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Review

Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges

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ABSTRACT

Over the past few decades there has been avid interest in developing *in situ* strategies for remediation of environmental contaminants. Major foci have been on persistent organic chemicals and metals. Phytoremediation, a strategy that uses plants to degrade, stabilize, and/or remove soil contaminants, has been extensively investigated. Rhizoremediation, a specific type of phytoremediation that involves both plants and their associated rhizosphere microbes, can occur naturally, or can be actuated by deliberately introducing specific microbes. These microbes can be contaminant degraders and/or can promote plant growth under stress conditions. Because initial phytoremediation research showed great promise as a cost-effective remedial strategy, considerable effort has been devoted to making the transition from the laboratory to commercialization. Despite our understanding of the mechanisms of remediation, and the success of studies in the laboratory and greenhouse, efforts to translate phytoremediation research to the field have proven challenging. Although there have been many encouraging results in the past decade, there have also been numerous inconclusive and unsuccessful attempts at phytoremediation in the field. There is a need to critically assess why remediation in the field is not satisfactory, before negative perceptions undermine the progress that has been made with this promising remedial strategy. Two general themes have emerged in the literature: (1) Plant stress factors not present in laboratory and greenhouse studies can result in significant challenges for field applications. (2) Current methods of assessing phytoremediation may not be adequate to show that contaminant concentrations are decreasing, although in many cases active remediation may be occurring. If phytoremediation is to become an effective and viable remedial strategy, there is a need to mitigate plant stress in contaminated soils. There is also a need to establish reliable monitoring methods and evaluation criteria for remediation in the field. This review will focus on the challenges and the potential of phytoremediation, particularly rhizoremediation, of organic contaminants from soils.

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1. Introduction

An increasingly industrialized global economy over the last century has led to dramatically elevated releases of anthropogenic chemicals into the environment. Prevalent contaminants include petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons, pesticides, solvents, metals, and salt. The resulting stresses on human and ecosystem health are well documented [1–3]. Although the use of plants to remediate radionuclide-contaminated soils was explored in the 1950s, the term phytoremediation was not invoked until the 1980s, and rapid expansion in this field only began in the last decade [4]. Phytoremediation has now emerged as a promising strategy for *in situ* removal of many contaminants [5–11]. Microbe-assisted phytoremediation, including rhizoremediation, appears to be particularly effective for removal and/or degradation of organic contaminants from impacted soils, particularly when used in conjunction with appropriate agronomic techniques [12–21]. The introduction will briefly review some of the laboratory and greenhouse research that preceded implementation of microbe-assisted phytoremediation in the field. The subsequent focus of the review will be on the challenges and potential of this remedial strategy for *in situ* removal of organic chemicals from contaminated sites.

1.1. Developing phytoremediation as a remedial strategy for organic contaminants

Prior to phytoremediation field trials, extensive research was performed in laboratories and greenhouses. Some of this work explored the effects of plants on removal of contaminants from spiked soil and soil excavated from contaminated sites [13,18,19,22,23]. Many of these experiments provided valuable insights into the types and specific mechanisms of phytoremediation of organic contaminants [5,11,24]. Some organic compounds can be transported across plant membranes. Of these, the low molecular weight compounds can often be removed from the soil and released through leaves via evapotranspiration processes (phytovolatilization). Some of the non-volatile compounds can be degraded or rendered non-toxic via enzymatic modification and sequestration *in planta* (phytodegradation, phytoextraction). Other compounds are stable in the plants and can be removed along with the biomass for sequestration or incineration.

Greenhouse experiments have also been conducted with spiked and/or excavated soil to determine how contaminated soils affect plant growth [5,13,19,24–27]. These experiments allowed researchers to explore methods for overcoming contaminant stress, without the confounding effects of field-dependent variables such as weather and nutrient limitation. It has been reported that plants can have more than 100 million miles of roots per acre, which suggests great potential for phytoremediation in natural environments [28]. One problem is that high concentrations of contaminants tend to inhibit plant growth, including root growth, in part due to oxidative stress [13,19,25,27]. The resulting stress will limit the rate of phytoremediation *in situ* [18,19,26]. Contaminated soils also tend to be

nutrient poor and/or lack microbial diversity, which contributes to sub-optimal plant biomass accumulation, as well as impeded rates of remediation [13,26,29,30].

When using spiked soils for remediation experiments in the greenhouse, the focus has often been on the ability of a given plant to survive and grow in the presence of a specific compound and/or to remediate it. However, soils at contaminated sites generally contain complex mixtures of chemicals, and often include both organic and inorganic components. In spiked soils, chemicals tend to be bioavailable, whereas contaminants in naturally weathered soils are often not readily bioavailable. For instance, germination and plant growth of seven plant species was assessed in soil spiked with a pure PAH mixture, soil spiked with coal tar, and weathered soil from a former coking plant [31]. These conditions led to significantly different results, which highlighted the need to perform greenhouse experiments with soils collected from contaminated sites before implementing a field-level remediation.

Concomitant with phytoremediation garnering widespread interest, the field of microbial bioremediation has also been expanding [32–36]. Contaminant-degrading microbes have been isolated from impacted soils and characterized [14,23,36], and it is postulated that contaminant-degrading bacteria can be found in virtually all soils [15]. Mechanistic studies using these microbial isolates have been performed on spiked and field-isolated soils [13,35,37]. Following isolation and characterization of contaminant-degrading microbes, attempts were made to inoculate contaminated field soils with the isolates; however, this remedial strategy has proven to be largely unsuccessful [11,14,34,35]. There are several potential reasons for this general lack of success [14,38]. These include the inability of introduced microbes to compete with existing microflora and microfauna in the soil environment; the inability of the microbes to grow to sufficient depths to reach sub-surface contaminants; insufficient nutrients in contaminated soils to support microbial growth; low bioavailability of contaminants; preferential utilization of carbon compounds other than the contaminant of interest; and the presence of toxicants at the site that inhibit microbial growth. One way to increase the potential of microbial remediation is to add natural analogues of contaminants to soil (analogue enrichment), which can stimulate bioremediation by inducing degradative pathways [39,40].

