1-1-2001

Advances in Phytoremediation

Annette C. Dietz

Jerald L. Schnoor

University of Iowa
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Annette C. Dietz and Jerald L. Schnoor
Department of Civil and Environmental Engineering, University of Iowa, Iowa City, Iowa, USA

Phytoremediation is the use of plants to remedy contaminated soils, sediments, and/or groundwater. Sorption and uptake are governed by physicochemical properties of the compounds, and moderately hydrophobic chemicals (logarithm octanol–water coefficients (Kow) = 1.0–3.5) are most likely to be bioavailable to roots, vascular plants. Some hydrophilic compounds, such as methyl-tert-butylether and 1,4-dioxane, may also be taken up by plants via hydrophobic bonding with transpiration water. Organic chemicals that pass through membranes and are translocated to stem and leaf tissues may be converted (e.g., oxidized by cytochrome P450s), conjugated by glutathione or amino acids, and compartmentalized in plant tissues as bound residue. The relationship between metabolism of organic xenobiotics and toxicity to plant tissues is not well understood. A series of chlorinated ethenes is more toxic to hybrid poplar trees (Populus deltoides x nigra, DN-34) than are the corresponding chlorinated ethanes. Toxicity correlates best with the number of chlorine atoms in each homologous series. Transgenic plants have been engineered to rapidly detoxify and transform such xenobiotic chemicals. These could be used in phytoremediation applications if issues of cost and public acceptability are overcome. Key words: hazardous wastes, hazardous wastes remediation, organic chemicals, phytoremediation, phytoxidation, plants, plant metabolism.

Sorption to Roots
Organic chemicals may sorb to roots and be transpired (volatilized) by plants. The first step is sorption to roots. When chemical contaminants in soil water or groundwater come into contact with roots, they may sorb or bind to the root structure and cell walls. Hemicellulose in the cell wall and the lipid bilayer of plant membranes can bind hydrophobic organic chemicals effectively. Such sorption should be relatively reversible and can be measured using standard sorption isotherms. Figure 1 is an example of a sorption isotherm after 48 hr for 1,4-dichlorobenzene in hydroponic solution with fresh hybrid poplar roots (Populus deltoides x nigra, DN-34) grown both in the laboratory and in the field at Amana, Iowa (19). The field roots contained higher lipid content and surface area, accounting for the enhanced partitioning with dichlorobenzene.

Briggs et al. (9) defined the root concentration factor (RCF) as the ratio of organic chemical sorbed on the root (milligrams per kilogram of fresh root tissue) to that in hydroponic solution (milligrams per liter). Thus, the slopes of the linear sorption isotherms in Figure 1 are measures of the RCF and have units of liters per kilogram. Briggs et al. measured the RCF of substituted phenyl ureas on barley roots and determined that hydrophobic organic chemicals were the most strongly sorbed. Hydrophobicity was related to the octanol–water partition coefficient (log Kow) of the organics, and log RCF was correlated with log Kow via a least squares regression equation. The greater the hydrophobicity of the chemical (as measured by the Log Kow), the greater its tendency to partition out of the aqueous phase and onto roots. Burken and Schnoor (5) published a similar relationship for organic contaminants typically found at waste sites, using hybrid poplar roots grown hydroponically. Both relationships indicate that organic chemicals with log Kow > 3.0 are highly sorbed by roots.

Log (RCF – 3.0) = 0.65 log Kow – 1.57 (5)
Log (RCF – 0.82) = 0.77 log Kow – 1.52 (9)

These two equations are plotted in Figure 2, together with data from selected organic chemicals on poplar roots from Burken and Schnoor (5). Selected organic chemicals, their physicochemical properties, and measured RCF values on hybrid poplar roots...
**Uptake and Translocation**

Rooted vascular plants must take up water and nutrients for growth. Nutrients are transported into cells through channels in membranes or via membrane-bound proteins that bind the chemical and transport it into the cell (active transport). Base metal cations (Ca$^{2+}$, Mg$^{2+}$, K$^+$, and Na$^+$) are taken up by active transport mechanisms. Organic chemicals can be taken up by plants via diffusion (passive uptake) through cell walls and membranes. In this case there may exist an optimum hydrophobicity that allows the chemical to bind to the lipid bilayer of the membrane but not too strongly for transport to be facilitated.

