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MINI-REVIEW

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Phytoremediation of toxic aromatic pollutants from soil

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Abstract The enormous growth of industrialization, and the use of numerous aromatic compounds in dyestuffs, explosives, pesticides and pharmaceuticals has resulted in serious environmental pollution and has attracted considerable attention continuously over the last two decades. Many aromatic hydrocarbons, nitroaromatic compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, diauxins and their derivatives are highly toxic, mutagenic and/or carcinogenic to natural microflora as well as to higher systems including humans. The increasing costs and limited efficiency of traditional physicochemical treatments of soil have spurred the development of new remediation technologies. Phytoremediation is emerging as an efficient treatment technology that uses plants to bioremediate pollutants from soil environments. Various modern tools and analytical devices have provided insight into the selection and optimization of remediation processes by various plant species. Sites heavily polluted with organic contaminants require hyperaccumulators, which could be developed by genetic engineering approaches. However, efficient hyperaccumulation by naturally occurring plants is also feasible and can be made practical by improving their nutritional and environmental requirements. Thus, phytoremediation of organics appears a very promising technology for the removal of contaminants from polluted soil. In this review, certain aspects of plant metabolism associated with phytoremediation of organic contaminants and their relevant phytoremediation efforts are discussed.

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Introduction

Over the past centuries, rapid growth of population, mining, industrialization, etc., have significantly contributed to extensive soil contamination. Various physical, chemical and biological processes have been employed for effective remediation of contaminated soil. The remediation strategy depends on the nature of the contaminant(s). Soils contaminated with different organic compounds can be treated by thermal desorption, soil washing, incineration and some landfilling. Various organic contaminants, primarily petroleum hydrocarbons, aromatic hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), nitroaromatic compounds (NACs) and chloroaromatics, etc., are amenable to microbiological treatment. The microbial remediation of various environmental pollutants cannot be exploited successfully under field conditions. Drastic changes in weather over time would not allow the microorganisms to remove pollutants totally from the environment, thus precluding the reclaiming of natural soil amendments. Phytoremediation is the term used to describe those methodologies that employ living higher organisms, which include green vegetation, plants, aquatic plants, trees and grasses, to remove toxic compounds. This technology has the advantage of in situ treatment of contaminated soils, sediments, groundwater, surface water and external atmosphere (Shimp et al. 1993; Cunningham et al. 1995; Macek et al. 2000).

The specific advantages, limitations and economics of phytoremediation have been extensively reviewed (Cunningham et al. 1995; Pletsch et al. 1999; Burken et al. 2000; Macek et al. 2000). Current research efforts are now focused on expanding phytoremediation to address soil and atmospheric pollutants, as plants have a large capacity to bind with organic compounds from the air (Salt et al. 1998). The major targets of phytoremediation are toxic heavy metals and pollutants that persist in soil for hundreds of years at smelter and mining sites, gas manufacturing plant sites, ammunition waste sites, landfills, nuclear waste dumps, over-fertilized farmland, stagnant marshland, rich effluent, and sludge from agricultural, industrial and municipal wastes. These sites contain a number of toxic compounds as indicated above. Some classes of organic compounds are relatively more rapidly degraded in the rhizospheric region; these include polychlorinated pesticides (PCP), polychlorinated biphenyls (PCBs), petroleum hydrocarbons, nitroaromatic compounds, explosives, surfactants and organophosphate insecticides (Mackova et al. 1998; Pradhan et al. 1998; Yateem et al. 1999; Alkorta and Garbisu 2001). Usually, plants use photosynthetic energy to scavenge these organic compounds from the soil and to concentrate them in their biomass according to nutritional requirements (Krämer and Chardonnens 2001). The stimulation of removal of pollutant contaminants from soil could be brought about through several mechanisms of plant/soil interaction, including: (1) increase in soil microbial activity, (2) increase in microbial association with the root and toxic compounds, and (3) changes in the physical and chemical properties of the contaminated soil. The development of methods to remediate soils and groundwater contaminated with toxic pollutants and other organic residues has been an area of intense research interest for several decades (Aprill and Sims 1990; Cunningham et al. 1995; Shanks and Morgan 1999; Macek et al. 2000; Meagher 2000). Some of these investigations have been targeted towards rapid disappearance of organic pollutants in aquatic plant systems (Schnoor et al. 1995; Burken et al. 2000) and an improved understanding of plant metabolism allowing the true potential of phytoremediation to be realized.