A convergence of phytoremediation and microbial bioremediation strategies led to a more successful approach to remediation of contaminants, particularly organic compounds. Microbe-assisted phytoremediation, with both naturally occurring microbes and deliberately stimulated via seed inoculation, has been investigated in the laboratory, greenhouse and field [10,13–15,18,19,22,41]. A variety of contaminant-degrading enzymes can be found in plants, fungi, endophytic bacteria and root-colonizing bacteria. These include peroxidases, dioxygenases, P450 monooxygenases, laccases, phosphatases, dehalogenases, nitrilases, and nitroreductases [5,13,15,42–58] (Table 1). Although there are some organisms that can completely degrade a specific organic contaminant (e.g., *Sphingobium chlorophenolicum* strain ATCC 39723 can mineralize pentachlorophenol [47,59]), individual species generally do not contain entire degradation pathways.

Table 1
Plant and microbial enzymes with a role in degradation of organic compounds. Microbial sources are designated (B) for bacterium, or (F) for fungus. All fungi except for *Aspergillus* are white rot fungi.

Enzyme family	Catalytic action	Examples of known sources
Various plant enzymes for uptake, transport, sequestration and degradation	General uptake and degradation	All plants [11,45]
Dehalogenase	Hydrolyzes chlorine and fluorine from halogenated aliphatic hydrocarbons (e.g., trichloroethylene) and aromatic hydrocarbons (e.g., PCBs, DDT)	<i>Xanthobacter autotrophicus</i> (B) [46] Hybrid poplar (<i>Populus</i> spp.) [5] <i>Sphingobium chlorophenolicum</i> (B) [47]
Laccase	Degradation of various aromatic compounds	Alfalfa (<i>Medicago sativa</i>) [48] <i>Trametes versicolor</i> (F) [49] <i>Coriolopsis polyzona</i> (F) [49]
Dioxygenase	Degradation of various aromatic compounds	<i>Pseudomonas</i> sp. (B) [50] <i>Mycobacterium</i> sp. (B) [50]
Peroxidase	Degradation of various aromatic compounds; reductive dehalogenation of aliphatic hydrocarbons	Horseradish (<i>Armoracia rusticana</i>) [5] <i>Phanerochaete chrysosporidium</i> (F) [49,51,52] <i>Phanerochaete laevis</i> (F) [53] Alfalfa (<i>Medicago sativa</i>) [48]
Nitrilase	Cleaves cyanide groups from aromatic and aliphatic nitriles	Willow (<i>Salix</i> spp.) [5] <i>Aspergillus niger</i> (F) [54]
Nitroreductase	Reduces nitro groups on nitroaromatic compounds (e.g., 2,4,6-trinitrotoluene); removes N from ring structures	<i>Comamonas</i> sp. (B) [55] <i>Pseudomonas putida</i> (B) [56] Hybrid poplar (<i>Populus</i> spp.) [5]
Phosphatase	Cleaves phosphate groups from organophosphates (e.g., pesticides)	Giant duckweed (<i>Spirodela polyrhiza</i>) [5]
Cytochrome P450 monooxygenase	Hydroxylation of aromatic and aliphatic hydrocarbons	Most aerobic bacteria, all fungi and all plants [45,57,58]

However, microbial consortia in the rhizosphere can work in tandem to effectively degrade these compounds [7,13,14,60].

One type of microbe-assisted phytoremediation is rhizoremediation, defined as degradation of contaminants in the rhizosphere. Rhizoremediation is emerging as one of the most effective means by which plants can effect the remediation of organic contaminants, particularly large recalcitrant compounds. In this case, complex interactions involving roots, root exudates, rhizosphere soil and microbes result in degradation of organics to non-toxic, or less-toxic, compounds. As much as 40% of a plant's photosynthate can be deposited in the soil as sugars, organic acids, and larger organic compounds [61]. These compounds are commonly used as carbon and energy sources by soil microbes [13,15,29,62]. On a per gram basis, rhizosphere soil has 10–100 times more microbes than unvegetated soil [63]. In soil containing large volumes of roots, microbial populations can reach titres of $\sim 10^{12}$ cells/g of soil [64]. This microbial consortia can provide various benefits to plants, including the synthesis of compounds that protect the plants by decreasing plant stress hormone levels; chelators for delivering key plant nutrients; protection against plant pathogens; and degradation of contaminants before they can negatively impact the plants [7,13,43,55,65,66].

The successful application of rhizoremediation is largely dependent on the capacity of contaminant degraders or plant growth promoting microbes to efficiently colonize growing roots [67]. Numerous bacterial traits, involving a multitude of genes, are required for effective root colonization [14,67–71]. These include production of thiamine and biotin, synthesis of the O-antigen of lipopolysaccharide, amino acid synthesis, and an efflux pump induced by isoflavonoids. The importance of microbial motility has been demonstrated using aflagellate mutants and rhizobacteria with diminished motility relative to wild-type colonizers [71]. Chemotaxis toward specific root exudate compounds is a key factor in efficient root colonization, although the chemotactic response can be elicited by different compounds depending on the colonizing species [69,72].

One important group of plant compounds in root colonization are complex aromatic compounds such as flavonoids and coumarins. Notably, there is little accumulation of these compounds in soil, because they are consumed by soil microflora that degrade them and use the reduced carbon and nitrogen [13,15,29,61,62]. It is fortuitous that these aromatic plant compounds are structurally similar to many organic contaminants such as polychlorinated biphenyls (PCBs), PAHs, and PHC, thereby providing a means to exploit natural processes in the rhizosphere for the remediation of contaminants [40].

