Advances in Phytoremediation

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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 = 1.0–3.5) are most likely to be bioavailable to rooted, vascular plants. Some hydrophilic compounds, such as methyl-*tert*-butylether and 1,4-dioxane, may also be taken up by plants via hydrogen 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, phytotoxicity, plants, plant metabolism. — *Environ Health Perspect* 109(suppl 1):163–168 (2001).
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Phytoremediation is the use of vegetation for *in situ* treatment of contaminated soils, sediments, and water. It is applicable at sites containing organic, nutrient, or metal pollutants that can be accessed by the roots of plants and sequestered, degraded, immobilized, or metabolized in place. In the last few years a greater understanding has been achieved regarding the uptake and metabolism of organic xenobiotic chemicals by plants, especially chlorinated solvents, some pesticides, and explosives compounds (*1–8*). These chemicals contaminate a large number of hazardous waste sites. In this review we focus on recent advances in the understanding of sorption, uptake, phytotransformation, and toxicity of such chemicals, especially chlorinated aliphatics.

Phytoremediation is popular because of its cost-effectiveness, aesthetic advantages, and long-term applicability (*2*). Applications include hazardous waste sites where other methods of treatment are too expensive or impractical, low-level contaminated sites where only "polishing treatment" is required over long periods of time, and sites where phytoremediation can be used in conjunction with other technologies as a final cap. Limitations of the technology include the potential for introducing the contaminant or its metabolites into the food chain, long cleanup times required to achieve regulatory action levels, and toxicity encountered in establishing and maintaining vegetation at waste sites.

Plants have shown the capacity to withstand relatively high concentrations of organic xenobiotic chemicals without toxic effects (*5,9*), and in some cases they can take up and convert chemicals quickly to less toxic metabolites (*3,10–13*). In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates and enzymes and the resulting buildup of organic carbon in the soil (*1,14,15*). When toxicity is an issue, nutrients and soil amendments can be added to ameliorate toxicity and establish vegetation at waste sites. Once the plants are established and contaminant concentrations are somewhat diminished, vigorous growth and remediation can occur.

For metal contaminants, plants show the potential for phytoextraction (uptake and recovery of metals into above-ground biomass), filtering metals from water onto root systems (*16*) or stabilizing wastes by hydraulic and erosional control at the site (phytostabilization) (*16–18*). Table 1 provides a summary of some phytoremediation applications and plants that have been used.

Organic Chemicals and Sorption to Roots

Organic chemicals may sorb to roots and be taken up, translocated, metabolized, or 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 K_{ow}) of the organics, and log RCF was correlated with log K_{ow} via a least squares regression equation. The greater the hydrophobicity of the chemical (as measured by the Log K_{ow}), 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 K_{ow}s > 3.0 are highly sorbed by roots.

$$\text{Log (RCF} - 3.0) = 0.65 \log K_{ow} - 1.57 \quad (5)$$

$$\text{Log (RCF} - 0.82) = 0.77 \log K_{ow} - 1.52 \quad (9)$$

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

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(*Populus deltoides x nigra,* DN-34) are shown in Table 2. The tendency of hydrophobic chemicals to partition into organic phases is not the only mechanism at play in binding of chemicals to roots. Specific sorption at chemical sites and enzymatic transformation by membrane-bound proteins are other mechanisms of potential importance. In Table 2, pentachlorophenol and 1,2,4-trichlorobenzene are highly sorbed to root tissues (RCF > 10 L/kg) because of their hydrophobicity, but aniline and phenol bind tightly to roots because of specific sorption and enzymatic transformation.