Contaminant attenuation in phytoremediation

The introduction of plants to the polluted site(s) has the potential to yield several indirect contaminant attenuation mechanisms, which assist in the removal of toxic substances/management of polluted sites. However, contaminant attenuation mechanisms involved in phytoremediation are complex and not limited only to the direct metabolism of contaminants by plants. Certain indirect attenuation mechanisms are involved in phytoremediation, such as the metabolism of contaminants by plant-associated microbes, and plant-induced changes in the contaminated environment. In terrestrial species, transport of contaminants to the plant is dominated by the uptake of water by roots, and distribution within the plant relies on xylem or phloem transport (Shimp et al. 1993; Macek et al. 2000). Various terms, reflecting each specific attenuation mechanism, have been extensively used to better describe specific applications of phytoremediation. These include phytoextraction, phytodegradation, phytotransformation, phytovolatilization and rhizodegradation (Burken et al. 2000; Meagher 2000).

Phytoextraction

The term refers to the use of pollutant-accumulating plants to remove metals or organics from soil by concentrating them in harvestable parts of the plant. The process often occurs with heavy metals, radionuclides and certain organic compounds that are resistant to plant metabolism, by uptake and translocation of such compounds in the soil by plant tissue in a recoverable form. Such hyperaccumulation is only possible when plants grow vigorously and produce over 3 t dry matter/hectare (Shimp et al. 1993; Salt et al. 1998; Yateem et al. 1999) able to accumulate large concentrations of the contaminant(s) in the harvestable plant tissue (>1,000 mg/kg). Various naturally occurring metal hyperaccumulators are present in nature that can accumulate 10–500 times higher levels of elements than crop plants. After a certain time period, the plants are harvested and disposed of or processed by incineration or, in the case of organic pollutants, composted for recycling. After incineration of plants, careful disposal in a hazardous waste landfill that provides effective site decontamination of bioavailable contaminants including PAHs and NACs is required (Huang et al. 1997; Pradhan et al. 1998; Pletsch et al. 1999; Macek et al. 2000). The non-bioavailability of other pollutants can be made more bioavailable by adding chemical additives such as biosurfactant, EDTA, etc., to the soil (Sandermann 1994; Maier et al. 2001).

Phytodegradation/phytotransformation

Phytodegradation/phytotransformation refers to a process beyond uptake and storage of contaminants. Phytodegradation has been studied extensively to understand the fate of herbicides in crop plants. Several reports on herbicide phytodegradation have been extended to cell cultures of non-crop species including hybrid poplar trees (Bockers et al. 1994; Thompson et al. 1998). In phytodegradation/phytotransformation, contaminants are taken up from soil/water, metabolized in plant tissues and broken up to less toxic or non-toxic compounds within the plant by several metabolic processes via the action of compounds produced by the plant (Shimp et al. 1993; Salt et al. 1998; Burken et al. 2000; Macek et al. 2000; Meagher 2000). The overall metabolic process involved in phytodegradation is in some ways analogous to human metabolism of xenobiotic chemicals; thus, a ‘green liver’ conceptual model is often used to describe phytodegradation (Sandermann 1994). The uptake of hydrophobic organic chemicals is very efficient while extremely hydrophobic or hydrophilic compounds are not very good candidates for phytoremediation. Such contaminants cannot be easily translocated within the plant, as they are either bound strongly to the surface of the roots or are not sorbed by roots and are actively transported through plant membranes (Burken et al. 2000; Meagher 2000).

Phytovolatilization

Another form of phytotransformation is phytovolatilization, in which volatile chemicals or their metabolic chemical compounds are released into the atmosphere through plant transpiration. Certain organic pollutants that are recalcitrant in the sub-surface environment react rapidly in the atmosphere with hydroxyl radical, an oxidant formed in the photochemical cycle. Very few contaminants are sufficiently water soluble, non-toxic to plants, and volatile enough to reach atmospheric concentrations that would be of concern by evapotranspiration (Davis et al. 1996). The process has been observed for contaminants such as trichloroethylene (TCE), PCBs and total petroleum hydrocarbon (Sandermann 1994; Schwab and Banks 1994; Davis et al. 1996; Burken and Schnoor 1999; Macek et al. 2000). Transfer of contaminants from the soil or groundwater to the atmosphere is not as desirable as in situ degradation, but it may be preferable to prolonged exposure in the soil environment and the risk of groundwater contamination.