One reason rhizoremediation occurs naturally is because flavonoids and other compounds released by roots can stimulate growth and activity of PCB and PAH degrading bacteria [13,26,62,73–75]. Furthermore, root growth and death promotes soil aeration, which can enhance oxidative degradation of recalcitrant organic compounds [14,62]. Notably, some plant species appear to increase the numbers of degradative microbes in a large volume of soil that extends beyond the rhizosphere [29,75]. Although rhizoremediation occurs naturally, it can also be optimized, by deliberate manipulation of the rhizosphere. This can be accomplished by using suitable plant–microbe pairs. These can be either combinations of plants and plant growth promoting rhizobacteria (PGPR), or combinations of plants and contaminant-degrading microbes. For example, a grass species combined with a naphthalene degrading microbe protected the grass seed from the toxic effects of naphthalene, and the growing roots propelled the naphthalene degrading bacteria into soil that would have been too deep to penetrate in the absence of roots [14]. Some of the main processes involved in rhizodegradation of PHC are summarized in Fig. 1. For more comprehensive discussions of the biological, chemical, biochemical and physical processes involved in phytoremediation and rhizoremediation of organic contaminants, the reader is directed to reviews of these processes [11,33,42,44,61,74,77,79].

Because remediation with transgenic organisms is largely untested in the field, this topic is not covered in great detail in this review. Nonetheless, the potential for using transgenic

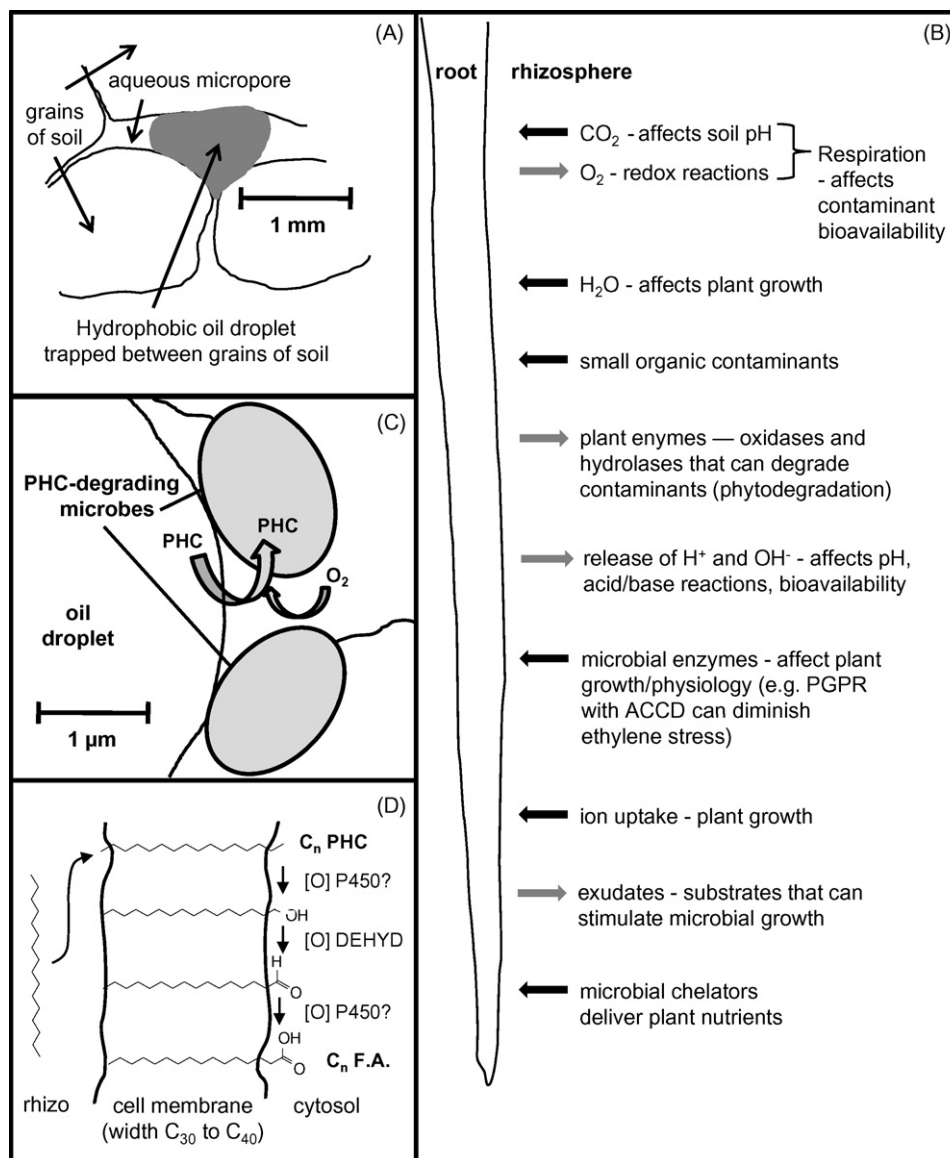


Fig. 1. Rhizoremediation of PHC. (A) *Bioavailability of PHC*: hydrophobic oil droplets are bound to soil particles or physically trapped in micropores and are not always easily bioavailable in bulk soil. Bioavailability depends on complex interactions between chemical, biochemical, physical, and environmental parameters in the microenvironment [8,9,11,76,77]. (B) *General processes affecting rhizoremediation*: plant roots support microbial growth at the root surface and in the rhizosphere. Roots create channels in soil that allow for movement of O_2 and H_2O , and that are wide enough for “trapped” contaminants to become accessible to microbes [26]. PGPR, plant growth promoting rhizobacteria; ACCD, 1-aminocyclopropane-1-carboxylate deaminase. (C) *Aerobic PHC degradation*: at the PHC–water interface, microbes use adhesion methods and/or biosurfactants [14,33,78]. The microbial surface has membrane-bound oxygenases used for the first step in degradation. The first steps of the degradative pathway incorporate two O atoms into the PHC to form fatty acid analogues. These microbes then grow and multiply at the surface. Any given microbe can only degrade part of the petroleum (i.e., some PHC components of the complex mixture) and theoretically, it takes 150 mg N and 30 mg P for microbes to convert 1 g PHC to microbial biomass [33]. As the petroleum droplet is degraded, different microbes continue the degradation process. (D) *Possible microbial oxygenation pathway of PHC to form a fatty acid*: rhizo, rhizosphere; P450, cytochrome P450; DEHYD, dehydrogenase; F.A., fatty acid.

plants and/or microbes to remediate organic contaminants has been extensively explored in the laboratory (Table 2) [34,36, 42,80–88]. Some examples include introducing genes encoding the biosynthetic pathway of biosurfactants to increase bioavailability of contaminants for microbes in the rhizosphere; adding genes to microbes or plants to enhance resistance to contaminant or environmental stressors; engineering plants and microbes that overexpress genes encoding enzymes involved in degradation pathways (e.g., cytochrome P450 genes); using plants engineered to release specific exudates that can induce degradative pathways in rhizomicrobia; and engineering plants with increased capacity for uptake, transport and sequestration of contaminants.