Some contaminants are transformed rapidly at the root surface by extracellular enzymes or by membrane-bound enzymes. Amines ($–NH_2$) and hydroxy (–OH) functional groups are transformed enzymatically. These compounds and their metabolites (especially aniline) bind irreversibly to roots and are chemically transformed. They are not desorbed appreciably because they are bound and transformed by the root tissue (*4,19*). Other examples include the reduction and transformation of nitroaromatic explosive compounds such as 2,4,6-trinitrotoluene (*4,6*). Nitroaromatics may bind tightly to roots and be transformed as the nitro group ($–NO_2$) is reduced to amino or hydroxyamino functionalities. More research is needed to understand these transformations and answer whether plant products are more or less toxic in the environment than the original free chemical.

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, toulene, 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.

Uptake of chemicals into plants through roots depends on the plant's uptake efficiency, the transpiration rate, and the concentration of chemical in soil water (*20*):

$$U = (TSCF)\ (T)\ (C),$$

where U is the the rate of chemical uptake by plant in milligrams per day, TSCF is the efficiency of uptake (dimensionless), T is the transpiration rate in liters per day, and C is the soil water concentration of chemical in milligrams per liter.

Uptake efficiency for rooted vascular plants (with chemicals that are not transformed immediately) is defined as the transpiration

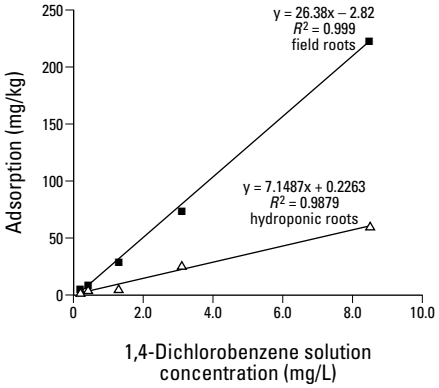

Figure 1. Isotherm for the sorption of 1,4-dichlorobenzene on hybrid poplar roots (*Populus deltoides x nigra*, DN-34). Hydroponically grown roots and roots extracted from 5-year-old trees in the field (1.0 g of fresh roots in 10-mL scintillation vials) were exposed to 0.1–10 mg/L of cold chemical or ^{14}C-radiolabeled chemical. Sorption was measured by difference in solution after equilibrium concentrations were 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 (*19*).

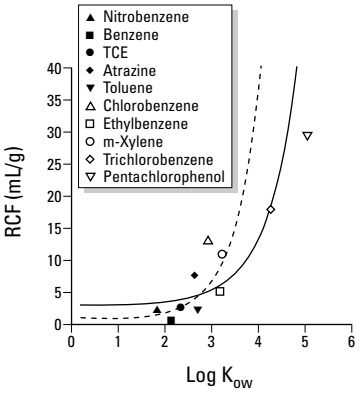

Figure 2. RCF as a function of the Log K_{ow} for selected xenobiotic chemicals. The solid line is the best fit expression for the chemicals shown with hybrid poplar trees (*5*). The dotted line represents the results from Briggs et al. (*9*) for substituted phenylureas on barley for comparison purposes.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

Table 1. Typical plants used in various phytoremediation applications.

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

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 K_{ow} = 1.0–3.5).

$$TSCF = 0.756 \exp[-(\log K_{ow} - 2.50)^2 / 2.58] \quad (9)$$

$$TSCF = 0.784 \exp[-(\log K_{ow} - 1.78)^2 / 2.44] \quad (5)$$

Recent reports have indicated that neutral, water-soluble chemicals with low hydrophobicities (log K_{ow} < 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 K_{ow} 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 CO_2 and less toxic aerobic 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 (*23*). 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 (*23*). P450 is involved in epoxide formation, whereas glutathione *S*-transferase (GST) catalyzes reactions with glutathione (*25*). TCE is one of the more 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

Table 2. Measured TSCF and RCF for some typical contaminants.[a]