Direct uptake of organics by plants is a surprisingly efficient removal mechanism from shallow contaminated sites with moderately hydrophobic organic chemicals (log $K_{ow}$ ~ 1 – 3.5). These include most benzene, toluene, ethylbenzene, and xylene (BTEX) chemicals, chlorinated solvents, and short-chain aliphatic chemicals. Hydrophobic chemicals (log $K_{ow}$ > 3.5) are bound so strongly to the surface of roots and soils that they cannot be translocated easily within the plant, and chemicals that are quite water soluble (log $K_{ow}$ < 1.0) are not sufficiently sorbed to roots nor actively transported through plant membranes (9). Hydrophobic chemicals (log $K_{ow}$ > 3.5) are candidates for phytostabilization and/or rhizosphere bioremediation by virtue of their long residence times in the root zone.

**Figure 1.** Isotherm for the sorption of 1,4-dichlorobenzene on hybrid poplar roots (Populus deltoides x nigra, DN-34). Hydroponically grown roots and roots extracted from 5-year-old trees in the field (1.0 g of fresh roots in 10-mL scintillation vials) were exposed to 0.1–10 mg/L of cold chemical or $^{14}$C-radiolabeled chemical. Sorption was measured by difference in solution after equilibrium was achieved (usually 48 hr) and by radiochemical methods. The RCF is the slope of the line: 26.4 mL/g for field roots and 7.1 mL/g for hydroponic roots (10).

**Figure 2.** RCF as a function of the Log $K_{ow}$ for selected xenobiotic chemicals. The solid line is the best fit expression for the chemicals shown with hybrid poplar trees (5). The dotted line represents the results from Briggs et al. (9) for substituted phenylureas on barley for comparison purposes. Data modified from Briggs et al. (9) and Burken and Schnoor (5).

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**Table 1.** Typical plants used in various phytoremediation applications.

<table>
<thead>
<tr>
<th>Application</th>
<th>Media</th>
<th>Contaminants</th>
<th>Typical plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytotransformation</td>
<td>Soil, groundwater, landfill leachate, land application of wastewater</td>
<td>Herbicides; chlorinated aliphatics (e.g., TCE); aromatics (e.g., BTEX); ammonium wastes (TNT, RDX, HMX, perchlorate); nutrients (nitrate, ammonium, phosphate)</td>
<td>Phreatophytic trees (Salix family, including poplar, willow, cottonwood), grasses (rye, fescue, Bermuda grass, sorghum, switchgrass, Reed canary grass), legumes (clover, alfalfa, cowpeas)</td>
</tr>
<tr>
<td>Rhizosphere bioremediation</td>
<td>Soil, sediments, land application, confined disposal facilities</td>
<td>Biodegradable organics (BTEX, THP, PAHs, PCBs, pesticides)</td>
<td>Grasses with fibrous roots (Bermuda, fescue, rye), phenoilics releasers (mulberry, apple, osage orange), phytotrophic trees</td>
</tr>
<tr>
<td>Phytostabilization</td>
<td>Soils</td>
<td>Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U); hydrophobic organics that are not biodegradable</td>
<td>Phreatophytic trees for hydraulic control, grasses with fibrous roots for erosion control</td>
</tr>
<tr>
<td>Phytoextraction</td>
<td>Soil, sediments, brownfields</td>
<td>Metals (Pb, Cd, Zn, Ni, Cu)</td>
<td>Indian mustard (Brassica juncea), sunflowers (Helianthus spp.), Thlaspi canadensis</td>
</tr>
<tr>
<td>Rhizofiltration</td>
<td>Groundwater, wastewater through constructed wetlands</td>
<td>Metals (Pb, Cd, Cu, Ni, Zn); radionuclides, hydrophobic organics</td>
<td>Aquatic plants: emergents (bullrush, cattail, coontail, pond weed, arrowroot), submerged (algae, stonewort, parrot feather, Hydra spp.)</td>
</tr>
<tr>
<td>Phytovolatilization</td>
<td>Soils and sediments</td>
<td>Selenium, arsenic, mercury, volatile organic compounds (e.g., MTBE)</td>
<td>Brassica juncea; wetlands plants; phreatophytic trees for groundwater capture</td>
</tr>
</tbody>
</table>