2. Potential for field applications

2.1. Advantages of phytoremediation and rhizoremediation

There are many advantages of phytoremediation and rhizoremediation that other remedial strategies do not provide [5,11,13,89]. Conventional *ex situ* methods, such as excavation and incineration, off-site storage, soil washing, and *in situ* capping for stabilization are generally much more expensive than *in situ* phytoremediation and rhizoremediation. The estimated cost of land filling or incineration of a ton of soil is between \$200–\$1500, significantly higher than the \$10–\$50/ton estimate for rhizoremediation [89,90]. With increasing fuel costs, the disparity is

Table 2

Examples for phytoremediation and rhizoremediation of organic contaminants using genetically modified organisms. Most of this information is from laboratory and greenhouse experiments. Plant experiments were performed hydroponically, unless otherwise noted. None of the transgenic species were field tested.

Transgenic species	Gene source	Gene(s)	Enzyme(s)	Organic contaminant	Results	References
Potato (<i>Solanum tuberosum</i>)	Rat	<i>CYP1A1</i>	Cytochrome P450 monooxygenase	Herbicides	Increased tolerance to atrazine and chlortoluron, assumed to be via metabolism to less-toxic derivatives	[80]
Tobacco (<i>Nicotiana tabacum</i>)	Human	<i>CYP2E1</i>	Cytochrome P450 monooxygenase	Halogenated hydrocarbons	Dramatically enhanced metabolism of trichloroethylene; increased uptake and debromination of ethylene dibromide	[81]
Hybrid poplar (<i>Populus tremula</i> × <i>Populus alba</i>)	Rabbit	<i>CYP2E1</i>	Cytochrome P450 monooxygenase	Volatile hydrocarbons	Enhanced removal and degradation of trichloroethylene, vinyl chloride, carbon tetrachloride, benzene and chloroform; enhanced removal of gaseous trichloroethylene, chloroform and benzene	[82]
Rice (<i>Oryza sativa</i>)	Human	<i>CYP1A1</i>	Cytochrome P450 monooxygenase	Herbicides	Enhanced metabolism of atrazine, norflurazon and chlortoluron (should also metabolize PAHs)	[83]
Rice (<i>O. sativa</i>)	Human	<i>CYP1A1</i> , <i>CYP2B6</i> , <i>CYP2C19</i>	Cytochrome P450 monooxygenases	herbicides	Enhanced metabolism of atrazine, norflurazon and metol achlor from soil (should also metabolize PAHs)	[84]
Tobacco (<i>N. tabacum</i>)	<i>Enterobacter cloacae</i>	<i>onr</i>	Pentaerythritol tetranitrate reductase	Explosives	Enhanced denitration of glycerol trinitrate	[85]
<i>Pseudomonas fluorescens</i>	<i>Burkholderia</i> sp.	<i>bph</i> operon	Suite of enzymes for the complete PCB degradation pathway	PCBs	Enhanced rate of degradation of numerous PCBs (resting cell assays)	[86]
<i>P. fluorescens</i> (psychrotolerant strain)	<i>Burkholderia</i> sp.	<i>dnt</i> genes	Suite of enzymes for degradation of 2,4-dinitrotoluene to pyruvate and propionyl-CoA	2,4-Dinitrotoluene	Complete degradation of 2,4-dinitrotoluene as a co-substrate at temperatures as low as 10 °C	[87]
<i>P. fluorescens</i>	<i>Burkholderia cepacia</i>	<i>tomA</i>	Toluene- <i>o</i> -monooxygenase	Trichloroethylene	Sixty-three percent degradation of trichloroethylene after four days in wheat rhizosphere	[88]

certain to increase. Protocols are relatively easy to implement, and after initial site preparation and planting, the maintenance costs for phytoremediation and rhizoremediation are minimal. An additional benefit to *in situ* phytoremediation and rhizoremediation is that organic materials, nutrients and oxygen are added to soil via plant and microbial metabolic processes. This improves the overall quality and texture of soil at remediated sites. Plants also provide groundcover, and their roots help to stabilize soil, which mitigates erosion from both wind and water. There is no size restriction for sites utilizing phytoremediation, and this strategy can be employed in any geographical area that can support plant growth. An additional advantage, albeit an unscientific one, is that there is high public acceptance for phytoremediation, which makes it an attractive option for industry and regulators.

2.2. Promising results from the field

Although the successes of laboratory and greenhouse experiments are rarely realized to the same extent in the field, there have been encouraging results that justify continued use of phytoremediation. For example, phytoremediation over a two year period decreased the total PHC concentration by 30%, which was double that of non-vegetated soils at a highly contaminated site [29]. A field study conducted on a site contaminated by a crude oil spill showed a 42% decrease in total PHC concentration using ryegrass (*Lolium annual*), and a 50% decrease using St. Augustine grass (*Stenotaphrum secundatum*), after 21 months [91]. At a site impacted by a crude oil spill, three years of phytoremediation with a combination of grasses and fertilizer led to a decrease in PAH concentrations, including some of the recalcitrant components [30]. In soil contaminated with aged creosote, degradation of the PAHs acenaphthene, fluorene, fluoranthene, pyrene and chrysene occurred in the rhizosphere of

tall fescue over a three year period [92]. Relative to unvegetated plots, tall fescue enhanced the degradation of most PAHs tested. In a 60 day trial, 96% of 2,4,6-trinitrotoluene was removed from a test plot by maize (*Zea mays*) [38].