Chemical	Log K_{ow}	Solubility—log C_w^{sat} at 25°C (mol/L)	Henry's Constant K_H', at 25°C (dimensionless)	Vapor pressure –log P^0 at 25°C (atmospheres)	TSCF[a]	RCF[a] (L/kg)
Benzene	2.13	1.64	0.2250	0.90	0.82	1
Toluene	2.69	2.25	0.2760	1.42	0.81	3
Ethylbenzene	3.15	2.80	0.3240	1.90	0.80	2
m-Xylene	3.20	2.77	0.2520	1.98	0.78	11
TCE	2.33	2.04	0.4370	1.01	0.75	3
Aniline*	0.90	0.41	2.2×10^{-5}	2.89	0.32	420
Nitrobenzene	1.83	1.77	0.0025[b]	3.68	0.82	3
Phenol**	1.45	0.20	$>1.0 \times 10^{-5}$	3.59	0.48	11.6
Pentachlorophenol	5.04	4.27	1.5×10^{-4}	6.75	0.04	30
Atrazine	2.69	3.81	1×10^{-7}	9.40	0.57	8
1,2,4-Trichlorobenzene	4.25	3.65	0.1130	3.21	0.04	19
1,4-Dioxane	–0.27	Miscible	2.0×10^{-4}	0.05	0.72	<1
Methyl-*tert*-butyl ether	1.1	0.36	0.56	0.49	0.65	<1
RDX	0.87	4.57	—	—	0.25	3.1

[a]Measured data from hydroponic studies with hybrid poplars. [b]Data from Burken and Schnoor (*5*), Lang (*19*), Aitchison et al. (*21*), and Winnike (*22*).
*pKa = 4.87. **pKa = 9.99.

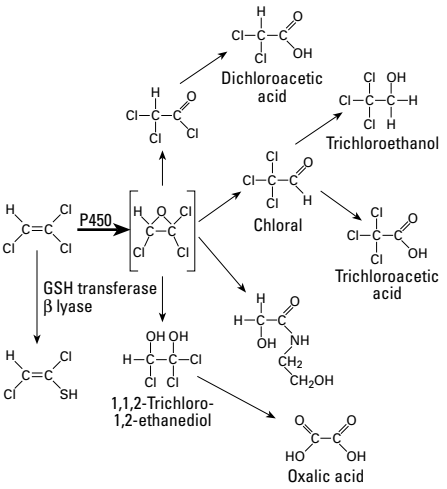

Figure 3. TCE metabolism in mammalian systems (*23*).

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, $–NH_2$, 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 monooxygenases 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 through 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 transformation 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 termed 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.

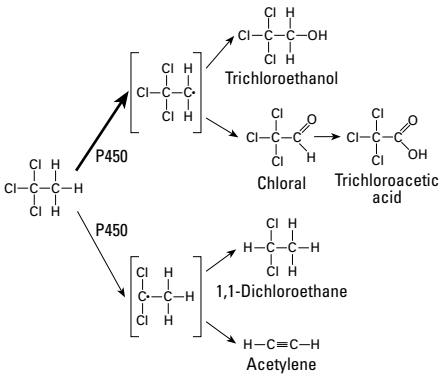

Figure 4. Metabolism of 111TCA in mammalian systems.

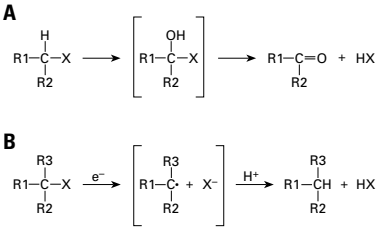

Figure 5. Oxidative (*A*) and reductive (*B*) dehalogenation activity mediated by P450s (*41*).

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)

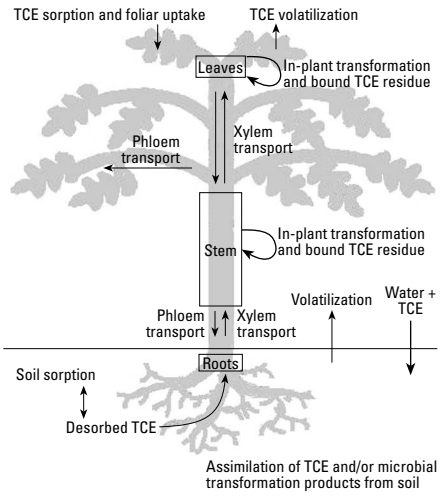

Figure 6. Potential uptake and transformation pathways of TCE in a plant–soil phytoremediation system (*45*).