Rhizodegradation

The remediation process in which the contaminant is transformed by microbes in the rhizosphere (i.e., the microbe-rich zone in intimate contact with the vascular root system of the plant) is referred to as rhizodegradation/rhizosphere bioremediation. In the rhizosphere, soil redox conditions, organic content, moisture, and other soil properties are manipulated by the activity of plant roots. Rhizodegradation is responsible for the enhanced removal of total petroleum hydrocarbons from soil by deep-rooted trees and other annual species (Schwab and Banks 1994; Carman et al. 1998). The fate of PAHs and other organic contaminants in the environment is associated with both abiotic and biotic processes, including chemical oxidation, bioaccumulation and microbial transformation. Microbial activity has been deemed the most influential and significant cause of PAH removal (Cerniglia 1997). Recent studies have indicated that stimulation of microbial activity in the rhizosphere of plants can also stimulate biodegradation of various toxic organic compounds (Siciliano et al. 1998; Liste and Alexander 2000; Daane et al. 2001). The general rhizosphere effect is well known and has been described as the zone of soil under the direct influence of plant roots, which usually extends a few millimeters from the root surface and is a dynamic environment for microorganisms (Curl and Truclove 1986). The rhizosphere microbial community is comprised of microorganisms with different types of metabolism and adaptive responses to variation in environmental conditions (Fig. 1). Microbial activity is thus generally higher in the rhizosphere due to the presence of readily biodegradable substrates that are exuded from the plant (Paul and Clark 1989).

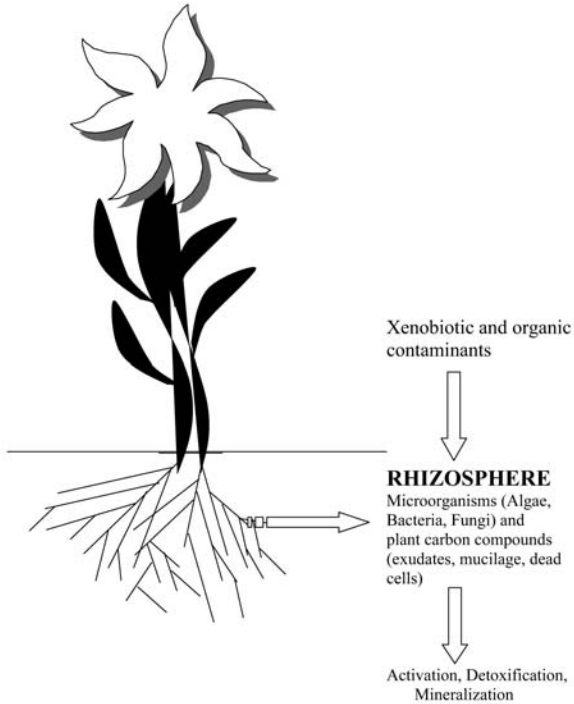

Fig. 1 Rhizodegradation of toxic contaminants by rhizospheric microorganisms

Contaminant metabolism during phytoremediation in plants

The specific interactions of a pollutant with soil, water and plants will vary depending on the chemical properties of the contaminant, the physiological properties of the introduced plant species and the contaminated medium. Organic compounds can be translocated to other plant tissues (Salt et al. 1998) and subsequently volatilized; they may undergo partial or complete degradation or they may be transformed to less phytotoxic compounds and bound in plant tissues. Collectively, these properties determine whether a contaminant is subjected to phytoextraction, phytodegradation, phytovolatilization or rhizodegradation, although in all cases, the process of phytoremediation begins with contaminant transport to the plant. In general, most organics appear to undergo some degree of transformation in plant cells before being sequestered in vacuoles or bound to insoluble cellular structures such as lignin. In wetland plant species, contaminants can enter through the roots or can partition from the water column directly into plant tissues (Fig. 1). The metabolism of certain non-agricultural contaminants such as PAHs, TCE, 2,4,6-trinitrotoluene (TNT), glyceroltrinitrate (GTN) and other cholorinated compounds has been documented (Trapp 1995; Yateem et al. 1999; Macek et al. 2000; Alkorta and Garbisu 2001). Uptake in terrestrial plants has been studied for many plant-contaminant combinations, and quantitative models to predict uptake rates have been documented (Trapp 1995). These models, which are based on flow in the transpira-

