Phytoremediation

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Abstract

Phytoremediation, the use of plants and their associated microbes for environmental cleanup, has gained acceptance in the past 10 years as a cost-effective, noninvasive alternative or complementary technology for engineering-based remediation methods. Plants can be used for pollutant stabilization, extraction, degradation, or volatilization. These different phytoremediation technologies are reviewed here, including their applicability for various organic and inorganic pollutants, and most suitable plant species. To further enhance the efficiency of phytoremediation, there is a need for better knowledge of the processes that affect pollutant availability, rhizosphere processes, pollutant uptake, translocation, chelation, degradation, and volatilization. For each of these processes I review what is known so far for inorganic and organic pollutants, the remaining gaps in our knowledge, and the practical implications for designing phytoremediation strategies. Transgenic approaches to enhance these processes are also reviewed and discussed.

Contents

INTRODUCTION

Phytoremediation: Advantages, Limitations, Present Status

Phytoremediation: the use of plants and their associated microbes for environmental cleanup

TCE: trichloroethylene

TNT: trinitrotoluene

PAH: polycyclic aromatic hydrocarbon

MTBE: methyl tertiary butyl ether

Phytoremediation is the use of plants and their associated microbes for environmental cleanup (99, 107, 108). This technology makes use of the naturally occurring processes by which plants and their microbial rhizosphere flora degrade and sequester organic and inorganic pollutants. Phytoremediation is an efficient cleanup technology for a variety of organic and inorganic pollutants. Organic pollutants in the environment are mostly man made and xenobiotic to organisms. Many of them are toxic, some carcinogenic. Organic pollutants are released into the environment via spills (fuel, solvents), military activities (explosives, chemical weapons), agriculture (pesticides, herbicides), industry (chemical, petrochemical), wood treatment, etc. Depending on their properties, organics may be degraded in the root zone of plants or taken up, followed by degradation, sequestration, or volatilization. Organic pollutants that have been successfully phytoremediated include organic solvents such as TCE (the most common pollutant of groundwater) (90, 111), herbicides such as atrazine (22), explosives such as TNT (61), petroleum hydrocarbons such as oil, gasoline, benzene, toluene, and PAHs (4, 93, 110), the fuel additive MTBE (26, 59, 128), and polychlorinated biphenyls (PCBs) (53).

Inorganic pollutants occur as natural elements in the earth's crust or atmosphere, and human activities such as mining, industry, traffic, agriculture, and military activities promote their release into the environment, leading to toxicity (91). Inorganics cannot be degraded, but they can be phytoremediated via stabilization or sequestration in harvestable plant tissues. Inorganic pollutants that can be phytoremediated include plant macronutrients such as nitrate and phosphate (60), plant trace elements such as Cr, Cu, Fe, Mn, Mo, and Zn (76), nonessential elements such as Cd, Co, F, Hg, Se, Pb, V, and W (15, 60), and radioactive isotopes such as ^{238}U, ^{137}Cs, and ^{90}Sr (34, 35, 87).

Phytoremediation can be used for solid, liquid, and gaseous substrates. Polluted soils and sediments have been phytoremediated at military sites (TNT, metals, organics), agricultural fields (herbicides, pesticides, metals, selenium), industrial sites (organics, metals, arsenic), mine tailings (metals), and wood treatment sites (PAHs) (8, 41, 93, 101, 129). Polluted waters that can be phytoremediated include sewage and municipal wastewater (nutrients, metals), agricultural runoff/drainage water (fertilizer nutrients, metals, arsenic, selenium, boron, organic pesticides, and herbicides), industrial wastewater (metals, selenium), coal pile runoff (metals), landfill leachate, mine drainage (metals), and groundwater plumes (organics, metals) (38, 42, 52, 60, 74, 101). Plants can also be used to filter air, both outdoors and indoors, from, e.g., NO_x, SO_2, ozone, CO_2, nerve gases, dust or soot particles, or halogenated volatile hydrocarbons (64, 86).

Phytoremediation has gained popularity with government agencies and industry in the past 10 years. This popularity is based in part on the relatively low cost of phytoremediation, combined with the limited funds available for environmental cleanup. The costs associated with environmental remediation are staggering. Currently, $6–8 billion per year is spent for environmental cleanup in the United States, and $25–50 billion per year worldwide (47, 122). Because biological processes are ultimately solar-driven, phytoremediation is on average tenfold cheaper than engineering-based remediation methods such as soil excavation, soil washing or burning, or pump-and-treat systems (47). The fact that phytoremediation is usually carried out in situ contributes to its cost-effectiveness and may reduce exposure of the polluted substrate to humans, wildlife, and the environment. Phytoremediation also enjoys popularity with the general public as a "green clean" alternative to chemical plants and bulldozers. Thus, government agencies like to include phytoremediation in their cleanup strategies to stretch available funds, corporations (e.g., electric power, oil, chemical industry) like to advertise their involvement with this environment-friendly technology, and environmental consultancy companies increasingly include phytoremediation in their package of offered technologies.

The U.S. phytoremediation market now comprises ~$100–150 million per year, or 0.5% of the total remediation market (D. Glass, personal communication). For comparison, bioremediation (use of bacteria for environmental cleanup) comprises about 2% (47). Commercial phytoremediation involves about 80% organic and 20% inorganic pollutants (D. Glass, personal communication). The U.S. phytoremediation market has grown—two- to threefold in the past 5 years, from $30–49 million in 1999 (47). In Europe there is no significant commercial use of phytoremediation, but this may develop in the near future because interest and funding for phytoremediation research are increasing rapidly, and many polluted sites in new European Union countries (Eastern Europe) await remediation. Phytoremediation may also become a technology of choice for remediation projects in developing countries because it is cost-efficient and easy to implement.

Phytoremediation has advantages but also limitations. The plants that mediate the cleanup have to be where the pollutant is and have to be able to act on it. Therefore, the soil properties, toxicity level, and climate should allow plant growth. If soils are toxic, they may be made more amenable to plant growth by adding amendments, as described below. Phytoremediation is also limited by root depth because the plants have to be able to reach the pollutant. Root depth is typically 50 cm for herbaceous species or 3 m for trees, although certain phreatophytes that tap into groundwater have been reported to reach depths of 15 m or more, especially in arid climates (88). The limitations of root depth may be circumvented by deep planting of trees in boreholes (up to 12 m) or pumping up polluted groundwater for plant irrigation. Depending on the biological processes involved, phytoremediation may also be slower than the more established remediation methods like excavation, incineration, or pump-and-treat systems. Flow-through phytoremediation systems and plant degradation of pollutants work fairly fast (days or months), but soil cleanup via plant accumulation often takes years, limiting applicability. Phytoremediation may also be limited by the bioavailability of the pollutants. If only a fraction of the pollutant is bioavailable, but the regulatory cleanup standards require that all of the pollutant is removed, phytoremediation is not applicable by itself (43). Pollutant bioavailability may be enhanced to some extent by adding soil amendments, as described below.

Nonbiological remediation technologies and bio/phytoremediation are not mutually exclusive. Because pollutant distribution and concentration are heterogeneous for many sites, the most efficient and cost-effective remediation solution may be a combination of different technologies, such as excavation of the most

Rhizofiltration: use of plants in hydroponic setup for filtering polluted water

Phytoextraction: use of plants to clean up pollutants via accumulation in harvestable tissues

contaminated spots followed by polishing the site with the use of plants. Such an integrated remediation effort requires a multidisciplinary team of knowledgeable scientists.