3. Challenges

3.1. Stressors and physical restrictions

Although there is increasing interest in *in situ* remediation of contaminants, the number of papers pertaining to phytoremediation appears to be declining, suggesting a decrease in the activity for this area of research [93]. Numerous reports of unsuccessful and inconclusive field trials provide some insight into the most prevalent challenges that have been encountered [26,94,95]. A frequently cited disadvantage of phytoremediation, compared to strategies such as excavation and *ex situ* treatment, is that the rate of remediation is much slower. In part, this is because plant growth is hard to achieve in heavily impacted soils, thereby limiting catabolism of contaminants [13,19,25,27]. Another challenge is that there are stressors that affect phytoremediation in the field that are not encountered in the laboratory or greenhouse. These include variations in temperature, nutrients and precipitation; herbivory (insects and/or animals); plant pathogens; competition by weed species that are better adapted to the site [91]. Any of these abiotic or biotic stressors can diminish or prevent plant growth in the field, and this will negatively impact on phytoremediation. For example, attempted phytoremediation of PHC and other organics at a hydrocarbon burn facility (part of a fire-fighting training facility) failed due to drought and competition from weeds [96]. Although weed species can also remediate organic compounds, excessive competition from weeds is usually viewed as a

project failure and uptake and/or degradation of contaminants by weeds were not investigated or reported in this case.

In addition to contaminant and environmental stressors in the field, there are physical challenges that limit the use of phytoremediation. For instance, increasing the moisture content of hydrophobic PHC-contaminated soils can be problematic once they have become dry, which can prevent germination and seedling growth. There are also challenges when the contaminated soil is deeper than the rooting zone; e.g., average root depth of herbaceous plants is ~50 cm [11]. This necessitates excavation prior to phytoremediation. Trees have deeper roots (average ~3 m, with much longer roots in some species) which can facilitate remediation at greater depths without excavation [11,13]. Dendroremediation (phytoremediation using trees) of explosives (e.g., 2,4,6-trinitrotoluene) and trichloroethylene from soil and groundwater has shown great promise [1,5,97]. The challenge is that it can be difficult to establish trees in contaminated soils, and once established, they take several years to attain sufficient biomass for efficient rates of phytoremediation [98]. Further, when remediation is complete, there may be a disposal problem if the roots and wood are deemed to be contaminated.

3.2. Complexity of phytoremediation in the field

A challenge frequently encountered in field studies is uneven distribution of contaminants, including “hot spots”, across a site [26,38,91,95]. In laboratory and greenhouse experiments, soils are generally well mixed, to achieve a uniform matrix. This may not be possible in the field, even if the site is extensively tilled prior to planting. The spatial heterogeneity of initial contaminant levels results in data scatter, which can make it difficult to statistically show significant treatment effects for field trials. This is also problematic in terms of regulatory standards because remediation success is often judged on a point-by-point basis rather than an average of data points from across the site. If one point fails to meet the regulatory targets, the whole site fails [99,100].

In addition to contaminant concentration, factors such as root structure, soil structure, organic composition of the soil, soil pH, moisture content and microbial activity often exhibit spatial variability at a given site, and can change over time [91,101]. For example, water added to hydrophobic PHC-contaminated soil can lack uniform distribution, leading to uneven moisture content across the site [26,32]. This can affect plant and microbial growth, and therefore, remediation potential. One way to mitigate the problem of spatial variability is to increase the number of samples analyzed per treatment plot. However, because most field studies have constraints including time and resources, the required extensive sampling is often not a realistic option [91]. This can lead to highly variable data that are difficult to interpret.

Agronomic techniques, such as tilling and addition of nutrients and organic matter, are often employed prior to planting [30,74]. These practices are generally designed to improve soil texture and composition to facilitate plant and/or microbial growth. They can, however, cause changes in soil pH and oxygen content, which can affect the bioavailability, and hence degradation, of contaminants even in bulk (non-rhizosphere) soil. Nutrients in the bulk soil, such as nitrogen and phosphorus, will positively affect the indigenous microbial populations, including contaminant degraders [74]. Aeration of field soil increases the soil oxygen concentration, which promotes oxidative degradation of numerous organic contaminants [18,19]. Photo-oxidation of previously buried organics can also occur. This explains why there is often a decrease in contaminant concentrations in bulk soil (generally used as the negative control) as well as in treatment plots. This can further confound attempts to statistically show that phytoremediation is superior to natural attenuation, particularly in the initial stages of a field trial.

The complexity of the rhizosphere in terms of biological interactions, geometry and temporal/spatial heterogeneity has been examined [79,102]. Determining the boundary between rhizosphere and bulk soil is almost impossible. Furthermore, it is impractical to definitively separate fine roots, soil directly in contact with the roots, and rhizosphere soil into different samples for analyses. This can present an analytical challenge for researchers endeavoring to show PHC and PCB remediation because rhizoremediation of these compounds is thought to occur at the root–soil boundary (rhizoplane) [26,74]. Less remediation is thought to occur in the rhizosphere (~2 mm zone extending from the roots), and much less in bulk soil.

There is evidence that some plants can provide the impetus for movement of hydrophobic organics such as PAHs from bulk soil into the rhizosphere [22]. Mobilization of PAHs may be accelerated by the release of organic acids from roots, which putatively increases PAH solubility and bioavailability. Furthermore, lipophilic contaminants such as PAHs adsorb strongly to roots [73,103]. This effect is more pronounced with increasing plant age [103]. If a soil sample contains root material, an increase in PAHs may be detected, even if total PAH concentration is decreasing in the soil [22]. In this case, one would expect a decrease in PAH concentration in bulk soil. That is, as contaminants migrate to plant roots, there will be an apparent increase in concentration in the rhizosphere, even if degradation is actively occurring in the root zone. From the above, it can be concluded that a multitude of variables can contribute to ambiguous field results.