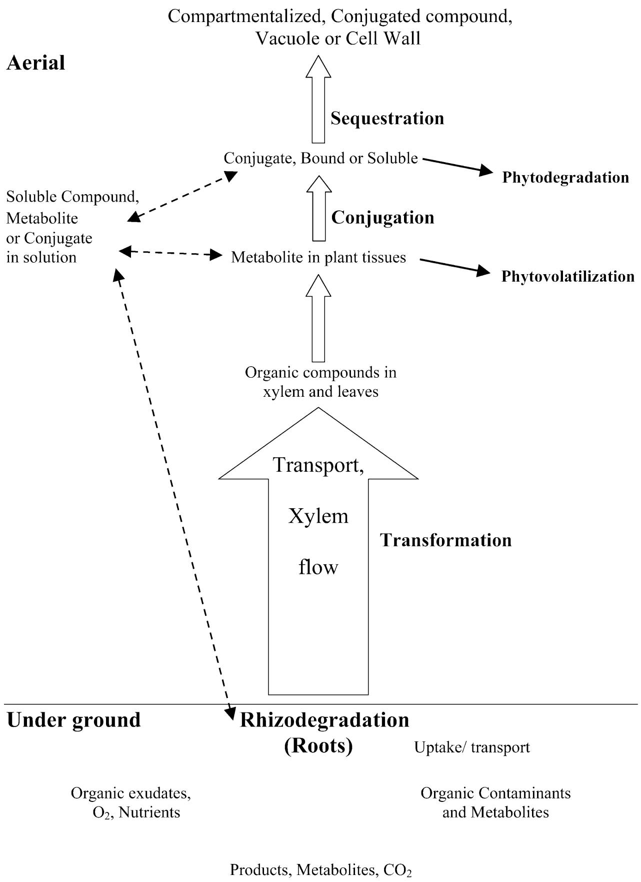

Fig. 2 Schematic representation of mechanism of organic contaminants removal and 'green liver' model for metabolism of xenobiotics in plants. *Dashed lines* Possible transport mechanism in aquatic plants, *solid/hollow arrows* established contaminant transportation routes in plant during phytoremediation

tion stream and organic partition relationships, have been used in the development of transpirational stream concentration factors (TSCF) for various xenobiotic compounds (Burken and Schnoor 1998). The 'green liver' model is often used to describe the fate and disposition of organic contaminants (xenobiotic compounds) within plants (Sandermann 1994). Metabolism of foreign compounds in plant systems is generally considered to be a 'detoxification' process that is similar to the metabolism of xenobiotic compounds in humans (Burken et al. 2000), hence the name 'green liver'. The detoxification of xenobiotics is carried out in three stages: transformation, conjugation and sequestration (Fig. 2). Certain initial reactions such as reduction, oxidation, and hydrolysis give rise to compounds that are amenable to subsequent conjugation reactions. The oxidation reactions are the predominant transformation reactions in the metabolism of pesticides; however, these reactions have also been found for certain nitroaromatic compounds (Komoßa et al. 1995). The xenobiotic compound undergoes conjugation with an organic molecule of plant origin, which plays a key role in the metabolism of xenobiotic compounds by the plant because it leads to a reduction in toxicity to the plant (Trapp 1995). Following conjugation, the resulting compound can undergo various sequestration processes. The storage of certain conjugates in plant organelles such as vacuoles is a common mechanism, whereupon the compound is no longer capable of interfering with cell function. The xenobiotic conjugates can also be incorporated into biopolymers such as lignin where they are characterized as nonextractable/bound residues, whereas in aquatic/wetland plants they can be excreted for storage outside the plant (Fig. 2).