This review aims to give a broad overview of the state of the science of phytoremediation, with references to other publications that give more in-depth information. After an introduction to the various phytoremediation technologies, the plant processes involved in uptake, translocation, sequestration, and degradation of organic and inorganic pollutants are reviewed in the context of phytoremediation. Finally, new developments including genetic engineering are discussed with respect to their prospects for phytoremediation.

Phytoremediation Technologies and Their Uses

Plants and their rhizosphere organisms can be used for phytoremediation in different ways (see **Figure 1**). They can be used as filters in constructed wetlands (60) or in a hydroponic setup (100); the latter is called rhizofiltration. Trees can be used as a hydraulic barrier to create an upward water flow in the root zone, preventing contamination to leach down, or to prevent a contaminated groundwater plume from spreading horizontally (90). The term phytostabilization denotes the use of plants to stabilize pollutants in soil (13), either simply by preventing erosion, leaching, or runoff, or by converting pollutants to less bioavailable forms

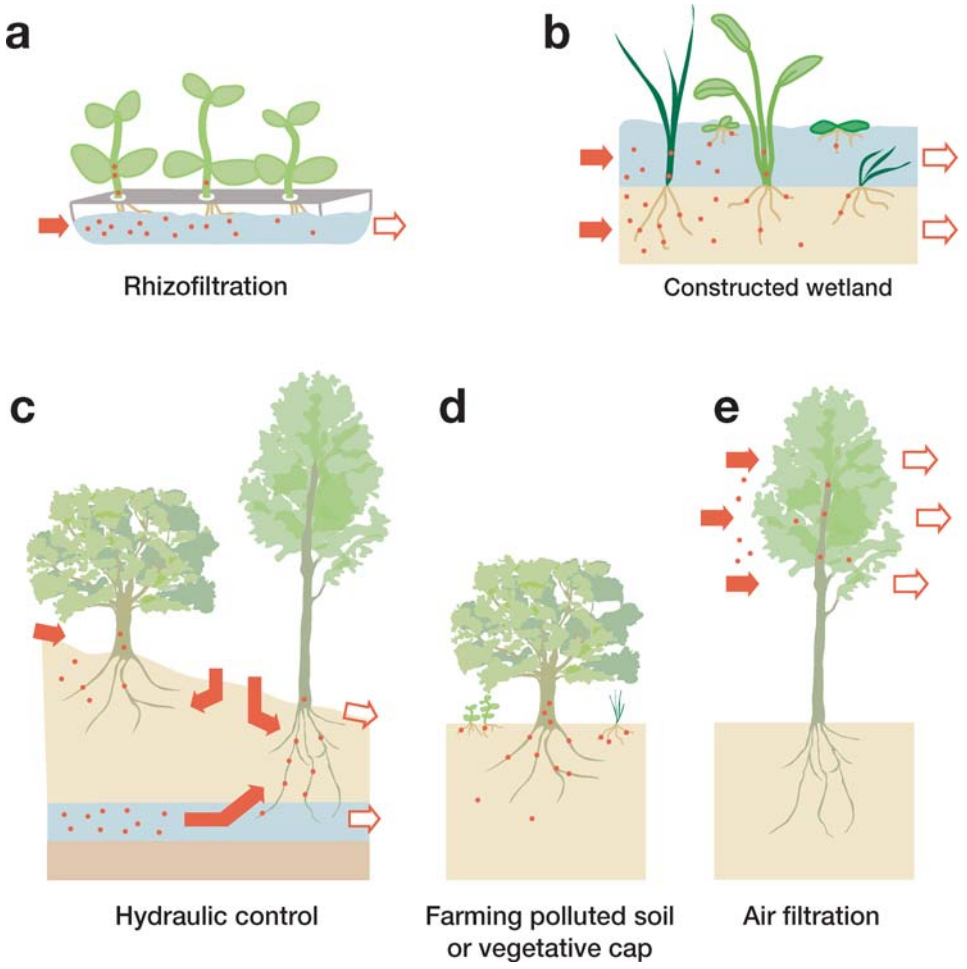

Figure 1

Phytoremediation technologies used for remediating polluted water, soil, or air. The red circles represent the pollutant.

(e.g., via precipitation in the rhizosphere). Plants can also be used to extract pollutants and accumulate them in their tissues, followed by harvesting of the (above ground) plant material. This technology is called phytoextraction (15). The plant material can subsequently be used for nonfood purposes (e.g., wood, cardboard) or ashed, followed by disposal in a landfill or, in the case of valuable metals, recycling of the accumulated element. The latter is termed phytomining (23).

Plants can facilitate biodegradation of organic pollutants by microbes in their rhizosphere (see **Figure 2**). This is called phytostimulation or rhizodegradation (82). Plants can also degrade organic pollutants directly via their own enzymatic activities, a process called phytodegradation (82). After uptake in plant tissue, certain pollutants can leave the plant in volatile form; this is called phytovolatilization (118). These various phytoremediation technologies are not mutually exclusive; for instance, in a constructed wetland, accumulation, stabilization and volatilization can occur simultaneously (52). Because the processes involved in phytoremediation occur naturally, vegetated polluted sites have a tendency to clean themselves up without human interference. This so-called natural attenuation is the simplest form of phytoremediation and involves only monitoring.

The different phytoremediation technologies described above are suitable for different classes of pollutants. Constructed wetlands have been used for a wide range of inorganics including metals, Se, perchlorate, cyanide, nitrate, and phosphate (52, 60, 92), as well as certain organics such as explosives and herbicides (60, 63, 83, 110). Rhizofiltration in an indoor, contained setup is relatively expensive to implement and therefore most useful for relatively small volumes of wastewater containing hazardous inorganics such as radionuclides (35, 87). The principle of phytostabilization is used, e.g., when vegetative caps are planted on sites containing organic or inorganic pollutants, or when trees are used as hydraulic barriers to prevent leaching or runoff of organic or inorganic contaminants. Trees can also be used in so-called buffer strips to intercept horizontal migration of polluted ground water plumes and redirect water flow upward (82). Natural attenuation is suitable for remote areas with little human use and relatively low levels of contamination. Phytoextraction is mainly used for metals and other toxic inorganics (Se, As, radionuclides) (9, 15). Phytostimulation is used for hydrophobic organics that cannot be taken up by plants but that can be degraded by microbes. Examples are PCBs, PAHs, and other petroleum hydrocarbons (62, 93). Phytodegradation works well for organics that are mobile in plants such as herbicides, TNT, MTBE, and TCE (21, 128). Phytovolatilization can be used for VOCs (128) such as TCE and MTBE, and for a few inorganics that can exist in volatile form, i.e., Se and Hg (52, 105).

Different phytotechnologies make use of different plant properties and typically different plant species are used for each. Favorable plant properties for phytoremediation in general are to be fast growing, high biomass, competitive, hardy, and tolerant to pollution. In addition, high levels of plant uptake, translocation, and accumulation in harvestable tissues are important properties for phytoextraction of inorganics. Favorable plant properties for phytodegradation are large, dense root systems

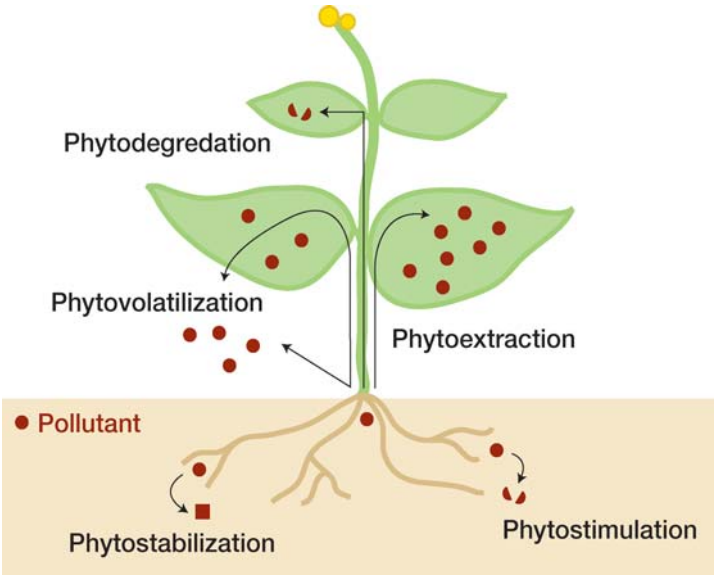

Figure 2

Possible fates of pollutants during phytoremediation: the pollutant (represented by *red circles*) can be stabilized or degraded in the rhizosphere, sequestered or degraded inside the plant tissue, or volatilized.