3.3. Regulatory acceptability

The issue of regulatory acceptability for phytoremediation of hazardous waste has been reviewed [104]. The primary obligations of government regulators are to ensure public safety and to protect the environment. They must be convinced that a given remedial strategy will diminish contaminant toxicity, mobility and/or concentration before it is approved for use in the field. Bioaccumulation of hazardous compounds in plants is one consideration. Provisions may be required for removal of contaminated plant materials as part of a remedial plan when the plants do not catabolize the contaminants. Another consideration is the potential ecological risks of introducing non-native plant and microbial species into field sites [11,105,106]. These species can spread from the contaminated site, displacing, or hybridizing with, native species.

Phytoremediation of organic compounds often takes place in the rhizosphere of fine roots that have a high turnover rate. These roots do not typically survive a full growing season and the decaying root tissue is converted into bulk soil during humification [62,74]. Organic matter can also be introduced to a site from other debris from the plants used for phytoremediation, as well as peat moss, animal manure, woodchips/sawdust and biosolids, which are sometimes added to the soil prior to planting. These sources of organic material can be modified to form soil organic matter. This is desirable, as it improves soil quality at sites that usually consist of highly degraded soil. Hydrocarbons can also be contributed by microbial populations. For example, diagenesis of bacteriophenacetrol, a widely occurring component of prokaryotic bacterial membranes, can result in the formation of a numerous hopanoic acids and hydrocarbons, including hopane [107]. Unfortunately, contributions of organic matter from these sources can obfuscate sample analyses because of the structural similarities to compounds being remediated. For example, in the case of PHC remediation, currently accepted analytical protocols such as those outlined by the United States Environmental Protection Agency and Canadian Council of Ministers of the Environment (CCME) do not distinguish between petrogenic and phytogenic carbon

compounds [108,109]. This means that naturally occurring organic matter that is co-extracted with organic contaminants can easily lead to overestimation of PHC levels in soil samples.

3.4. Use of genetically modified organisms in the field

Although promising results have been obtained using genetically modified organisms in the laboratory and greenhouse, regulatory restrictions for *in situ* applications have prevented any substantial accumulation of field data [42,89]. If genetically modified organisms are used without adequate containment systems, there is the potential for inserted genetic material to be transferred to indigenous populations. As well, recombinant strains that contain antibiotic resistance genes from the cloning procedures cannot be released in the environment [86]. There has also been low public acceptance of genetic engineering, particularly in Europe [110]. Although it has been postulated that public opposition to the use of genetically modified organisms is based on irrational fear rather than scientific evidence, regulatory bodies often defer to public opinion when formulating policies [111–113].

Even if the problems of regulatory restrictions and public acceptance can be overcome, there are challenges that are likely to hinder the application of genetically modified organisms for phytoremediation and rhizoremediation in the field. Genetically engineered microbial strains often fail to compete with native microbes in the rhizosphere, and their numbers dwindle to levels that cannot effectively support remediation [7,114]. Degradation of organic contaminants generally requires the concerted action of numerous enzymes and it is generally impractical to introduce all the genes required for degradation of an organic contaminant into a single plant or microbial genome [7]. It can be difficult to stably maintain even a single gene in transformed or recombinant organisms and the desired trait, such as enhanced degradative capacity, is often lost [113,115]. In plants, silencing of transgenes, via mechanisms such as cytosine methylation, make the use of this technology inherently unpredictable [116].

4. Overcoming the challenges

4.1. Strategies for minimizing ecological risk

One of the best ways to minimize the ecological risks and regulatory problems associated with introducing non-native biota (including genetically modified species) into an ecosystem is to use native species for phytoremediation [21]. This not only includes plant species, but also microbial species that are used to facilitate plant growth and/or degrade contaminants. Using native plant species can serve a dual purpose of remediation and native habitat reconstruction/reclamation, which may be required following successful remediation [3,117]. In addition to the perceived ecological advantages, this also appears to be one of the best strategies for microbe-assisted phytoremediation because native species are already adapted to the conditions at a given site [21].

To minimize ecological risks from non-native (transgenic and non-transgenic) phytoremediation species, it is often necessary to employ a biological containment system [105,106]. For example, genes can be introduced to prevent propagation, or to render a species overly sensitive to abiotic stressors such as temperature changes or chemicals [105,118]. Ideally, multiple transgenes are employed to prevent gene flow between the introduced species and other species in the environment. To reinforce the containment system, mitigator genes linked to the primary transgene can be added [105]. Mitigator genes confer non-deleterious traits to the phytoremediation species, but are harmful to related species should gene transfer occur. Alternatively, they can prevent the

phytoremediation species from successfully competing outside the contaminated site. An effective mitigator gene for use in plants is one that results in overexpression of a cytokinin oxidase [119]. Overproduction of this enzyme decreases levels of isopentenyl- and zeatin-type cytokinins, which leads to significant decreases in shoot biomass and increases in root biomass [120]. This has the dual benefit of rendering the plant unfit to compete with wild-type plants, while enhancing phytoremediation potential. One example for containment of microbial strains is the introduction of a killing gene such as porin-inducing protein in conjunction with a repressor for the lethal function [106]. Expression of the killing gene is dependent upon the presence or absence of a given contaminant in the environment. To reinforce containment using this system, the microbes can be genetically engineered such that expression of a repressor and synthesis of an essential metabolite are under the control of the same promoter [106]. In the absence of the contaminant, the killing protein is synthesized and synthesis of a key metabolite ceases, leading to death of the introduced microbial strain.

