.M., Germida, J.J., 2000. PhytoPet© – A database of plants that play a role in the phytoremediation of petroleum hydrocarbons. In: Proceedings of the Second Phytoremediation Technical Seminar – June 13, 2000, Vancouver, BC. Environment Canada, Ottawa. pp. 29-40.
29. Ferro, A., Kennedy, J., Doucette, W., Nelson, S., Jauregui, G., McFarland, B. and Bugbee, B. 1997. Fate of benzene in soils planted with alfalfa: uptake, volatilization, and degradation. *In* Phytoremediation of Soil and Water Contaminants. ACS Symposium Series 664. *Edited by* E.L. Kruger, T.A. Anderson and J.R. Coats. American Chemical Society, Washington, D.C. pp. 223-237.
30. Flathman, P.E. and Lanza, G.R. 1998. Phytoremediation: current views on an emerging green technology. J. Soil Contam. 7: 415 – 433.
31. Genouw, G., de Naeyer, F., van Meenen, P., vam de Werf, H., de Nijs, W. and Verstraete, W. 1994. Degradation of oil sludge by landfarming - a case-study at the Ghent harbour. Biodegrad. 5: 37-46.
32. Goodin, J.D. and Webber, M.D. 1995. Persistence and fate of anthracene and benzo(*a*)pyrene in municipal sludge treated soil. J. Environ. Qual. 24: 271-278.
33. Grosser, R.J., Warshawsky, D. and Vestal, J.R. 1995. Mineralization of polycyclic and *n*-heterocyclic aromatic compounds in hydrocarbon-contaminated soils. Environ. Toxicol. Chem. 14: 375-382.
34. Gunther, T., Dornberger, U. and Fritsche, W. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere. 33: 203-215.
35. Haby, P. A. and D. E. Crowley. 1996. Biodegradation of 3-Chlorobenzoate as affected by rhizodeposition and selected carbon substrates. J. Environ. Qual. 25, 304-310.
36. Heitkamp, M.A. and Cerniglia, C.E. 1989. Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from a pristine ecosystem. Appl. Environ. Microbiol. 55: 1968-1973.
37. Houghton, J.E., Shanley M.S., 1994. Catabolic potential of pseudomonads: A regulatory perspective. In: Chaudbry, G.R. (Ed.). Biological Degradation and Bioremediation of Toxic Chemicals, Timber Press, Portland, OR. pp. 11-32.
38. Hrudey, S.E., Pollard, S.J., 1993. The challenge of contaminates sites: remediation approaches in North America. Environ. Rev. 1, 55-72.
39. Hynes, R., Germida, J.J., and Farrell, R.E. 2002. (Submitted to Internat. J. Phytoremed.)
40. Interstate Technology and Regulatory Cooperation (ITRC) Work Group–Phytoremediation Work Team, Phytoremediation Decision Tree, 1999.
41. Jordahl, J.L., Foster, L., Schnoor, J.L., Alvarez, P.J.J., 1997. Effect of hybrid poplar

trees on microbial populations important to hazardous waste bioremediation. Environ. Toxicol. Chem. 16, 1318-1321.

42. Kanaly, R., Bartha, R., Fogel, S., Findlay, M., 1997. Biodegradation of [^{14}C]benzo[a]pyrene added in crude oil to uncontaminated soil. Appl. Environ. Microbiol. 63, 4511-4515.
43. Kordel, W., Dassenakis, M., Lintelmann, J., Padberg, S., 1997. The importance of natural organic material for environmental processes in water and soils. Pure Appl. Chem. 69, 1571-1600.
44. Lin, Q. and Mendelssohn, I.A. 1998. The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. Ecol. Engin. 10: 263-274.
45. Loehr, R.C. and Webster, M.T. 1996. Performance of long-term, field-scale bioremediation processes. J. Hazard. Mater. 50: 105-128.
46. Miya, R.K., M.K. Firestone., 2000. Phenanthrene degrader community dynamics in rhizosphere soil from a common annual grass. J. Environ. Qual. 29, 584-592.
47. Nichols, T.D., Wolf, D.C., Rogers, H.B., Beyrouty, C.A., Reynolds, C.M., 1997. Rhizosphere microbial populations in contaminated soils. Water Air Soil Pollut. 95, 165-178.
48. Paul, E.A., Clark, F.E., 1989. Occurrences and distribution of soil organics. In: Paul, E.A., Clark, F.E. (Eds.). Soil Microbiology and Biochemistry. Academic Press, San Diego. pp. 81-84.
49. Qiu, X., Leland, T.W., Shah, S.I., Sorensen, D.L. and Kendall, E.W. 1997. Field study: grass remediation for clay soil contaminated with polycyclic aromatic hydrocarbons. *In* Phytoremediation of Soil and Water Contaminants. ACS Symposium Series 664. *Edited by* E.L. Kruger, T.A. Anderson and J.R. Coats. American Chemical Society, Washington, D.C. pp. 186-199.
50. Radwan, S., Sorkhoh, N. and El-Nemr, I. 1995. Oil biodegradation around roots. Nature. 376: 302.
51. Reilley, K.A., Banks, M.K. and Schwab, A.P. 1996. Organic chemicals in the environment: dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual. 25: 212-219.
52. Robson, D.B., J.D. Knight, Farrell, R.E., and Germida, J.J. 2002. (Submitted to Internat. J. Phytorem.)
53. Rogers, H.B., Beyrouty, C.A., Nichols, T.D., Wolf, D.C. and Reynolds, C.M. 1996. Selection of cold-tolerant plants for growth in soils contaminated with organics. J.Soil Contam. 5: 171-186.
54. Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L. and Carreira, L.H. 1995. Phytoremediation of organic and nutrient contaminants. Environ. Sci. Technol. 29: 318-323.
55. Schwab, A.P., Al-Assi, A.A. and Banks, M.K. 1998. Adsorption of naphthalene onto plant roots. J. Environ. Qual. 27: 220-224.
56. Schwendinger, R.B. 1968. Reclamation of soil contaminated with oil. J.Inst. Petrol. 54: 182-197.

Back
to previous page

57. Shuttleworth, K.L. and Cerniglia, C.E. 1996. Bacterial degradation of low concentrations of phenanthrene and inhibition by naphthalene. Microb. Ecol. 31: 305-317.
58. Siciliano, S.D. and Germida, J.J., 1998. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environ. Rev. 6, 65-79.
59. Simonich, S.L. and Hites, R.A. 1994a. Importance of vegetation in removing polycyclic aromatic hydrocarbons from the atmosphere. Nature. 370 : 49-51.
60. Simonich, S.L. and Hites, R.A. 1994b. Vegetation-atmosphere partitioning of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 28: 939-943.
61. Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A., 1998. Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ.
62. Walton, B.T., Hoylman, A.M., Perez, M.M., Anderson, T.A., Johnson, T.R., Guthrie, E.A., and Christman, R.F. 1994a. Rhizosphere microbial communities as a plant defense against toxic substances in soils. *In* Bioremediation Through Rhizosphere Technology. ACS Symposium Series 563. *Edited by* T.A. Anderson and J.R. Coats. American Chemical Society, Washington, DC. pp. 82-92.
63. Watkins, J.W., Sorensen, D.L. and Sims, R.C. 1994. Volatilization and mineralization of naphthalene in soil-grass microcosms. *In* Bioremediation Through Rhizosphere Technology. ACS Symposium Series 563. *Edited by* T.A. Anderson and J.R. Coats. American Chemical Society, Washington, D.C. pp. 123-131.