Rhizodegradation/ phytostimulation: degradation of pollutants in the rhizosphere due to microbial activity

Phytodegradation: breakdown of pollutants by plant enzymes, usually inside tissues

Phytovolatilization: release of pollutants by plants in volatile form

VOC: volatile organic compound

PCB: polychlorinated biphenyl

and high levels of degrading enzymes. A large root surface area also favors phytostimulation, as it promotes microbial growth; furthermore, production of specific exudate compounds may further promote rhizodegradation via specific plant-microbe interactions (93).

In constructed wetlands for phytoremediation, a variety of emergent, submerged, and floating aquatic species are used. Popular genera/species are cattail (*Typha* sp.), parrot feather (*Myriophyllum* sp.), *Elodea* sp., *Azolla* sp., duckweed (*Lemna* sp.), water hyacinth (*Eichhornia crassipes*), and *Spartina* sp. Poplar (*Populus* sp.) and willow (*Salix* sp.) can be used on the edges of wetlands. For brackish water, certain species of *Spartina* are useful, as well as pickleweed (*Salicornia* sp.) and saltgrass (*Distichlis spicata*) (74). For inorganics, the floating species water hyacinth, Azolla, and duckweed are popular because they are good metal accumulators and can be harvested easily; cattail and poplar are also used because they are tolerant, grow fast, and attain a high biomass. Aquatic plants that work well for organics remediation include parrot feather and Elodea (83) because they have high levels of organic-degrading enzymes. Rhizofiltration involves aeration and therefore is not limited to aquatic species; it often makes use of terrestrial species with large roots and good capacity to accumulate inorganics, such as sunflower (*Helianthus annuus*) or Indian mustard (*Brassica juncea*) (35).

In a vegetative cap for phytostabilization, a combination of trees and grasses may be used. Fast-transpiring trees such as poplar maintain an upward flow to prevent downward leaching, while grasses prevent wind erosion and lateral runoff with their dense root systems. Grasses tend to not accumulate inorganic pollutants in their shoots as much as dicot species (12), minimizing exposure of wildlife to toxic elements. Poplar trees are very efficient at intercepting horizontal groundwater plumes and redirecting water flow upward because they are deep rooted and transpire at very high rates, creating a powerful upward flow (27, 82).

Popular species for phytoextraction are Indian mustard and sunflower because of their fast growth, high biomass, and high tolerance and accumulation of metals and other inorganics (15, 107). A special category of plants are the so-called hyperaccumulators: plant species that accumulate one or more inorganic elements to levels 100-fold higher than other species grown under the same conditions (19). Hyperaccumulators have been reported for As, Co, Cu, Mn, Ni, Pb, Se, and Zn (7, 11, 77). These elements are typically hyperaccumulated up to 0.1–1% of dry weight even from low external concentrations. Despite these properties hyperaccumulators are not very popular for phytoremediation because they are often slow growing and attain low biomass. So far only one hyperaccumulator species, the Ni hyperaccumulator *Alyssum bertolonii*, has been used for phytoremediation in the field (23, 73). The recently discovered As hyperaccumulating fern *Pteris vittata* may also show promise for phytoextraction of As (77).

For phytostimulation of microbial degraders in the root zone, grasses such as fescue (*Festuca* sp.), ryegrass (*Lolium* sp.), *Panicum* sp., and prairie grasses (e.g., *Buchloe dactyloides*, *Bouteloua* sp.) are popular because they have very dense and relatively deep root systems and thus a large root surface area (4). Mulberry trees also enjoy popularity for use in phytostimulation because of their reported ability to produce phenolic compounds that stimulate expression of microbial genes involved in PCB and PAH degradation (44, 72, 93). For phytodegradation of TCE and atrazine, poplar has been the most popular and efficient species so far, owing to its high transpiration rate and capacity to degrade and/or volatilize these pollutants (22, 110).

Poplar is also the most-used species for phytovolatilization of VOCs because of its high transpiration rate, which facilitates the movement of these compounds through the plant into the atmosphere. For volatilization of inorganics, only Se has been investigated in detail. In general, plant species that take up and volatilize sulfur compounds also accumulate and volatilize Se well because S and Se are chemically similar and their metabolism occurs via the same pathways (2). Members of the

Brassica genus are particularly good volatilizers of Se (117). Among the aquatic species tested, rice, rabbitfoot grass, Azolla, and pickleweed were the best Se volatilizers (52, 74, 97, 133).

Finally, when choosing plant species for a certain site, it is advisable to include species that grow locally on or near the site. These species are competitive under the local conditions and, if they are growing on the site, can tolerate the pollutant.

BIOLOGICAL PROCESSES AFFECTING PHYTOREMEDIATION

Phytoremediation effectively removes pollutants, but in many cases the underlying biological mechanisms remain largely unknown. To increase the efficiency of phytoremediation technologies, it is important that we learn more about the biological processes involved. These include plant-microbe interactions and other rhizosphere processes, plant uptake, translocation mechanisms, tolerance mechanisms (compartmentation, degradation), and plant chelators involved in storage and transport. Other processes that need more study are movement of pollutants through ecosystems via the soil-water-plant system to higher trophic levels. In the following sections we follow the path of pollutants toward, into, and within the plant during phytoremediation. For each step I discuss what is known and not known about factors influencing remediation, potential limiting steps for organic and inorganic pollutants, and the practical implications for phytoremediation. Also, I discuss transgenic approaches that have been or may be used to enhance phytoremediation efficiency at each step.

Pollutant Bioavailability

For plants and their associated microbes to remediate pollutants, they must be in contact with them and able to act on them. Therefore, the bioavailability of a pollutant is important for its remediation. Pollutant bioavailability depends on the chemical properties of the pollutant, soil properties, environmental conditions, and biological activity. Soils with small particle size (clay) hold more water than sandy soils, and have more binding sites for ions, especially cations (CEC) (116). The concentration of organic matter (humus) in the soil is also positively correlated with CEC, as well as with the capacity to bind hydrophobic organic pollutants. This is because humus mainly consists of dead plant material, and plant cell walls have negatively charged groups that bind cations, as well as lignin that binds hydrophobic compounds (21).

CEC: cation exchange capacity

Two important chemical properties of a pollutant that affect its movement in soils are hydrophobicity and volatility. Hydrophobicity is usually expressed as the octanol:water partition coefficient, or log K_{ow} (121). A high log K_{ow} corresponds with high hydrophobicity. Extremely hydrophobic molecules such as PCBs, PAHs, and other hydrocarbons (log $K_{ow} > 3$) are tightly bound to soil organic matter and do not dissolve in the soil pore water. This lack of bioavailability limits their ability to be phytoremediated, leading to their classification as recalcitrant pollutants. Nonaqueous liquids may sink down to the ground water and, depending on whether they are more or less dense than water, end up below the aquifer (DNAPLs) or on top of the aquifer (LNAPLs). Organics with moderate to high water solubility (log $K_{ow} < 3$) will be able to migrate in the soil pore water to an extent that is inversely correlated with their log K_{ow}.