Abbreviations: BTEX, benzene, toluene, ethylbenzene, and total xylene. HMX, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine. MTBE, methyl-tert-butyl ether. PAHs, polycyclic aromatic hydrocarbons. PCBs, polychlorinated biphenyls. RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine. TCE, trichloroethylene. TNT, 2,4,6-trinitrotoluene. TPH, total petroleum hydrocarbons.
stream concentration factor (TSCF). TSCF is the ratio of the concentration in the transpiration stream of the plant to the concentration in soil water, and TSCF depends on physicochemical properties, chemical speciation, and the plant itself. Some measured values appear in Table 2. TSCF can vary from zero (no uptake) to 1.0 (uptake at the same concentration as the soil water concentration). Chemicals that react biochemically at the root–water interface do not follow the above relationship because uptake is determined by site binding and biochemical reaction and not by the rate of passage through membranes into the transpiration stream. Transpiration rate is a key variable that determines the rate of chemical uptake for a given phytoremediation application and depends on the plant type, leaf area, nutrients, soil moisture, temperature, wind conditions, and relative humidity. High transpiration corresponds to rapid uptake, and this is why fast-growing phreatophytes (e.g., hybrid poplars and willows) are frequently employed in phytoremediation applications.

TSCFs have been measured for herbicide-related chemicals (substituted phenylureas and o-methylcarbamoyloximes) with crop species (barley) by Briggs and coworkers (9). Burken and Schnoor (5) measured a wide variety of chemicals found at hazardous waste sites with hybrid poplar trees. Both relationships predict a large uptake for chemicals in the moderately hydrophobic range (log Kow = 1.0–3.5).

\[
\text{TSCF} = 0.756 \exp[-(\log K_{ow} - 2.50)/2.58]
\]

(9)

\[
\text{TSCF} = 0.784 \exp[-(\log K_{ow} - 1.78)/2.44]
\]

(5)

Recent reports have indicated that neutral, water-soluble chemicals with low hydrophobicities (log Kow < 1.5) may still be taken up by rooted vascular plants in some cases. Aitchison et al. (21) showed that the heterocyclic ether 1,4-dioxane is rapidly taken up and translocated by hybrid poplar cuttings. The TSCF was approximately 0.72, even though its log Kow is extremely low (–0.27), and it does not bind significantly to roots. It is suggested that chemicals such as 1,4-dioxane and methyl-tert-butyl ether (22) may be taken up via hydrogen bonding with water molecules into the transpiration stream.

**Enzymatic Transformations**

Phytotransformation refers to the uptake of organic contaminants from soil and groundwater and the subsequent metabolism or transformation by plants. Once an organic chemical is taken up and translocated, it undergoes one or more phases of transformation (11):

- Phase I—Conversion: oxidations, reductions, hydrolysis.
- Phase II—Conjugation: with glutathione, sugars, amino acids.
- Phase III—Compartmentation: Conjugates from phase II are converted to other conjugates and deposited in plant vacuoles or bound to cell wall and lignin.

Phase III conjugates are sometimes termed “bound residues” because of their inability to be extracted by chemical methods. These conjugates are likely covalently bound to stable tissues in the plant. However, one concern is whether under different conditions, such as in the gut of a worm or herbivore, there could be lignases or other enzymes able to sever covalent bonds and liberate the parent compound or toxic conjugate from the bound residue.