Enzyme involvement in phytoremediation

During phytoremediation, oxygenation is a common process in pesticide and herbicide metabolism and it is an important mode of attachment when certain microorganisms encounter what are often highly lipophilic compounds (Cole 1983). Studies to determine the specific enzymes responsible for oxidative transformations have not yet been performed. According to current understanding of the metabolism of several NACs, cytochrome P450 enzymes are localized primarily in the microsomes (endoplasmic reticulum) of the plant cell and need molecular oxygen as a secondary substrate, with NADPH or NADH as cofactors (Hatzios and Penner 1982; Sandermann 1994). Recent investigations have shown that plants appear to contain sets of specific metabolic isoenzymes and their corresponding genes (Komoßa et al. 1995; Pena and Seguin 2001; Shang et al. 2001). The metabolic pathways in animals and plants seem to be similar, although more complex metabolic processes occur in plants than in animals, with an important difference from animal metabolism being especially in the formation of bound residues. This belief is based on the production of similar TCE metabolites in both plants and mammals. Many of the enzyme systems involved in metabolism of TCE in mammals are also found in plants (e.g., cytochrome P450 oxygenases and glutathione S-transferases) (Cunningham and Ow 1996). TCE metabolism in poplar may be similar to the mammalian breakdown of TCE (Newman et al. 1998). A TCE metabolite, trichloroethanol, further glycosylated into SS-D-glucoside, did not persist in plant tissues after removal of the plant from the contaminated site, indicating further metabolism (Shang et al. 2001). The exact pollutant attenuation metabolic mechanism(s) of enzymatic degradation is currently unknown. Results from degradation of certain xenobiotics by poplar trees have shown the potentiality of dehalogenase enzyme (Schnoor et al. 1995), which oxidizes alkanes, alkenes, and methanes and their halogenated analogues. Dehalogenase(s) will ultimately mineralize TCE to CO_2 via an oxidative pathway. Various other enzymes such as peroxygenases, cytochrome P450, peroxidase, glutathione-S-transferases, carboxylesterases, O-glucosyltransferases, O-malonyltransferases, N-glucosyltransferases and N-malonyltransferases are involved in the oxidation of xenobiotics in plant cells, transport of intermediates and further com-

Table 1 Some of the enzymes involved in phytoremediation

Plant(s)	Enzyme(s) produced	Pollutant(s) degraded	References
Populus sp., *Myriohyllium spicatum*, *Lemna minor*, *Algae nitella* etc.	Nitroreductases	Nitroaromatics (explosives etc.)	Hatzios and Penner 1982; Sanderman 1994
Myriohyllium spicatum, *Algae nitella*	Lactases	Munitions attenuation	Hatzios and Penner 1982; Sanderman 1994
Populus sp., *Myriohyllium* sp., *Algae nitella*, *Algae spirogyra*	Dehalogenases	Chlorinated solvents, ethylene containing compounds	Hatzios and Penner 1982; Schnoor et al 1995; Cunnigham and Ow 1996
Armoracia rusticana	Peroxidases	Phenols	Hatzios and Penner 1982; Cunningham et al 1995; Cunnigham and Ow 1996

partmentation processes (Sandermann 1994; Macek et al. 2000; Krämer and Chardonnens 2001). Table 1 lists some of the enzymes known to be active in phytoremediation systems and some plants that contain them. A need still exists to screen more plants for these and other enzymes, such as phosphatases for organophosphates, aromatic dehydrogenases for chlorinated aromatics (DDT, PCBs, etc) and O-demethylases for pentadimethaline, alachlor and metolachor, for their potential usefulness in phytoremediation.