4.2. Strategies for diminishing stress that limits plant growth in the field

Successful phytoremediation is dependent upon high root biomass production. Plants growing in contaminated soils often have to cope with the combined stress of nutrient deficiency and chemical toxicity. Biosynthesis of stress ethylene can ensue, leading to plant growth inhibition and diminished plant biomass (especially for roots) [121]. A successful strategy for overcoming the challenge of plant stress in phytoremediation is to use plant growth promoting rhizobacteria that express 1-aminocyclopropane-1-carboxylic acid deaminase [16,17]. This enzyme hydrolyzes 1-aminocyclopropane-1-carboxylic acid, the immediate precursor to ethylene in plants, thereby lowering the rate of ethylene biosynthesis [122]. Importantly, PGPR only lower the level of deleterious stress ethylene, without affecting the small burst of ethylene produced at the onset of stress, which is believed to activate key plant defense responses [121]. Consequently, PGPR can enhance germination and plant growth rates under stress conditions, particularly when used in conjunction with contaminant-tolerant plant species [16,17,121]. Notably, PGPR promote rapid accumulation of root biomass that can provide a sink for soil contaminants [16,18,19,23,25]. Many of these rhizobacteria (e.g., *Pseudomonas* spp.) can act both as plant growth promoters and contaminant degraders [42,65,123]. PGPR can also act as biocontrol agents, by mitigating the effects of pathogenic organisms [124–126]; or they can act as biofertilizers that directly, or indirectly, result in an increase in nutrient uptake by plants [127]. Thus, it is likely that PGPR could contribute to phytoremediation processes via multiple modes of action.

PGPR-enhanced phytoremediation systems have been developed that appear to overcome the challenge of plant stress in contaminated soils [18,19,25,128,129]. Using grass and grain seeds (*Festuca arundinacea*, *Lolium multiflorum*, *Secale cereal*, *Pennisetum glaucum*, *Hordeum vulgare*, *Poa pratensis*, and *Elymus canadensis*) treated with various naturally occurring, non-pathogenic *Pseudomonas* strains, soils containing organic compounds have been successfully remediated in the greenhouse and in the field. Although phytoremediation was observed in the absence of PGPR seed treatments, the addition of PGPR consistently enhanced remediation rates. In the greenhouse, 86% of creosote in spiked soil (2 g/kg soil) and 50% of PAHs from an industrial brownfield soil (500 mg/kg soil) were removed during eight and four month growth periods, respectively [18,129]. Over two four month growth periods, over 90% of PHC (50 g/kg soil) were removed from weathered soil contaminated with oil sludge [19]. Compounds remediated in the

Table 3

PHC remediation in field experiments using PGPR-enhanced phytoremediation. The oil refinery land farm site had repeated applications of petroleum sludge over a period of 20 years (PHC \cong 15%). The constructed biopile contained soil with low levels of PHC (\sim 1%) that had been moved to this location for treatment. Values indicate percent remediation, which is the decrease in the levels of PHC in soils remediated for 120 days with PGPR-enhanced phytoremediation, relative to samples taken at the onset of the season for each year. AR, annual ryegrass; TF, tall fescue; and B, barley. Values are \pm S.E.; $n \geq 10$.

Year	Landfarm	Constructed biopile	Former oil well
2004	44 \pm 5 (AR)	N/A	N/A
2005	35 \pm 6 (AR/TF)	35 \pm 2 (AR/TF)	N/A
2006	22 \pm 3 (AR/TF/B)	26 \pm 1 (AR/TF)	N/A
2007	16 \pm 2 (AR/TF/B)	19 \pm 3 (AR/TF)	35 \pm 2 (AR/TF)

greenhouse experiments included highly toxic and recalcitrant PAHs such as chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(ah)pyrene, benzo(ghi)perylene, indo(123-cd)pyrene and hydrophobic, recalcitrant PHC from CCME fraction 3 (compounds containing 16–34 carbons; often highly toxic) and fraction 4 (compounds containing 34–50 carbons) [18,19,129]. Notably, PAHs and PHC were degraded in the soil, which eliminates the need to dispose of plants after field experiments. After developing this system in the laboratory and greenhouse, field trials were performed. Similar results were observed in the field (Table 3) [20,128].

In contrast to root-colonizing microbes, endophytes colonize internal plant tissues, including root, leaf and vascular tissues [113]. Inoculating plants with endophytes can circumvent some of the challenges that limit effectiveness of root colonizers, including dependence on specific soil pH, temperature and soil moisture content for optimum growth, and competitive pressures in the rhizosphere. Naturally occurring endophytic bacteria have been found to degrade organic contaminants such as nitro-substituted explosives [130]. A genetically modified endophytic strain of *Burkholderia cepacia* with the ability to degrade toluene was constructed by transferring the plasmid pTOM from a root-colonizing strain of the same species. The endophyte not only diminished toluene phytotoxicity in its host, yellow lupine (*Lupinus luteus*), but also decreased the amount of toluene released via evapotranspiration [131]. When hybrid poplars (*Populus trichocarpa* \times *deltoides*) were inoculated with another toluene-degrading endophytic strain of *B. cepacia*, similar results were observed [132]. Notably, this endophyte did not become established *in planta*. Rather, the plasmid coding for toluene degradation was transferred to various endogenous endophytes, regardless of whether or not toluene was present in the environment. Although the use of endophytes holds great promise for degrading contaminants and mitigating plant stress, field trials will be required to assess the effectiveness of this approach *in situ*, and they will be subject to most of the challenges outlined in the previous section.

4.3. Improved methods for monitoring, sampling and analyzing experimental data from the field

With greater interest in using phytoremediation in the field, the need to regulate and assess remedial success came to the fore in the 1990s [104]. There have been attempts by government agencies to address the inconsistencies in experimental protocols, particularly regarding which analytical parameters need to be measured to demonstrate adequate performance of remediation systems in the field. An increasing number of researchers involved in phytoremediation of soil PHC are employing protocols developed by the Remediation Technologies Development Forum, a group with government, industry and academic partners [133].