Chlorinated aliphatic compounds such as trichloroethylene (TCE) have been reported to be mineralized to CO₂ and less toxic aromatic metabolites (trichloroethanol, trichloroacetic acid, and dichloroacetic acid) by Newman et al. (3). These products are consistent with those found in the human liver for TCE destruction by cytochrome P450 (P450), which is an abundant enzyme in plants as well as humans (23). Thus, plants are sometimes viewed as “green livers” in terms of their enzyme biochemistry.

**Nitroreductase and Laccase enzymes in plants** can break down ammunition wastes such as 2,4,6-trinitrotoluene (TNT) and may incorporate the broken ring structures into new plant material or organic detritus that becomes a part of soil organic matter (2). Detoxification mechanisms may transform the parent chemical to nonphytotoxic metabolites stored in plant tissues. A thorough understanding of pathways and end products of enzymatic processes will simplify toxicity investigations of *in situ* phytoremediation.

**Phytotransformation Enzymology and Biochemistry**

Plant degradation of many organic compounds follows pathways similar to those observed in other eukaryotes (24). Research on chlorinated aliphatic degradation in humans has focused mainly on their activation and resulting toxicity, carcinogenicity, or mutagenicity. The metabolism of these compounds can vary, even within a homologous series, but many go through oxidation to form a radical. This has been noted for carbon tetrachloride and to a lesser extent in other chlorinated methanes (25). The major dechlorination pathway for chlorinated ethylenes involves the formation of epoxides, with polychlorinated ethenes such as TCE and tetrachloroethylene (PCE) alternatively being conjugated to glutathione (26). P450 is involved in epoxide formation, whereas glutathione S-transferase (GST) catalyzes reactions with glutathione (25). TCE is one of the most studied compounds: metabolites commonly reported in experiments with rodents are chloral hydrate, trichloroethanol, dichloroacetic acid, and trichloroacetic acid (26).

Transformation pathways of TCE in mammals are shown in Figure 3. The epoxide

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Table 2. Measured TSCF and RCF for some typical contaminants.²

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Log Kow</th>
<th>Solubility—log Csat</th>
<th>Henry’s Constant Kf at 25°C (dimensionless)</th>
<th>Vapor pressure (log P at 25°C (atmospheres))</th>
<th>TSCF²</th>
<th>RCF² (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>2.13</td>
<td>1.64</td>
<td>0.2250</td>
<td>0.98</td>
<td>0.82</td>
<td>1</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.69</td>
<td>2.75</td>
<td>0.7960</td>
<td>1.42</td>
<td>0.81</td>
<td>3</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>3.15</td>
<td>2.80</td>
<td>0.3240</td>
<td>1.90</td>
<td>0.80</td>
<td>2</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>3.20</td>
<td>2.77</td>
<td>0.2520</td>
<td>1.98</td>
<td>0.78</td>
<td>11</td>
</tr>
<tr>
<td>TCE</td>
<td>2.33</td>
<td>2.04</td>
<td>0.4370</td>
<td>1.01</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>Aniline*</td>
<td>0.91</td>
<td>0.41</td>
<td>2.2 × 10⁻⁶</td>
<td>2.89</td>
<td>0.32</td>
<td>420</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>1.83</td>
<td>1.77</td>
<td>0.0025³</td>
<td>3.68</td>
<td>0.82</td>
<td>3</td>
</tr>
<tr>
<td>Phenol**</td>
<td>1.45</td>
<td>0.20</td>
<td>&gt;1.0 × 10⁻³</td>
<td>3.59</td>
<td>0.48</td>
<td>11.6</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>5.04</td>
<td>4.27</td>
<td>1.5 × 10⁻⁴</td>
<td>6.75</td>
<td>0.04</td>
<td>30</td>
</tr>
<tr>
<td>Atrazine</td>
<td>2.59</td>
<td>3.81</td>
<td>1.0 × 10⁻⁷</td>
<td>9.40</td>
<td>0.57</td>
<td>8</td>
</tr>
<tr>
<td>1,2,4-Trichlorobenzene</td>
<td>4.25</td>
<td>3.85</td>
<td>0.1130</td>
<td>3.21</td>
<td>0.04</td>
<td>19</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>−0.27</td>
<td>Miscible</td>
<td>2.0 × 10⁻⁴</td>
<td>0.05</td>
<td>0.72</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Methyl-tert-butyl ether</td>
<td>1.1</td>
<td>0.36</td>
<td>0.56</td>
<td>0.49</td>
<td>0.65</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Measured data from hydroponic studies with hybrid poplars. *Data from Burken and Schnoor (5), Lang (19), Aitchison et al. (27), and Winnike (23).