Phytoremediation and genetically engineered plants

Adverse environmental conditions and hyperaccumulation of contaminants in the soil may not allow survival of efficient natural plants in natural ecosystems; hence, efficiency of phytoremediation may be lower in comparison to laboratory conditions. The hyperaccumulation of various contaminants via phytoremediation is likely to require the use of plants engineered to at least tolerate and ideally to hyperaccumulate several xenobiotics. Various biotechnological techniques are capable not only of developing an efficient hyperaccumulator plant for environmental cleanup but can also fulfill the idea of a 'magic tree' for efficient cleanup of the environment from chemical pollution. To some extent, molecular biology approaches have already been in use to evaluate phytoremediation and reveal elimination of toxicity from contaminated sites (Rugh et al. 1998; Bizily et al. 1999; Krämer and Chardonnens 2001). This approach has been used to produce transgenes to shorten the juvenile phase, alter lignin biosynthesis and increase cellulase accumulation in forest trees (Pena and Seguin 2001). A few recent advances have come to light in the development of a transgenic approach for metal phytoremediation that are effective in plants with potential for environmental application (Rugh et al. 1998; Bizily et al. 1999). The forest tree yellow poplar (*Liriodendron tulipifera*) was targeted for transformation due to its desirable structural and biological characteristics and for its demonstrated facility for genetic manipulation (Wilde et al. 1992; Rugh et al. 1998). Axenic tumor cultures of poplar cells clone H11-11 were grown in the presence of radiolabelled TCE (Gorden et al. 1998). In total, 70–90% TCE was transpirated under laboratory conditions; however, greenhouse and field studies resulted in less than 5% of total TCE transpiration. Transgenic tobacco plants expressing P450 2E1 metabolized the environmental pollutants TCE and ethylene dibromide (EDB) at an enhanced rate. The largest increase in TCE metabolism when compared with the control plants was found in roots. This finding is understandable because the MAC promoter used is expressed most strongly in the roots (Comai et al. 1990). By using rat P450 1A1 fused to yeast NADPH-P450 oxidoreductase, Shiota et al. (1994) made transgenic tobacco plants that metabolized the herbicide chlortoluron. Like other cytochrome P450 enzymes, P450 2E1 requires NADPH oxidoreductase and also cytochrome B5 for efficient transfer of electrons from NADPH (Shiota et al. 1994; Chen et al. 1996). The genes encoding oxidoreductase from plants have been cloned from several plant species and have also been sequenced. The mammalian and plant oxidoreductases are similar enough that a human P450 can interact with a tobacco oxidoreductase. Introduction of human P450 2E1 into tobacco plants resulted in a significant increase in metabolism of both TCE and EDB (Doty et al. 2000). French et al. (1999) developed a transgenic plant that was able to degrade explosive nitrate esters and NACs by introducing the bacterial enzyme pentaerythritol tetranitrate reductase. Such investigations have shown that plants can be genetically engineered to address the most widespread of groundwater contaminants, such as halogenated hydrocarbons, nitroaromatic hydrocarbons and other contaminants. Such novel phytoremediation technology might not be sufficiently developed solely by classical gene-by-gene genetic engineering, but by combining the genome(s) of a potential hyperaccumulator with a related species of non-accumulator with high-biomass, availability of genetic information, and marker-assisted breeding, could be a promising approach in the future.

Phytoremediation in field studies

Full-scale applications of phytoremediation are currently limited to only a small number of projects. There are various aspects that need to be considered before and during implementation of phytoremediation. Over the past decade, increased attention has been given to phytoremediation of PAHs, nitroaromatic compounds and various xenobiotics in the field. As discussed, a number of plants are present in natural ecosystems that can degrade compounds at a number of sites contaminated with different environmental pollutants. Initially, a high planting density is implemented as a high amount of evapotranspiration and total root mass is desired. It is often recommended to irrigate the contaminated site on the order of 10–20 inches per year, predominantly for terrestrial application. Certain contaminated sites, like mines and smelters, require low pH and increased water-holding capacity to ensure healthy growth of vegetation (Hughes et al. 1997). The United States Army is endeavoring to clean TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from contaminated wetlands and from groundwater contaminated with residues of the explosives TNT, RDX, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and dinitrotoluene using a variety of plants. The screening and sampling of numerous plant species growing on explosive-contaminated soils has yielded considerable information contributing to understanding TNT uptake by plants in the natural environment. Species that took up, and tested positive for, RDX and other explosives in the Joliet Army Ammunition Plant, Illinois and Iowa Army Ammunition Plant (IAAP), Iowa included block locust (*Robinia pseudoacacia*), red cedar (*Janiperus virginiana*), smartweed, bromegrass (*Bromus inermis*), Pigweed (*Amaranthus* sp.), reed canary grass (*Phalaris arundianasea*), Canadian golden rod (*Solidago canadensis*) and ragweed (*Ambrosia antemislelfolia*) (Schneider et al. 1996). However, only ragweed tested positive for TNT uptake or the presence of its metabolites 2- and 4-aminodinitrotoluene. The field sampling was consistent with laboratory studies with respect to TNT uptake and transformation, revealing that TNT and identifiable metabolites do not translocate from root tissue. Results of field and lab studies were partially in agreement for translocation of RDX, although lower concentrations were observed in the field than predicted from earlier lab studies.

