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Phytodegradation of organic compounds

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The phytodegradation of organic compounds can take place inside the plant or within the rhizosphere of the plant. Many different compounds and classes of compounds can be removed from the environment by this method, including solvents in groundwater, petroleum and aromatic compounds in soils, and volatile compounds in the air. Although still a relatively new area of research, there are many laboratories studying the underlying science necessary for a wide range of applications for plant-based remediation of organic contaminants.

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Abbreviations

MTBE	methyl-tert-butyl ether
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyl
TCE	trichloroethylene
TNT	2,4,6-trinitrotoluene
TPH	total petroleum hydrocarbon

Introduction

Phytoremediation is the use of plants to remediate contaminants in the environment. With the high costs of site remediation, it is important that we continue to develop and refine innovative, low-cost methods for cleaning the environment. Phytoremediation also has the benefit of contributing to site restoration when remedial action is ongoing. The phytoremediation of organic compounds can take place from the soil, air, groundwater or surface water. The action of plants can include the degradation, adsorption, accumulation and volatilization of compounds or the enhancement of soil rhizosphere activity. Which plant activity occurs can depend not only on the medium to be remediated and the type of plant used, but also on the physical properties of the contaminant. Thus, two different compounds in the same medium can interact with a plant in very different ways. In this review, we classify compounds of a similar nature together and discuss how recent studies have helped to elucidate plant interactions with those compounds.

A summary of the contaminants and plants discussed is given in Table 1.

Phytoremediation of solvents

The phytoremediation of solvents is almost at the point of being considered an accepted technology, even as new information on the fate of solvents in plants continues to come to light. Shang and Gordon [1] showed that the groundwater contaminant trichloroethylene (TCE) taken up by suspension cell cultures of hybrid poplar becomes part of the non-volatile, un-extractable portion of the cells. In whole plant studies, however, Ma and Burken [2••] demonstrated that the stems and trunks of plants release TCE to the atmosphere. To better understand the fate of TCE in whole plant systems, one of us (Newman) is collaborating with Burken to better define the mass balance and determine all fates of TCE in plants.

Other groundwater contaminants also continue to be studied. Ma and Burken [3] looked at the diffusion of compounds such as 1,1,2,2-tetrachloroethane and carbon tetrachloride from the transpiration stream of mature trees on contaminated sites and found that stem concentrations can be used as indicators of aquifer concentrations. The fate of ethylene dibromide (EDB) and TCE has also been studied in the tropical tree *Leuceana leucocephala* [4] and the plant was shown to be highly effective at taking up both compounds. Bromide levels in the hydroponic solutions of plants exposed to EDB showed a marked increase, indicating a dehalogenation activity in the plants. Laboratory studies with a hairy root culture of *Brassica napus* exposed to 2,4-dichlorophenol resulted in greater than 90% removal of the compound from solution over a range of pH values [5]. The plant-based treatment of benzotriazoles, corrosion inhibitors used in glycol-based deicing fluids, has also been studied. Castro *et al.* [6] observed that benzotriazoles appeared to be actively taken up and then incorporated into plant tissue by sunflowers (*Helianthus annuus*) grown in hydroponic solution.

Advances in the phytoremediation of methyl-tert-butyl ether (MTBE) have progressed more slowly. Although previous studies have shown that a wide variety of plants are capable of taking up the compound, little is known about its fate. Trapp *et al.* [7] looked at the fate of MTBE in a variety of plants including trees, grasses and herbs,

Table 1

Summary of contaminants and plants discussed in the text*.

Class	Contaminant	Plants studied	Effect studied
Chlorinated	TCE	Poplar	Metabolism [1], volatilization from trunk [2••]
	CT, tetrachloroethane	Poplar [3]	Volatilization from trunk
	TCE	*Leuceana* [4], poplar rhizosphere [26]	Metabolism
	2,4-Dichlorophenol	*Brassica* [5]	Metabolism
De-icing agents	Benzotriazoles	*Hellianthus* [6]	Metabolism
Gasoline additivies	MTBE	Herbaceous plants [7], pine [10••]	Metabolism
	MTBE	Poplar [9]	Volatilization from trunk
Pesticides	EDB	*Leuceana* [4]	Metabolism
	Trifluralin, lindane	Rye [11]	Uptake
	DDE	*Curcurbita* [12,13••,15••,16]	Uptake
	Simazine	Parrot feather, canna	Uptake [14]
Energetics	TNT	Parrot feather [18], *Arabidopsis* [21]	Metabolism
	HMX	Poplar [22], bean, alfalfa, canola [23]	Metabolism
	TNT, RDX, HMX	Poplar endophytes	Metabolism
	Perchlorate	Tobacco [24], poplar [25]	Metabolism
PAH/TPH		Sorghum [27], alfalfa [39], clover, rye [40], poplar [42,43], willow [44]	Rhizosphere effects
	Pyrene	Rye [28], fescue [30••], *Kandelia* [42], *Bruguiera* [47]	Rhizosphere effects
	Gasoline	Pothos [48]	Atmospheric remediation
	Jet fuel	Fescue [33]	Rhizosphere effects
	TPH	Poplar [42,43]	Rhizosphere effects
	Mineral oil	Willow [44]	Rhizosphere effects
	Diesel, heavy oil	Grasses native to California (US) [35]	Rhizosphere effects
PCBs		*Brassica* [41]	Rhizosphere effects