Log K_{ow}: the octanol:water distribution coefficient, a measure for pollutant hydrophobicity

DNAPL: dense nonaqueous phase liquid

LNAPL: light nonaqueous phase liquid

Pollutant volatility, expressed as Henry's law constant (H_i), is a measure of a compound's tendency to partition to air relative to water (26). Pollutants with $H_i > 10^{-4}$ tend to move in the air spaces between soil particles, whereas pollutants with $H_i < 10^{-6}$ move predominantly in water. If H_i is between 10^{-4} and 10^{-6}, compounds are mobile in both air and water. Both water-mobile and air-mobile organic contaminants can diffuse passively through plants. While the fate of water-mobile organics is phytodegradation or sequestration, volatile organics can be rapidly volatilized by plants without chemical modification (18).

Inorganics are usually present as charged cations or anions, and thus are hydrophilic. The bioavailability of cations is inversely correlated with soil CEC. At lower soil pH, the bioavailability of cations generally increases due to replacement of cations on soil CEC sites by H^+ ions (116). The bioavailability of ions is also affected by the redox conditions. Most terrestrial soils have oxidizing conditions, and elements that can exist in different oxidation states will be in their most oxidized form [e.g., as selenate, arsenate, Cr(VI), Fe^{3+}]. In aquatic habitats more reducing conditions exist, which favor more reduced elemental forms [e.g., selenite, arsenite, Cr(III), Fe^{2+}]. The oxidation state of an element may affect its bioavailability (e.g., its solubility), its ability to be taken up by plants, as well as its toxicity. Other physical conditions that affect pollutant migration and bioavailability are temperature and moisture. Higher temperatures accelerate physical, chemical, and biological processes in general. Precipitation will stimulate general plant growth, and higher soil moisture will increase migration of water-soluble pollutants. The bioavailability of pollutants may also be altered by biological activities, as described in the next section. In polluted soils the more bioavailable (fraction of) pollutants tend to decrease in concentration over time due to physical, chemical, and biological processes, leaving the less or nonbioavailable (fraction of) pollutants. Consequently, pollutants in aged polluted soils tend to be less bioavailable and more recalcitrant than pollutants in soil that is newly contaminated, making aged soils more difficult to phytoremediate (93).

EDTA: ethylene diamine tetra acetic acid

Understanding the processes affecting pollutant bioavailabilty can help optimize phytoremediation efficiency. Amendments may be added to soil that make metal cations more bioavailable for plant uptake. For instance, adding the natural organic acids citrate or malate will lower the pH and chelate metals such as Cd, Pb, and U from soil particles, usually making them more available for plant uptake. The synthetic metal chelator EDTA is also extremely efficient at releasing metals from soil. This principle is used in chelate-assisted phytoextraction where EDTA is added to soil shortly before plant harvesting, greatly increasing plant metal uptake (108). Before chelate-assisted phytoextraction is used in the field, it is important to do a risk assessment study to determine possible effects of the chelator on metal leaching. In other situations it may be desirable to decrease metal bioavailability if metals are present at phytotoxic levels or in phytostabilization. In such cases lime may be mixed in with the soil to increase the pH or organic matter to bind metals (12, 20). Adding organic matter also decreases the bioavailability of hydrophobic organics, whereas adding surfactants (soap) may increase their bioavailability. For organics that can exist in more or less protonated forms with different charges, manipulation of soil pH can also affect their solubility and ability to move into plants. Finally, water supply may be optimized to facilitate pollutant migration while preventing leaching or runoff.

Rhizosphere Processes and Remediation

Rhizosphere remediation occurs completely without plant uptake of the pollutant in the area around the root. The rhizosphere extends approximately 1 mm around the root and is under the influence of the plant. Plants release a variety of photosynthesis-derived organic compounds in the rhizosphere that can serve as carbon sources for heterotrophic fungi and bacteria (16). As much as 20% of carbon fixed by a plant may be released from its roots (93). As a result, microbial densities are 1–4 orders of magnitude higher in rhizosphere soil than in bulk soil, the so-called general rhizosphere effect (108). In turn, rhizosphere microbes can promote plant health by stimulating root growth (some microorganisms produce plant growth regulators), enhancing water and mineral uptake, and inhibiting growth of other, NO pathogenic soil microbes (65).

In rhizosphere remediation it is often difficult to distinguish to what extent effects are due to the plant or to the rhizosphere microbes. Laboratory studies with sterile plants

and microbial isolates can be used to address this question. Rhizosphere remediation may be a passive process. Pollutants can be phytostabilized simply via erosion prevention and hydraulic control as described above. There is also passive adsorption of organic pollutants and inorganic cations to the plant surface. Adsorption of lipophilic organics to lignin groups in the cell walls is called lignification (82). Rhizosphere remediation may also be the result of active processes mediated by plants and/or microbes. These processes may affect pollutant bioavailability, uptake, or degradation.

Pollutant bioavailability may be affected by various plant and/or microbial activities. Some bacteria are known to release biosurfactants (e.g., rhamnolipids) that make hydrophobic pollutants more water soluble (126). Plant exudates or lysates may also contain lipophilic compounds that increase pollutant water solubility or promote biosurfactant-producing microbial populations (113). Furthermore, plant- and microbe-derived enzymes can affect the solubility and thus the bioavailability of organic pollutants via modification of side groups (131).

Bioavailability of metals may be enhanced by metal chelators that are released by plants and bacteria. Chelators such as siderophores, organic acids, and phenolics can release metal cations from soil particles. This usually makes the metals more available for plant uptake (116) although in some cases it can prevent uptake (28). Furthermore, plants extrude H^+ via ATPases, which replace cations at soil CEC sites, making metal cations more bioavailable (116). Some plant roots release oxygen, such as aquatic plants that have aerenchyma (air channels in the stem that allow oxygen to diffuse to the root); this can lead to the oxidation of metals to insoluble forms (e.g., FeO_3) that precipitate on the root surface (60). Conversely, enzymes on the root surface may reduce inorganic pollutants, which may affect their bioavailability and toxicity (e.g., CrVI to CrIII) (76).

Organic pollutants may be degraded in the rhizosphere by root-released plant enzymes or via phytostimulation of microbial degradation. Examples of organics that are degraded in the rhizosphere by microbial activity include PAHs, PCBs, and petroleum hydrocarbons (62, 93). Plants can stimulate these microbial degradation processes. First, plant carbon compounds released into the rhizosphere facilitate a higher microbial density—the general rhizosphere effect. Second, secondary plant compounds released from roots may specifically induce microbial genes involved in degradation of the organic compound, or act as a cometabolite to facilitate microbial degradation (44, 72, 93). Better knowledge of these plant-microbe interactions is needed to more efficiently design phytoremediation strategies or engineer more efficient plant-microbe consortia.