Their intent was to develop protocols that would allow comparative tests of remedial strategies at numerous and varied geographical locations. These protocols, developed in the 1990s, recommend standards for plot size; number of replications; choice of plant species; plant and soil sampling procedures; microbial and hydrocarbon analyses; statistical treatment of data; time-points and end-point (three years). For example, the protocols recommend eight random sub-samples per plot (minimum plot size of 6 m²), combined into one composite sample for analysis, to help mitigate the effects of spatial heterogeneity at field sites [133].

Given the variability at field sites, evaluating data using averages of similar treatments can actually mask positive results. In one case, a slight difference in PHC concentration was observed between plots at one end of a field site, compared to the other end [134]. Averaging numerous data points obscures changes that occurred in individual plots. Significant variability (50% of the variation in a given field) often occurs within a 1 m radius (i.e., smaller than the average plot size) [101]. Thus, in addition to using composite samples for analyses, it may be useful to view the data as changes in contaminant concentration at approximately the same location in a given plot over time. That is, if one is sampling 20 points across a field at each time interval, one should compare remediation over time at each point as well as in the average across the site. As well, appropriate use of trends in temporal data can sometimes overcome statistical problems due to data scatter. For contaminants such as PCBs that can be phytoextracted, another approach has been proposed [135]. Rather than attempting to quantify decreases in soil contamination at the end of a growing season, assessing total plant uptake of the contaminant could be used as a measure of success in the field. Another consideration is the acceptable level of significance for statistical analyses of field data. To demonstrate a statistically significant effect of phytoremediation, the accepted standard is a value of 5% ($P \leq 0.05$). However, inherent variability is greater in field studies than in laboratory and greenhouse experiments. Thus, it may be useful for members of the scientific community, industry, and regulatory bodies to establish a value of 10% ($P \leq 0.1$) as the acceptable level of significance.

In the Remediation Technologies Development Forum protocols, a three year end-point is considered more realistic than expecting full, or even significant, remediation in a single growing season [133]. Establishing a longer time frame for field trials is advantageous for researchers because ambiguous results are often observed in short term field studies as a result of tilling and amending the soil at the onset of a field trial. The three year time frame also allows for assessment of the phytoremediation system and improvement of methods between field seasons. If problems in the field are encountered, laboratory and greenhouse experiments can be conducted to resolve the issues prior to the onset of the next field season.

One way to facilitate statistical analysis of field data is to use particularly recalcitrant compounds in the soil samples to normalize the data [37]. These internal markers (also called “conservative biomarkers”) are not generally degraded to any appreciable degree during phytoremediation. Concentrations of individual contaminants can be directly compared (normalized) to the internal marker. The relative ratio of a specific compound to the internal marker decreases as remediation occurs. Hopanes, compounds found in crude oil, can be used as biomarkers for PHC remediation, including PAHs [30,134]. Although using conservative biomarkers may be beneficial in some experimental systems, uncritical use of these compounds for normalization can be problematic because they can be degraded under certain conditions. For example, enhanced degradation of hopane has been observed in the presence of microbial cultures, and some

phytoremediation systems degrade highly recalcitrant hydrocarbons, which would prevent their use for normalization, or could lead to significant underestimates of remediation [18,19,37]. Because hopane has been found to degrade during phytoremediation, more recalcitrant compounds may be better to use (e.g., dimethyl chrysene).

Although methods to distinguish biologically derived organic compounds from organic contaminants are not widely employed in the field of phytoremediation, they are used for chemical fingerprinting to assess liability after accidental release of chemicals into the environment [136]. In this case, it becomes necessary to distinguish contamination from naturally occurring and anthropogenic hydrocarbons (i.e., background hydrocarbons). A number of hydrocarbon indices have been derived to assess the source of environmental hydrocarbons, including the carbon preference index, average chain length and various *n*-alkane/acyclic isoprenoid ratios [137]. These methods can also be used to distinguish plant-derived hydrocarbons from petrogenic hydrocarbons for assessing phytoremediation. For example, an abundance of odd-numbered *n*-alkane peaks in the range of C₂₅–C₃₁ are indicative of epicuticular waxes derived from leaf cuticles, whereas petroleum is comprised mainly of even-numbered carbon compounds [107]. The carbon preference index can be used to indicate the ratio of odd-numbered to even-numbered carbon compounds, thus indicating the fraction of phytogenic compounds in a sample. Biodegraded or uncombusted petroleum will often appear as a prominent unresolved complex mixture, containing C₁₇–C₃₅ compounds [107,137]. The distribution of *n*-alkanes tends towards decreasing abundance with increasing carbon number for petrogenic carbons (C₂₅ > C₂₇ > C₂₉ > C₃₁), with the reverse trend for phytogenic hydrocarbons [137].

Unfortunately, the methods to distinguish between the different sources of hydrocarbons involve sophisticated analyses, such as gas chromatography–mass spectrometry (GC–MS), that can be cost prohibitive. However, if these methodologies become adopted, they would likely be routinely employed at private analytical laboratories. Once established as standard protocols, economy of scale should considerably bring down the cost per sample. Furthermore, once new protocols are established, not all field samples would require GC–MS analyses: several samples from each time interval could be used to establish background PHC and normalize the rest of the data.

5. Conclusions

Although there is a need to be cautiously optimistic regarding widespread application of phytoremediation, and the limits of this remedial strategy need to be acknowledged, one should not be discouraged by the challenges that have been encountered while trying to bring this technology into common practice. Progress has been made in terms of overcoming some of the challenges, especially overcoming plant stress in the field. Rhizoremediation will continue to be adapted and improved by performing greenhouse and lab experiments in conjunction with field work to address problems that are currently being encountered. Establishing new protocols for sampling, chemical analysis, and interpretation of the data will be necessary to show effectiveness of phytoremediation and rhizoremediation. In particular, developing cost-effective methods to distinguish between petrogenic and phytogenic carbon compounds could revolutionize acceptability of this technology, and motivate regulators to change the guidelines for remediation of organic contaminants. Phytoremediation, and particularly rhizoremediation, have more potential than ever before, for becoming cost-efficient and effective ways of removing organic contaminants from the environment.

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