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Figure 3. TCE metabolism in mammalian systems (23).

Environmental Health Perspectives • VOLUME 109 • SUPPLEMENT 1 • March 2001
intermediate is highly transient and difficult to detect. Thus, its role in the overall metabolism of TCE is still controversial and relatively uncertain.

Chlorinated ethanes are less studied, although the major metabolites reported in rat urine are trichloroethanol and trichloroacetic acid (27). Rats exposed to 1,1,1-trichloroethane (111TCA) by inhalation under hypoxia were found to exhale acetylene (28). Whereas chlorinated ethylenes are converted by P450 through an epoxide intermediate, ethanes go through chlorine or hydrogen abstraction, producing a free radical carbanion intermediate. In general, chlorinated ethenes are more reactive than the ethane analogues. Potential transformation pathways for 111TCA are shown in Figure 4.

Phytotransformation has been studied most with pesticides in crop plants. These compounds undergo a series of metabolic processes. The first phase introduces functional groups such as –OH, –NH2, or –SH and can occur by oxidation, reduction, or hydrolysis (29). For highly lipophilic compounds, oxygenation is a typical reaction of this first phase, increasing solubility (30). Plant enzymes that typically catalyze phase I reactions are P450 monoxygenases and carboxylesterases (29).

The second phase involves conjugation with D-glucose, glutathione, or amino acids, resulting in soluble, polar compounds (31). Insoluble conjugates with cell wall components also form in plants. These can form by nonselective reactions with free radicals used in lignin synthesis or by more selective incorporation into hemicellulose (24). Insoluble conjugates are typically reported as bound residue because of difficulty in further characterization. Detoxification of herbicides in plants is attributed to conjugation with glutathione catalyzed by GST (32). Many herbicide safeners (chemicals applied before or in conjunction with herbicide application to protect crop species from herbicide damage) promote glutathione conjugation and detoxification by either increasing levels of glutathione or increasing activity of GST (33). Other enzymes that may be involved in phase II reactions include O- and N-glucosyltransferases and malonyltransferases (29).

The third phase of plant metabolism is compartmentation and storage. Unlike mammals, plants do not have a way to excrete unwanted compounds, so soluble metabolites are stored in the vacuole or as part of cell wall material. The transport of glutathione conjugates into the vacuole has been demonstrated in barley cell cultures (34). P450s are involved in both bacterial and eukaryotic transformation of chlorinated aliphatics (23,35). They also detoxify many pesticides in plants as part of phase I metabolism. Therefore, it is likely that plant metabolism of chlorinated aliphatics is also mediated by a P450.

In plants, most P450s are membrane bound in microsomes such as plastids or endoplasmic reticulum (36). Several can be induced by light (37), whereas others are induced by plant stresses such as wounding, pathogens, or xenobiotic compounds (38). Xenobiotic induction of P450s in animal systems (such as birds and fish) has led to its use as an indicator of environmental contamination (39–40).

More than 50 reactions in plants are catalyzed by P450s (38), including both oxidative and reductive dehalogenation, as shown in Figure 5 (41). Reductive dehalogenation of polyhalomethanes has been demonstrated in several P450s, suggesting that this may be a general reactivity, especially under low oxygen conditions (42).