Rhizosphere processes that favor phytoremedation may be optimized by the choice of plant species, e.g., plants with large and dense root systems for phytostimulation, or aquatic plants for metal precipitation. If a certain exudate compound is identified to enhance phytoremediation (e.g., a chelator or a secondary metabolite that stimulates microbial degradation) plants can be selected or genetically engineered to produce large amounts of this compound. In one such study, overexpression of citrate synthase in plants conferred enhanced aluminum tolerance, probably via enhanced citrate release into the rhizosphere, which prevented Al uptake due to complexation (28). In another approach to stimulate rhizosphere remediation, certain agronomic treatments may be employed that favor the production of general and specific exudate compounds, such as clipping or fertilization (72). Inorganic fertilizer is preferred over organic fertilizer (manure) for use in phytostimulation because the latter provides an easy-to-digest carbon source that microbes may prefer to use instead of the organic pollutant.

If the microbial consortia responsible for the remediation process are known, it may be possible to increase the abundance of these species by the choice of vegetation. An alternative approach is to grow these microbial isolates in large amounts and add them to the soil, a process called bioaugmentation. Introducing nonnative microbes to sites is considered ineffective

because they tend to be outcompeted by the established microbial populations. In another approach to optimize rhizosphere remediation, the watering regime may be regulated to provide an optimal soil moisture for plant and microbial growth. If redox reactions are involved in the remediation process, periodic flooding and draining of constructed wetlands may be effective to alternate reducing and oxidizing conditions (62).

Plant Uptake

Root concentration factor (RCF): the ratio of pollutant concentration in root relative to external solution, used as a measure for plant uptake

Uptake of pollutants by plant roots is different for organics and inorganics. Organic pollutants are usually manmade, and xenobiotic to the plant. As a consequence, there are no transporters for these compounds in plant membranes. Organic pollutants therefore tend to move into and within plant tissues driven by simple diffusion, dependent on their chemical properties. An important property of the organic pollutant for plant uptake is its hydrophobicity (17, 121). Organics with a log K_{ow} between 0.5 and 3 are hydrophobic enough to move through the lipid bilayer of membranes, and still water soluble enough to travel into the cell fluids. If organics are too hydrophilic ($\log K_{ow} < 0.5$) they cannot pass membranes and never get into the plant; if they are too hydrophobic ($\log K_{ow} > 3$) they get stuck in membranes and cell walls in the periphery of the plant and cannot enter the cell fluids. Because the movement of organics into and through plants is a physical rather than biological process, it is fairly predictable across plant species and lends itself well to modeling (26). The tendency of organic pollutants to move into plant roots from an external solution is expressed as the root concentration factor (RCF = equilibrium concentration in roots/equilibrium concentratrion in external solution).

In contrast, inorganics are taken up by biological processes via membrane transporter proteins. These transporters occur naturally because inorganic pollutants are either nutrients themselves (e.g., nitrate, phosphate, copper, manganese, zinc) or are chemically similar to nutrients and are taken up inadvertently (e.g., arsenate is taken up by phosphate transporters, selenate by sulfate transporters) (1, 112). Inorganics usually exist as ions and cannot pass membranes without the aid of membrane transporter proteins. Because uptake of inorganics depends on a discrete number of membrane proteins, their uptake is saturable, following Michaelis Menten kinetics (80). For most elements multiple transporters exist in plants. The model plant *Arabidopsis thaliana*, for instance, has 150 different cation transporters (6), and 14 transporters for sulfate alone (56). Individual transporter proteins have unique properties with respect to transport rate, substrate affinity, and substrate specificity (low affinity transporters tend to be more promiscuous) (80). These properties may be subject to regulation by metabolite levels or regulatory proteins (e.g., kinases). Furthermore, the abundance of each transporter varies with tissue-type and environmental conditions, which may be regulated at the transcription level or via endocytosis. As a consequence, uptake and movement of inorganics in plants are complex species- and conditions-dependent processes, and difficult to capture in a model.

When inorganic pollutants accumulate in tissues they often cause toxicity, both directly by damaging cell structure (e.g., by causing oxidative stress due to their redox activity) and indirectly via replacement of other essential nutrients (116). Organics tend to be less toxic to plants, partly because they are not accumulated as readily and because they tend to be less reactive. Thus, when soils are polluted with a mixture of organics and metals the inorganics are most likely to limit plant growth and phytoremediation. Phytoremediation of mixed pollutants (organics and inorganics) is an understudied area, but very relevant because many sites contain mixed pollution.

The presence of rhizosphere microbes can affect plant uptake of inorganics. For instance, mycorrhizal fungi can both enhance uptake of essential metals when metal levels are low and decrease plant metal uptake when metals are present at phytotoxic levels (46, 104). Also,

rhizosphere bacteria can enhance plant uptake of mercury and selenium (29). The mechanisms of these plant-microbe interactions are still largely unclear; microbe-mediated enhanced plant uptake may be due to a stimulatory effect on root growth, microbial production of metabolites that affect plant gene expression of transporter proteins, or microbial effects on bioavailability of the element (30).

Depending on the phytoremediation strategy, pollutant uptake into the plant may be desirable (e.g., for phytoextraction) or not (e.g., for phytostabilization). For either application, plant species with the desired properties may be selected. Screening studies under uniform conditions are a useful strategy to compare uptake characteristics of different species for different pollutants. Agronomic practices may also be employed to maximize pollutant uptake. Plant species may be selected for suitable rooting depth and root morphology (88). Furthermore, plant roots can be guided to grow into the polluted zone via deep planting in a casing, forcing the roots to grow downward into the polluted soil and to tap into polluted water rather than rainwater (88). Supplemental water (via irrigation) and oxygen (via air tube to roots) may also facilitate pollutant uptake, and soil nutrient levels may be optimized by fertilization. Not only will nutrients promote plant growth and thus uptake of the pollutant, they may also affect plant uptake of pollutants via ion competition at the soil and plant level. For instance, supplying phosphate will release arsenate from soils, making it more bioavailable; on the other hand, phosphate will compete with arsenate for uptake by plants because both are taken up by phosphate transporters (1).

It may also be possible to manipulate plant accumulation by genetic engineering. A transgenic approach that may be used to alter uptake of inorganic pollutants is overexpression or knockdown of membrane transporter proteins. This approach was used successfully to enhance accumulation of Ca, Cd, Mn, Pb, and Zn (5, 58, 123). The specificity of membrane transporters for different inorganics may also be manipulated via protein engineering (102). Furthermore, altering plant production of chelator molecules can affect plant metal accumulation (39, 49, 54, 134, 135). Hyperaccumulator species offer potentially interesting genetic material to be transferred to high-biomass species. Constitutive expression of a Zn transporter in the root cell membrane is one of the underlying mechanisms of the natural Zn hyperaccumulator *Thlaspi caerulescens* (94). Research is ongoing to isolate genes involved in metal hyperaccumulation and hypertolerance.

Chelation and Compartmentation in Roots

As mentioned above, plants can release compounds from their roots that affect pollutant solubility and uptake by the plant. Inside plant tissues such chelator compounds also play a role in tolerance, sequestration, and transport of inorganics and organics (103). Phytosiderophores are chelators that facilitate uptake of Fe and perhaps other metals in grasses; they are biosynthesized from nicotianamine, which is composed of three methionines coupled via nonpeptide bonds (57). Nicotianamine also chelates metals and may facilitate their transport (115, 127). Organic acids (e.g., citrate, malate, histidine) not only can facilitate uptake of metals into roots but also play a role in transport, sequestration, and tolerance of metals (70, 107, 127). Metals can also be bound by the thiol-rich peptides GSH and PCs, or by the Cys-rich MTs (24). Chelated metals in roots may be stored in the vacuole or exported to the shoot via the xylem. As described in more detail below, organics may be conjugated and stored or degraded enzymatically. An overview of these processes is depicted in **Figure 3**.