*This does not reflect what was done in previous studies and should not be considered an inclusive list of all plant and contaminants studied. CT, carbon tetrachloride; DDE, 2,2-*bis*(*p*-chlorophenyl)-1,1-dichlorethylene; EDB, ethylene dibromide; HMX, octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine.

and found no evidence for its metabolism by the plants; they proposed that volatilization was the main fate. It was also shown by Ramaswami *et al.* [8] that rhizosphere bacteria were not a significant source of MTBE degradation. In laboratory studies, Ma *et al.* [9] found that not only was MTBE released from the leaves, as reported in several previous studies, but it also diffused from the trunk of the plants. A more recent study of five mature Monterey pines exposed to MTBE in the aquifer reported much higher levels of tert-butyl alcohol (TBA) than MTBE through the transpiration stream, indicating that metabolism was taking place either in the rhizosphere or within the plant system [10••]. This study also showed a significant decrease in MTBE levels in the aquifer as it passed under the trees, indicating that phytoremediation may be a viable option for MTBE, even if the fate of the compound in the plant is not yet fully understood.

Phytoremediation of pesticides

The area of pesticide phytoremediation has recently yielded some interesting results. Li *et al.* [11] looked at the uptake of trifluralin and lindane by ryegrass: trifluralin appeared to be metabolized by the plant, whereas lindane had minimal metabolism and instead formed bound residues. A number of factors have been shown to effect the phytoremediation of pesticides. For example, White and colleagues [12,13••] showed that organic acids increased the uptake of 2,2-*bis*(*p*-chlorophenyl)-1,1-dichlorethylene (DDE) and Knuteson *et al.* [14] reported that younger plants (two weeks old) exhibited greater uptake of simazine (2-chloro-4,6-bis(ethylamino)-1,3,5-triazine) than plants that were just two weeks older. Although it has long been known that the ability to take up and translocate heavy metals is not only species-specific, but also ecotype specific, White and coworkers [15••,16] reported that the uptake of DDE was also subspecies specific, with certain cultivars showing significantly higher uptake. They concluded that this difference was the result of higher exudation rates of low molecular weight organic acids.

Phytoremediation of explosive compounds

The phytoremediation of explosive compounds is of great interest to the Department of Defense, as they look for innovative technologies to reduce the levels of contaminants on ranges while still keeping the ranges operational. In this regard, Wang *et al.* [17] studied the interactions between 2,4,6-trinitrotoluene (TNT) and *Myriophyllum aquaticum*, looking at transformation rates. Planting with Johnsongrass or wild ryegrass has been shown to decrease soil concentrations of TNT [18] and it has been reported that the use of plants to remediate surface waters contaminated with TNT could reduce toxicity in the system [19]. A newly discovered endophytic bacterium, *Methylobacterium* sp. (isolated from hybrid poplar), was shown by van Aken *et al.* [20••] to be capable of degrading TNT, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine (HMX); it may be that this bacterium has a major role in the degradation of explosive compounds in plants. However, Ekman *et al.* [21] found that the detoxification enzymes cytochrome P450, glutathione *S*-transferase, an ABC transporter, and a probable nitroreductase were all induced in *Arabidopsis* roots following TNT exposure, which opens the possibility of these enzymes being involved in plant degradation of TNT. In contrast to the promising results seen with TNT, Yoon *et al.* [22] showed that HMX has limited metabolism in plants, and could result in accumulation of the compound in plants. Likewise, Groom *et al.* [23] looked at HMX uptake using alfalfa (*Medicago sativa*), bush bean (*Phaseolus vulgaris*), canola (*Brassica rapa*), and other plants and saw the same limited uptake and metabolism in the plants, suggesting reduced potential for the phytoremediation of this compound.