The potential of phytoremediation and active remediation at the IAAP site were studied jointly by the United States Army Corps of Engineers, Waterways Experiments Station, and the University of Iowa (Thompson et al. 1998). The efficacy of poplar trees to remediate TNT and RDX contamination at the IAAP site was successfully tested (Thompson et al. 1998, 1999). Soils contaminated with pollutants such as TNT, TCE and PCP have been successfully remediated using parrot-feather (*Myriophyllum aquaticum*) (Macek et al. 2000). Pradhan et al. (1998) used phytoremediation as a primary remediation technology and as a final polishing step for treatment of soil contaminated with PAHs. Significant reduction in total PAH concentration was observed after 6 months of treatment with alfalfa (*Medicago sativa*), switch grass (*Panicum virgatum*) and Little bluestem grass (*Schizachyrium scoparium*). To investigate the prospects of phytoremediation, selected agricultural and indigenous terrestrial plants were examined for their capacity to

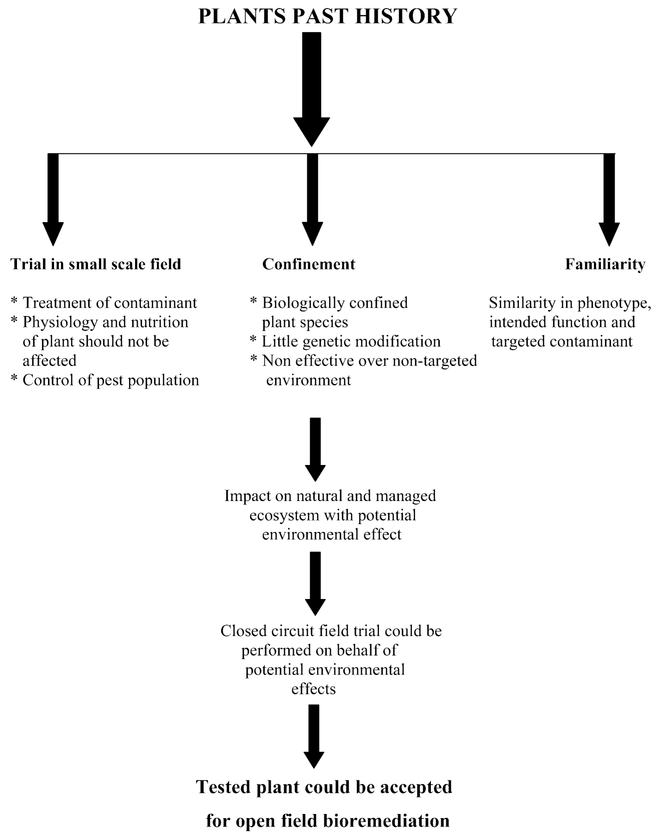

Fig. 3 Assessment of released natural/genetically engineered plants for bioremediation during field-testing

accumulate and degrade the explosive HMX on contaminated soil from an anti-tank firing range in Alberta, Canada (Groom et al. 2002). The pattern of its accumulation in plants collected from the range matched that of the agricultural plant species. Among the agricultural plant species, wheat and ryegrass demonstrated rapid growth in the presence of HMX and accumulated significant quantities of this explosive, identifying these plants as candidates for future field trials and applications. The work of Best et al. (1997) and Larson et al. (1999a, 1999b) also investigated the scope of phytoremediation, but focused on aquatic-based systems. They successfully identified plants with the ability to remove TNT and RDX from aquatic systems; however, the fate of transport and the metabolic pathways were not determined. Plants with the greatest potential for use in wetland systems were the submerged plants coontail and American pondweed and the emergent plants common arrowhead, reed canary grass and fox sedge. A 2-year study using box lysimeters was conducted under field conditions to examine the potential for phytoremediation of soil contaminated with TNT and 2,2′,5,5′-tetrabromobiphenyl using Johnson grass and Canadian wildrye (Sung et al. 2002). Levels of both compounds in the soil were found to drop below their detection limits after treatment. Lalande et al. (2003) studied the effect of annual ryegrass (*Lolium multiflorum*) on the dissipation of pyrene in a Cecil loamy soil in a

10 month experiment under field conditions in Clemenson, South Carolina. The pyrene level was found to decrease below its detection limit after 301 days indicating successful phytoremediation of this contaminant. In another study, phytoremediation with industrial hemp (*Cannabis sativa*) of soil contaminated with two PAHs (benzo[a]pyrene and chrysene) was studied. Hemp showed a very high tolerance to the contaminants and levels of both PAHs were significantly reduced in soil planted with hemp in pots (Campbell et al. 2002). Recently, the tropical leguminous tree *Leuceana leucocephala* was shown to take up and effectively metabolize TCE and EDB and it has also been successfully used for remediation of EDB-contaminated groundwater following an accidental EDB spill in Hawaii (Doty et al. 2003).