GSH: glutathione
PC: phytochelatin
MT: metallothionein protein

Chelation in roots can affect phytoremediation efficiency as it may facilitate root sequestration, translocation, and/or tolerance. Root sequestration may be desirable for phytostabilization (less exposure to wildlife) whereas export to xylem is desirable for phytoextraction. If chelation is desirable, it may be enhanced by selection or engineering of plants with higher levels of the chelator in question. Root

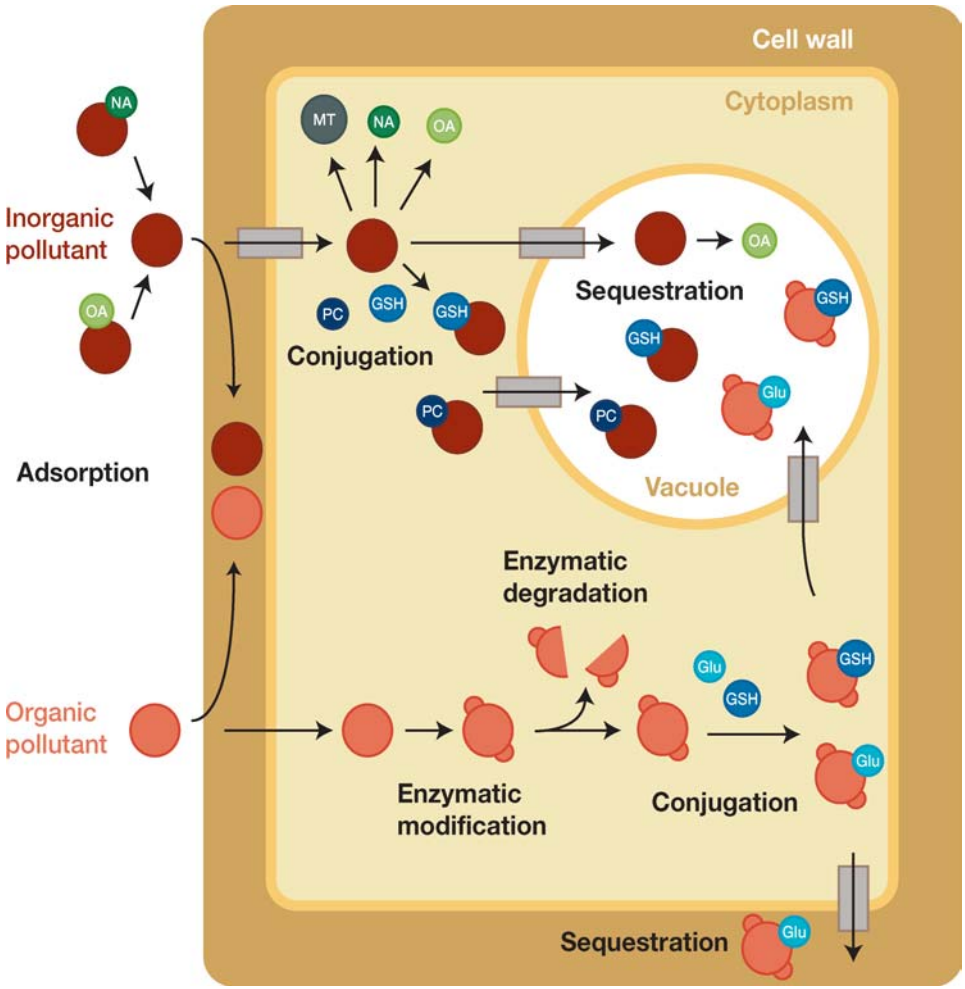

Figure 3

Tolerance mechanisms for inorganic and organic pollutants in plant cells. Detoxification generally involves conjugation followed by active sequestration in the vacuole and apoplast, where the pollutant can do the least harm. Chelators shown are GSH: glutathione, Glu: glucose, MT: metallothioneins, NA: nicotianamine, OA: organic acids, PC: phytochelatins. Active transporters are shown as boxes with arrows.

sequestration and export to xylem might be manipulated by overexpression or knockdown of the respective membrane transporters involved. Unfortunately, little is known about these tissue-specific transporters of inorganics. The completion of the sequencing of the *Arabidopsis* and rice genomes should accelerate the analysis of transporter gene families.

Translocation

Translocation from root to shoot first requires a membrane transport step from root symplast into xylem apoplast. The impermeable suberin layer in the cell wall of the root endodermis (Casparian strip) prevents solutes from flowing straight from the soil solution or root apoplast into the root xylem (116). Organic pollutants pass the membrane between root symplast and xylem apoplast via simple diffusion. The TSCF is the ratio of the concentration of a compound in the xylem fluid relative to the external solution, and is a measure of uptake into the plant shoot. Entry of organic pollutants into the xylem depends on similar passive movement over membranes as their uptake into the plant. Thus, the TSCF for organics shows a similar correlation with hydrophobicity as RCF: Compounds with a log K_{ow} between 0.5 and 3 are most easily transported to the xylem and translocated to the shoot (121).

Transpiration stream concentration factor (TSCF): the ratio of pollutant concentration in xylem fluid relative to external solution, used as a measure for plant translocation

Inorganics require membrane transporter proteins to be exported from the root endodermis into the root xylem. Some inorganics are chelated during xylem transport by organic acids (histidine, malate, citrate), nicotianamine, or thiol-rich peptides (67, 95, 115, 127). For most inorganics it is still unclear via which transporter proteins they are exported to the root xylem and to which—if any—chelators they are bound during transport. Better knowledge of the transporters and chelators involved in translocation of inorganics would facilitate the development of transgenics with more efficient phytoextraction capacity.

Bulk flow in the xylem from root to shoot is driven by transpiration from the shoot, which creates a negative pressure in the xylem that pulls up water and solutes (116). Plant transpiration depends on plant properties and environmental conditions. Plant species differ in transpiration rate, due to metabolic differences (e.g., C3/C4/CAM photosynthetic pathway) and anatomical differences (e.g., surface to volume ratio, stomatal density, rooting depth) (116). Species such as poplar are phreatophytes, or water spenders; they have long roots that tap into the ground water (27). Mature poplar trees can transpire 200–1000 liters of water per day (38, 132). In addition to plant species composition, vegetation height and density affect transpiration, as well as environmental conditions: Transpiration is generally maximal at high temperature, moderate wind, low relative air humidity, and high light (116). Consequently, phytoremediation mechanisms that rely on translocation and volatilization are most effective in climates with low relative humidity and high evapotranspiration.

Chelation and Compartmentation in Leaves

Import into leaf cells from leaf xylem involves another membrane transport step. Inorganics are taken up by specific membrane transporter proteins. Organics enter the leaf symplast from the shoot xylem by simple diffusion, the rate of which depends on the chemical properties of the pollutant, as discussed above. Once inside the leaf symplast, the pollutant may be compartmentized in certain tissues or cellular locations. In general, toxic pollutants are sequestered in places where they can do the least harm to essential cellular processes. At the cellular level, pollutants are generally accumulated in the vacuole or cell wall (21, 24). At the tissue level they may be accumulated in the epidermis and trichomes (50, 69).