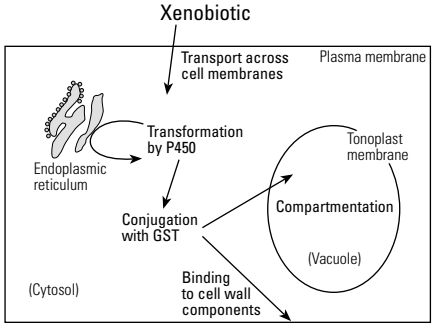

Figure 7. Likely cellular transport and metabolic processes in plants.

(glyphosate-tolerant) soybeans. Glyphosate (*N*-phosphonomethyl glycine) is the active ingredient in Roundup (Monsanto), a widely used herbicide effective against both grasses and broad-leaf weeds. The new line of soybeans contains a bacterial 5-enolpyruvylshikimate-3-phosphate synthase that is more resistant to glyphosate inhibition, allowing the modified soybean plants to withstand applications of the herbicide without reduction in yield (*50,51*).

Several transgenic plant species are being developed with phytoremediation applications in mind. Tobacco plants containing a human P450 2E1 were able to transform up to 640 times the amount of TCE compared with control plants (*52*). They also showed increased uptake and metabolism of ethylene dibromide, another halogenated hydrocarbon commonly found in groundwater. Higher tolerance to the explosives glycerol trinitrate and 2,4,6-trinitrotoluene was achieved by transgenic tobacco plants expressing a microbial pentaerythritol tetranitrate reductase (*53*). Denitration of glycerol trinitrate was also more rapid and complete in the transgenic seedlings. Although metals cannot be enzymatically degraded like organic contaminants, genetic engineering may improve phytoremediation of heavy metals. Rugh and coworkers at the University of Georgia have transferred a bacterial mercuric ion reductase into *Arabadopsis thaliana* and yellow poplar, thereby increasing mercuric ion tolerance and conversion to the less toxic elemental mercury form, which is volatilized from the transgenic plants (*54,55*).

These improvements have great potential for field applications, assuming that public acceptance of genetically modified organisms can be achieved. The potential for cross-fertilization of genetically engineered plants to wild types in the environment would need to be addressed. Sterile clones could be used because there is no need for plant reproduction in most phytoremediation applications.

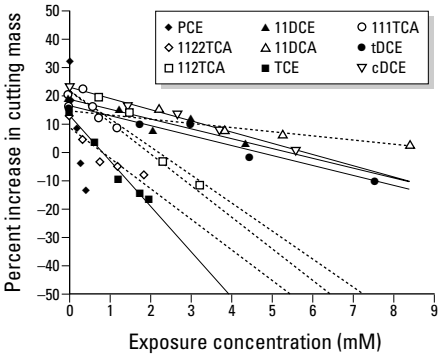

Figure 8. Abbreviations: 1122TCA, 1,1,2,2-tetrachloroethane; 11DCA, 1,1-dichloroethane. Concentration–response curves for *Populus deltoides x nigra* exposed to chlorinated ethenes (solid lines) and ethanes (dotted lines).

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 ethenes or ethanes. 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 dichlorinated ethenes [*cis*-dichloroethane (cDCE), *trans*-dichloroethylene (tDCE), and 1,1-dichloroethylene (11DCE)] and for two trichlorinated ethanes [111TCA and 1,1,2-trichloroethane (112TCA)]. Ethenes cause zero growth at lower concentrations than do similarly chlorinated ethanes. 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 carbanion intermediates formed from chlorinated ethanes. 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.

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