There are several potential mechanisms for the uptake and transformation of chlorinated aliphatics in a plant–soil system. These are summarized in Figure 6. Possible mechanisms include microbial transformation in the rhizosphere, uptake of the chemical and/or its metabolites into the roots, xylem transfer of the compounds to the leaves, volatilization from the leaves, foliar uptake of chemicals from the air, phloem transfer, and bound residue formation throughout the plant. All these mechanisms may prove important in phytoremediation of sites contaminated with chlorinated aliphatics.

Several researchers have studied the fate of TCE in plants, with varying amounts of phytovolatilization and phytotransformation reported (43). Many investigators have had difficulty isolating and identifying the metabolic products formed bound residue (44–46). However, Newman et al. (3) have reported TCE metabolism to trichloroethanol, trichloroacetic acid, and dichloroacetic acid in hybrid poplar. These results suggest that plant degradation of chlorinated aliphatics likely occurs by oxidative pathways similar to those of mammalian systems. Overall mass balances have been poor, indicating that other processes or further transformations may be occurring. Figure 7 shows a potential reaction sequence and binding of xenobiotics that may occur within cells.

Transgenic Plants

One of the most recent advances in phytoremediation is the development of genetically modified plants able to take up and degrade contaminants. With increased understanding of the enzymatic processes involved in plant tolerance and metabolism of xenobiotic chemicals, there is new potential for engineering plants with increased phytoremediation capabilities (47–49). This type of technology has already been used for several years in agricultural applications, such as Roundup Ready (Monsanto, St. Louis, MO).
Phytoremediation of organic chemicals

One major advantage of genetically engineered plants is that specific enzymes for degradation of a contaminant could be transferred to a plant species that is indigenous to an ecosystem or has other desirable remediation properties such as rapid growth, deep root structures, or high water uptake.

Toxicity Issues

The relationship between plant transformation of xenobiotics and phytotoxicity is not completely understood. In mammalian systems, the activation of TCE through the epoxide intermediate produces its carcinogenicity. Similarly, some phytotransformations may cause plant toxicity if further enzymatic activity cannot successfully break down metabolites or sequester them.

The relative effects of the nine chlorinated solvents on hybrid poplar were compared by plotting the percent increase in cutting mass versus hydroponic exposure concentration (Figure 8). In general, cuttings tolerated higher concentrations of solvents with fewer chlorine atoms within a series of homologous ethanes or ethenes. The number of chlorine atoms was more closely related to growth reduction than was the arrangement of the chlorine atoms, as observed by comparing lines for the three isomers of dichloroethanes [cis-dichloroethane (cDCE), trans-dichloroethene (tDCE), and 1,1-dichloroethene (11DCE)] and two trichloroethanes [111TCA and 1,1,2-trichloroethane (112TCA)]. Ethanes cause zero growth at lower concentrations than do similarly chlorinated ethenes. The reason for these trends is not yet known. It is plausible that the more highly chlorinated compounds require more enzymatic steps to metabolize them. Epoxide intermediates potentially formed from chlorinated ethenes may be more difficult to further metabolize than the possible carbonan intermediates formed from chlorinated ethenes. Further research is needed to elucidate the relationship between phytotransformation and phytotoxicity.

Conclusions

Phytoremediation has been advanced in the last few years by increased understanding of the mechanisms of plant uptake and the various types of enzymatic metabolism that occur. Sorption and uptake constants such as the RCF and TSCF may help model plant uptake rates of various chemicals, allowing more accurate prediction of treatment times required for phytoremediation technology. Research into enzymatic transformation pathways will help determine the ultimate fate of chemicals in a plant remediation system.

Recent studies with transgenic plants show that specific degradation capabilities may be added to plant species selected for other reasons. Further research into the biochemical processing of xenobiotic compounds will provide insight into phytotoxicity constraints, and genetic engineering may allow plants to tolerate higher concentrations of chemicals. This new knowledge will allow phytoremediation to be applied more widely and effectively.

References and Notes


Dietz and Schnoor