Diverse results have also been observed for the remediation of perchlorate. Although one study reported the accumulation of perchlorate in the leaves of tobacco plants [24] another showed that poplars were able to reduce perchlorate, albeit at a low level [25].

Phytoremediation of petroleum compounds

The rhizosphere degradation of chlorinated solvents under new plantations is limited, but work by Godsy *et al.* [26] indicates that as the trees age the microbial populations might change and foster anaerobic degradation. With compounds such as the polycyclic aromatic hydrocarbons (PAHs), petroleum compounds and polychlorinated biphenyls (PCBs), the role of the rhizosphere is much more critical.

Efforts to demonstrate plant or rhizosphere effects on petroleum degradation, particularly PAHs, continue. In a greenhouse study, Banks *et al.* [27] observed generally greater remediation efficiency in soil planted with four genotypes of *Sorghum bicolor* (L.) compared with unvegetated controls. Although the genotypes were selected for different root characteristics and root exudate levels, these differences were not reflected in remediation efficiencies. Measuring plant effects on PAH degradation in soil remains difficult, and our ability to measure phytoremediation treatment effects is confronted by numerous interactions among the soil-rhizosphere-microbial-plant system. Parts of the system interact on a continuous basis, interactions vary with conditions, such as moisture and temperature, as well as with the growth stage of both plants and microorganisms. These interactions make understanding and monitoring phytoremediation complex. Results from studies are mixed. Using small field plots in previously vegetated soils that had been amended with pyrene, mixed and then either revegetated with annual ryegrass (*Lolium multiflorum* Lam.) or kept fallow, Lalande *et al.* [28] reported greater pyrene loss from unvegetated treatments and suggested that microorganisms may use easily degradable organic matter as a carbon source rather than pyrene. Joner *et al.* [29] suggested that priming effects associated with experimental protocols were large, and may tend to mask beneficial effects from the synergistic effects of fertilizing and planting. Solid evidence for increased degradation with plants continues to be reported. Using ^{14}C-labeled pyrene, Chen *et al.* [30••] showed 38% and 30% pyrene mineralization in tall fescue (*Festuca arundinacea*) and switchgrass (*Panicum virgatum* L.), respectively, compared with 4.3% in the unplanted control.

Contamination with hydrocarbon-based compounds affects the carbon:nitrogen (C:N) ratio in soil and can lead to nitrogen immobilization. Adam and Duncan [31] evaluated leguminous plants in diesel-contaminated soil and found that contaminated soil had fewer nodules, but that these nodules were more developed. White *et al.* [32] investigated soil amendments with different C:N ratios for their effects on both seed germination and plant growth. Addition of boiler litter, which had a C:N ratio of approximately eight, resulted in lower soil total petroleum hydrocarbons (TPHs) compared to amendments with higher C:N ratios.

In field soils, numerous soil–plant interactions and soil conditions, including soil moisture and temperature, may influence contaminant fate. Plants can affect soil water content and movement in several ways. Karthikeyan *et al.* [33] used column studies to demonstrate that transport and biodegradation of jet fuel (JP-8) were affected by soil water, and that soil columns planted with fescue grass (*Festuca arundinacia*) had less leaching of JP-8. They suggested that plant transpiration and associated water movement could favor biodegradation by keeping mobile contaminants near the soil surface, and they developed a modeling approach to describe water and JP-8 transport [34] (Table 1).

Although field studies are more difficult to conduct and monitor than greenhouse studies, results are encouraging. Banks *et al.* [35] found that vegetative treatment of diesel- and heavy-oil-contaminated soil yielded both lower TPH values in the vegetated soil and reduced toxicity. They suggested that longer term treatment may be needed for further reductions in TPH concentration. Robson *et al.* [36] have provided a list of plants adapted to hydrocarbon-contaminated soils and cold regions.