When pollutants are sequestered in tissues, they are often bound by chelators or form conjugates (see **Figure 3**). Toxic inorganics are usually metals. Chelators that are involved in metal sequestration include the tripeptide GSH (γ-glu-cys-gly) and its oligomers, the PCs. XAS has shown that inorganics that were complexed by PCs in vivo include Cd and As (95); there may be others since PC synthesis is induced by various other metals (24). After chelation by GSH or PCs, an ABC-type transporter actively transports the metal-chelate complex to the vacuole, where it is further complexed by sulfide (24, 75). Organic acids such as malate and citrate are also likely metal (e.g., Zn) chelators in vacuoles, as judged from XAS (70). Ferritin is an iron chelator in chloroplasts (120). Additional metal-chelating proteins exist (e.g., MTs) that may play a role in sequestration and tolerance (e.g., of Cu) and/or in homeostasis of essential metals (48). There is still much to be discovered about the roles of these different chelators in transport and detoxification of inorganic pollutants.

XAS: X-ray absorption spectroscopy

Conjugation to GSH also plays a role in sequestration and tolerance of organic pollutants (78). A large family of GSTs with different substrate specificities mediate conjugation of organics to GSH in the cytosol (55, 68, 89). The glutathione S-conjugates are actively transported to the vacuole or the apoplast by ATP-dependent membrane pumps (79, 81, 109, 130). An alternative conjugation-sequestration mechanism for organics in plants involves coupling a glucose or a malonyl-group to the organic compound, followed by transport of the conjugate to the vacuole or the apoplast (25). These conjugation steps are mediated by

GST: GSH-S-transferases

a family of glucosyltransferases and malonyltransferases, and the transport steps by ATP-dependent pumps (21).

To be conjugated, the organic compound may need chemical modification to create suitable side groups for conjugation. These modification reactions can be oxidative or reductive. For example, cytochrome P450 monooxygenases catalyze an oxidative transformation, incorporating an O atom from oxygen into an organic molecule such as atrazine to create a hydroxyl side group (25). Nitroreductases are an example of enzymes that mediate a reductive transformation, converting a nitro group of, e.g., TNT to an amino group (83). Other enzymes that mediate modifications of organic pollutants include dioxygenases, peroxidases, peroxygenases, and carboxylesterases (21). Thus, accumulation of organic pollutants typically comprises three phases: chemical modification, conjugation, and sequestration (**Figure 3**). This sequence of events has been summarized as the "green liver model" because of its similarity to mammalian detoxification mechanisms (21, 109). Some natural functions of the enzymes and transporters involved are to biosynthesize and transport natural plant compounds such as flavonoids, alkaloids, and plant hormones, and to defend against biotic stresses (78, 98).

Uptake and accumulation in leaves without toxic effects are desirable properties for phytoextraction. To maximize these processes, plants may be selected or engineered that have higher levels of transporters involved in uptake of an inorganic pollutant from the xylem into the leaf symplast. Better knowledge of the transporters involved in the process would be helpful because this is still a largely unexplored area. Similarly, plants with high transporter activities from cytosol to vacuole can be more efficient at storing toxic inorganics (58, 114, 123). Sequestration and tolerance may also be enhanced by selection or engineering of plants with higher production of leaf chelators or conjugates. This can be mediated by higher levels of enzymes that produce these conjugates, e.g., enzymes synthesizing GSH, PCs, glucose, organic acids, or chelator proteins (49, 54, 134, 135). In addition, enzymes that couple the chelator or conjugant to the pollutant (GSH transferases, glucosyltransferases) may be overexpressed (40) or enzymes that modify organics to make them amenable to conjugation (32, 33, 51).

In all cases where potentially toxic pollutants are accumulated in plant tissues, phytoremediation in the field should include a risk assessment study because the plant material may pose a threat to wildlife. The degree of toxicity will depend on leaf concentration but also on the form of the pollutant that is accumulated. During accumulation the toxicity of the pollutant may change. To test the potential toxicity of the plant material, a laboratory digestibility study may be done using model organisms or in vitro simulations of animal digestion systems. In the field, exposure to wildlife may be minimized by, e.g., fencing, netting, noise, and scarecrows.

Degradation

Only organic pollutants can be phytoremediated via degradation. Inorganic elements are undegradable and can only be stabilized or moved and stored. In phytodegradation plant enzymes act on organic pollutants and catabolize them, either mineralizing them completely to inorganic compounds (e.g., carbon dioxide, water and Cl_2), or degrading them partially to a stable intermediate that is stored in the plant (82). This enzymatic degradation of organics can happen in both root and shoot tissue. Degradation within plant tissues is generally attributed to the plant, but may in some cases involve endophytic microorganisms (10).

Phytodegradation involves some of the same classes of enzymes responsible for accumulation in tissues. The modifying enzymes that create side groups on organics that increase solubility and enable conjugation also play a role in the initial steps of phytodegradation. Thus, enzyme classes involved in phytodegradation include dehalogenases, mono- and dioxygenases, peroxidases, peroxygenases, carboxylesterases, laccases, nitrilases, phosphatases, and nitroreductases (131). Also, if pollutants are only

partially degraded and the degradation products stored in plants, these are often conjugated and sequestered by the same mechanisms described above, involving GSH-S-transferases, malonyl- and glucosyltransferases, and ATP-dependent conjugate-transport pumps (21). These degradation products of pollutants that accumulate in vacuoles or apoplast of plant tissues are called bound residues (21). Atrazine and TNT are examples of organic pollutants that are partially degraded followed by storage of the degradation products as bound residues (14, 22). For TCE, different results were obtained in different studies: Overall, TCE appears to be in part volatilized by the plant, part is stored as bound residue, and part may be completely degraded (111). Phytoremediation of TCE is a much-studied process, and the remaining uncertainty about its fate illustrates that still much remains to be learned about the metabolic fate of organics in plants. Better knowledge in this respect would be beneficial not only for further improvement of phytoremediation efficiency, but also for better estimating the potential risks involved.

Phytodegradation of organic pollutants may be optimized by selecting or engineering plant species with higher activities of the enzymes thought to be involved and rate-limiting. There are some examples of promising transgenic approaches. The expression in plants of bacterial enzymes involved in reductive transformation of TNT (tetranitrate reductase or nitroreductase) resulted in enhanced plant tolerance and degradation of TNT (45, 51). Also, the constitutive expression of a mammalian cytochrome P450 in tobacco resulted in an up to 640-fold higher ability to metabolize TCE (33).

Volatilization

Phytovolatilization is the release of pollutants from the plant to the atmosphere as a gas. Inorganic Se can be volatilized by plants and microorganisms. Volatilization of Se involves assimilation of inorganic Se into the organic selenoaminoacids selenocysteine (SeCys) and selenomethionine (SeMet). The latter can be methylated to form dimethylselenide (DMSe), which is volatile (119). Volatilization of the inorganics As and Hg has been demonstrated for microorganisms, but these elements do not appear to be volatilized to significant levels by (nontransgenic) plants (105).

Many VOCs can be volatilized passively by plants. Volatile pollutants with a Henry's law constant $H_i > 10^{-6}$ that are mobile in both air and water can move readily from the soil via the transpiration stream into the atmosphere (18). In this way, plants act like a wick for VOCs to facilitate their diffusion from soil. Examples of organic pollutants that can be volatilized by plants are the chlorinated solvent TCE and the fuel additive MTBE (26, 90).

Because volatilization completely removes the pollutant from the site as a gas, without need for plant harvesting and disposal, this is an attractive technology. In the case of Se, the volatile form was also reported to be 2–3 orders of magnitude less toxic than the inorganic Se forms (119). Volatilization may be promoted in several ways. Although volatilization of VOCs is passive, the process may be maximized by using phreatophyte species with high transpiration rates and by promoting transpiration (preventing stomatal closure through sufficient irrigation). For Se, enzymes of the S assimilation pathway mediate Se volatilization, and overexpression of one of these, cystathionine-γ-synthase promotes Se volatilization (124). In another approach, the enzyme SeCys methyltransferase from a Se hyperaccumulator species was expressed in a nonaccumulator, also significantly enhancing Se volatilization (71). Volatilization of mercury by plants was achieved by introducing a bacterial mercury reductase (MerA). The resulting plants volatilized elemental mercury and were significantly more Hg-tolerant (105).