Our understanding of rhizosphere microbiology and its changes during phytoremediation is improving. Siciliano *et al.* [37••] observed greater levels of hydrocarbon-related catabolic genes (*ndoB*, *alkB* and *xylE*) in rhizosphere soils relative to bulk soil. Muratova *et al.* [38] used fluorescence microscopy to show that the microflora in the rhizosphere of bitumen-contaminated soil was greater than in unplanted soil, and that the community structure was also different. Differences between alfalfa (*Medicago sativa*) and reed (*Phragmites australis*) rhizospheres were also observed. Additionally, alfalfa enhanced the PAH-degrading population in the rhizosphere [39]. In a pot study, Joner and Leyval [40] found that in PAH-contaminated soil planted with a clover–ryegrass mix PAH concentrations decreased as a function of proximity to the roots, and that mycorrhiza generally enhanced plant growth and favored growth of clover at the expense of ryegrass. Singer *et al.* [41] repeatedly augmented Arochlor1242-contaminated soil with PCB-degrading bacteria, carvone and salicyic acid as enzyme inducers, a surfactant and minimal salts medium, and compared *Brassica nigra* to non-vegetated controls. The *B. nigra*-planted treatments resulted in 61% PCB removal in the top 6 cm after nine weeks of bioaugmentation, compared with 43% and 14% PCB removal in the 0–2 and 2–6 cm depths, respectively, in unplanted controls.

In addition to grasses and legumes, trees such as *Populus* are increasingly being considered for use in PAH and TPH degradation. In short-term tests, Wittig and colleagues [42,43] found that poplar cuttings grown in a PAH-amended sand-nutrient solution had similar shoot biomass, growth and leaf water content to the controls, but that transpiration, nutrient solution uptake, and root mass were reduced. The water content of the leaves was similar among plants from the PAH-contaminated soils and controls. The effects were also dependent on the PAH under study. Willow (*Salix viminalis* L. 'Orm') has been evaluated for the dissipation of mineral oil and PAHs in dredged sediment [44]. Mineral oil concentration decreased 57% after 1.5 years in the willow-planted treatment compared to 15% in the control. In the vegetated soil, mineral oil degradation was greater in the roots zone than in the total column of soil.

Because the volume of soil affected by rhizosphere processes is related to root morphology and size, there is continued interest in rooting patterns. Rentz *et al.* [45••] investigated five passive methods for increasing oxygen delivery to enhance root development and plant growth. Using commercial oxygen release compounds stimulated plant growth and root density in a pot study. These results suggest that there may be effective ways to enhance root growth in oxygen-limiting situations and to couple phytoremediation with other technologies to enhance both.

Ke *et al.* [46] investigated using mangrove (*Kandelia candel* and *Bruguiera gymnorrhiza*) wetland systems to treat pyrene-contaminated sediments. After six months, pyrene concentrations decreased in the mangrove treatments relative to the controls. Although there was a measurable increase in pyrene concentrations in the roots relative to the non-pyrene control roots, the increase was minor compared with the total pyrene removed from the sediment, suggesting that plant uptake was not a significant path for pyrene loss. The authors found that adding humic acid (HA) to the soil reduced both plant growth and pyrene loss, suggesting that pyrene binding to the HA limited its bioavailability [47].

Although most problems with petroleum compounds are associated with contaminated soils and groundwater, the atmospheric release of gasoline compounds also needs to be addressed. Oyabu *et al.* [48] investigated the ability of *Epipremnum aureum* (golden pothos) to remove gasoline from the air and found that the plants purification ability reached a maximum value 40 h after gasoline vapors were inroduced into the growth chamber. The authors did not separate soil effects from plant effects in their study.

Models of phytoremediation

Difficulties in measuring phytoremediation rates, predicting treatment times, and developing monitoring schemes are recognized as current limitations to using phytoremediation. Developing effective models may help to address these limitations and might help the research community to understand the interactions among the many processes that contribute to phytoremediation. Thoma and colleagues [49,50] developed a model for screening grass species for the phytoremediation of weathered petroleum. Models for calculating hydraulic control based on transpiration and groundwater characteristics have been discussed and evaluated, with the suggestion that field measurements be used to verify model predictions whenever possible [51]. International interest in using phytoremediation has lead to reviews that discuss both models and processes thought to be important [52], as well as performance evaluations of existing models against experimental datasets [53]. Matthews *et al.* [54] used a numerical groundwater flow model and evaluated how variations in hydrogeologic and seasonal growth parameters influenced the minimum plantation area needed for plume capture. They found that the aquifer horizontal hydraulic conductivity and saturated thickness directly influenced the plantation size.

Conclusions

Reviews and conference summaries of phytoremediation studies are appearing regularly and allow concise overviews of international phytoremediation efforts [55–63]. As more researchers, site owners and regulators become aware of the technology, there is a great potential for the application of this area of phytoremediation. Advances in groundwater and soil remediation continue to lead to a better understanding of the many processes by which plants can have a positive impact on contamination in the environment.

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