If a toxic volatile pollutant is emitted by plants during phytoremediation, the fate of the gas in the atmosphere should be determined as part of risk assessment. Such a study was done for volatile Se and Hg, and the pollutant was reportedly dispersed and diluted to such an extent that it did not pose a threat (74, 85).

NEW DEVELOPMENTS IN PHYTOREMEDIATION

In the past 10 years phytoremediation has gained acceptance as a technology and has been acknowledged as an area of research. There has already been a substantial increase in our knowledge of the mechanisms that underlie the uptake, transport, and detoxification of pollutants by plants and their associated microbes. Still, large gaps in our knowledge await further research, as indicated above. Phytoremediation efficiency is still limited by a lack of knowledge of many basic plant processes and plant-microbe interactions. There is also a need for more phytoremediation field studies to demonstrate the effectiveness of the technology and increase its acceptance.

Continued phytoremediation research should benefit from a (more) multidisciplinary approach, involving teams with expertise at all organization levels, to study the remediation of pollutants from the molecule to the ecosystem. Phytoremediation research at universities is generally carried out by scientists with expertise at a certain organizational level (e.g., plant molecular biology, plant biochemistry, plant physiology, ecology, or microbiology) and of a certain subset of pollutants (e.g., heavy metals, herbicides, TNT, or PAHs). Because research on phytoremediation of organics and inorganics requires different expertise they are carried out in different research communities, with more engineers studying organics and more biologists studying inorganics. These researchers do not interact optimally, in part because of a lack of phytoremediation conferences and scientific journals that cover inorganics and organics equally. Because 64% of polluted sites contain mixtures of organics and inorganics (36), phytoremediation would benefit from more collaborative studies by teams of researchers from different backgrounds, to combine expertise in phytoremediation of both types of pollution and at multiple organization levels.

Despite the remaining gaps in our knowledge, research has yielded much useful knowledge for phytoremediation, as described above. This has also resulted in practical phytoremediation resources, such as online databases of plant species that may be useful for cleanup of different types of pollutants (84) (PHYTOPET lists species particularly useful for cleanup of petroleum hydrocarbons and PHYTOREM lists plants that are recommended for metals and metalloids). The U.S. Environmental Protection Agency also maintains a phytoremediation Web site (http://www.clu-in.org) with a wealth of information for researchers and the general public (e.g., citizen's guides, phytoremediation resource guide) (37, 38).

Future field phytoremediation projects should benefit from (more) collaboration between research groups and industry so that they can be designed to address hypotheses and gain scientific knowledge in addition to meeting cleanup standards. Future field phytoremediation projects will also benefit from coordinated experimental design across projects so that results can be better compared.

An interesting development in phytoremediation is its integration with landscape architecture. Remediation of urban sites (parks, nature areas) may be combined with an attractive design so that the area may be used by the public during and after the remediation process while minimizing risk (66). Other sites that are phytoremediated may be turned into wildlife sanctuaries, like the Rocky Mountain Arsenal in Denver, once one of the most polluted sites in the United States (http://www.pmrma.army.mil/).

Another new development in phytoremediation is the use of transgenic plants. Knowledge gained from plant molecular studies in the past 10 years has led to the development of some promising transgenics that show higher tolerance, accumulation, and/or degradation capacity for various pollutants, as described above. So far, these transgenics have mainly been tested in laboratory studies using artificially contaminated medium rather than soils from the field, let alone field studies. However, this is starting to change. One field phytoremediation study using transgenic Indian mustard plants that overexpress enzymes involved in sulfate/

selenate reduction and in accumulation of GSH was just completed (96, 134, 135). Three types of transgenic Indian mustard plants that overexpress enzymes involved in sulfate/selenate reduction and in accumulation of GSH showed enhanced Se accumulation in the field when grown on soil polluted with Se, B, and other salts (G. Banuelos, N. Terry, D. LeDuc, E. Pilon-Smits & C. Mackey, unpublished results). Earlier, these same transgenics showed enhanced capacity to accumulate Se and heavy metals (Cd, Zn) from polluted soil from the field in greenhouse experiments (12, 125). Another field experiment testing Hg volatilizing (MerA) poplar trees is presently underway (D. Glass, personal communication).

In the coming years, mining of the genomic sequences from *Arabidopsis thaliana* and rice and availability of new genomic technologies should lead to the identification of novel genes important for pollutant remediation, including regulatory networks (e.g., transcription factors) and tissue-specific transporters. The expression of these genes may then be manipulated in high-biomass species for use in phytoremediation. Other new developments in plant genetic engineering are tailored transgenics that overexpress different enzymes in different plant parts (e.g., root-specific expression of one gene and shoot-specific expression of another) or that express a transgene only under certain environmental conditions (31). Also, genetic engineering of the chloroplast genome offers a novel way to obtain high expression without the risk of spreading the transgene via pollen (106). In another totally new approach, it was shown to be possible to genetically manipulate an endophytic microorganism, leading to enhanced toluene degradation (10).

As transgenics are being tested in the field and the associated risks assessed, their use may become more accepted and less regulated, as has been the case for transgenic crops. Also, as more information becomes available about the movement of pollutants in ecosystems and the associated risks, the rules for cleanup targets may be adjusted depending on future use of the site, bioavailability of the pollutant, and form of the pollutant. Because phytoremediation only remediates the bioavailable fraction of the pollution, stringent cleanup targets limit the applicability of this technology. If targets can be adjusted to focus on the bioavailable (i.e., toxic) fraction of the pollutant, phytoremediation could become more widely applicable. This would reduce cleanup costs and enable the cleanup of more sites with the limited funds available.

SUMMARY POINTS

1. Plants and their associated microbes can remediate pollutants via stabilization, degradation in the rhizosphere, degradation in the plant, accumulation in harvestable tissues, or volatilization.
2. Phytoremediation offers a cost-effective and environment-friendly alternative or complementary technology for conventional remediation methods such as soil incineration or excavation and pump-and-treat systems.
3. Although phytoremediation works effectively for a wide range of organic and inorganic pollutants, the underlying biological processes are still largely unknown in many cases. Some important processes that require further study are plant-microbe interactions, plant degradation mechanisms for organics, and plant transport and chelation mechanisms for inorganics.
4. New knowledge and plant material obtained from research is being implemented for phytoremediation in the field. The first field tests with transgenic plants are showing

promising results. As more results demonstrating the effectiveness of phytoremediation become available its use may continue to grow, reducing cleanup costs and enabling the cleanup of more sites with the limited funds available.

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***Burkholderia cepacia*, a bacterial endophyte of yellow lupine, was transformed with a plasmid from a related strain containing genes that mediate toluene degradation. After infection of lupine with the modified strain, the resulting plants were more tolerant to toluene and volatilized less of it through the leaves. This is the first example of genetic modification of an endophyte for phytoremediation.**

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This is one of the first examples of manipulation of plant rhizosphere processes.

This was one of the first investigations of plant-microbe interactions in the context of phytoremediation.

This is an example of a tailored transgenic that overexpresses more than one enzyme and expression is targeted to specific tissues to give maximal effect.

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This shows potential for tailoring transporters to specifically take up metals of interest while excluding other metals.

Introduction of the bacterial *MerA* gene resulted in enhanced Hg tolerance in *Arabidopsis*.

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