So far, no genetically engineered plants have been used commercially in phytoremediation. Reasons include the various safety aspects and also appropriate approval from regulatory bodies for outdoor use of such plants. Transgenic plants introduced into the environment might be able to move in a variety of environment media (air, soil and water). Several questions concerning the effect of introduced genetically modified plants arise whenever the intended introduction differs substantially from that with an established record of safety. The release of transgenic plants into the environment will depend on a number of factors, including their familiarity, the nature of the genetic modification, the ability to confine plants, and the perceived environmental impact (Fig. 3).

Conclusion

The major impact on the society today of environmental contamination can be viewed as an ecological malaise. Phytoremediation is an emerging technology that is certainly on the verge of being a big part of the solution to the contamination problem and hence can be prescribed as ‘environmental medicine’. Phytoremediation of aromatic pollutants is a multidisciplinary treatment technique with the central thrust on plant physiology. Thus, knowledge of the natural habitat of the degradative plant population is essential before a cost-effective, ecologically safe and environmentally sound bioremediation plan can be submitted. The limitation with this technology is that it has not been demonstrated conclusively at many sites and in full-scale projects. Aspects such as the role of enzymes, metabolites, microorganisms, plant selection and genetic engineering have to be better understood. Phytoremediation of aromatic pollutants aims to degrade them into less toxic/non toxic harmless compounds and limit their further movement by sequestration and accumulation. Deep groundwater contaminants can be treated by irrigation on constructed wetlands. Several plant species, in particular poplar trees, have shown promising potential for phytoremediation of many highly toxic and recalcitrant organic compounds such as PCBs, PAHs, nitroaromatic explosives, etc. This plant-mediated technology could be used as an in situ, non-invasive and aesthetic solution with the additional advantages of versatility and low cost. It can be employed in high-risk contamination zones by planting specific species for preventive measures. Although total field bioremediation is often a difficult task whether using natural or genetically engineered plants, phytoremediation can often be considered as either the ‘only’ or ‘polishing step’ solution to a contamination problem.

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References

Alkorta I, Garbisu C (2001) Phytoremediation of organic contaminants in soils. Bioresour Technol 79:273–276

Aprill W, Sims RC (1990) Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere 20:253–265

Best EHP, Zappi ME, Fredrickson HL, Sprecher SL, Larson SL, Ochman M (1997) Screening of aquatic and wetland plant species for the phytoremediation of explosives-contaminated groundwater from the Iowa Army Ammunition Plant. Ann N Y Acad Sci 829:179–194

Bizily S, Rugh CL, Summers AO, Meagher RB (1999) Phytoremediation of methylmercury pollution: *Mer B* expression in *Arabidopsis thaliana* confers resistance to organomercurials. Proc Natl Acad Sci USA 96:6808–6813

Bockers M, Rivero C, Thielde B, Janowski T, Schmidt B (1994) Translocation and metabolism of 3,4-dichloroaniline in soybean and wheat plants. J Biosci 49:719–726

Burken JG, Schnoor JL (1998) Predictive relationships for uptake of organic contaminants by hybrid poplar trees. Environ Sci Technol 32:3379–3385

Burken JG, Schnoor JL (1999) Distribution and volatilization of organic contaminants following uptake by hybrid poplar trees. Int J Phytoremed 1:139–152

Burken JG, Shanks JV, Thompson PL (2000) Phytoremediation and plant metabolism of explosives and nitroaromatic compounds. In: Spain JC, Hughes JB, Knackmuss HJ (eds) Biodegradation of nitroaromatic compounds and explosives. Lewis, Washington, D.C., pp 239–275

Campbell S, Paquin D, Awaya JD, Li QX (2002) Remediation of benzo[a]pyrene and chrysene-contaminated soil with industrial hemp (*Cannabis sativa*) Int J Phytoremed 4:157–168

Carman E, Crossman T, Gatliff E (1998) Phytoremediation of no. 2 fuel-oil contaminated soil. J Soil Contam 7:455–466

Cerniglia CE (1997) Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J Ind Microbiol Biotechnol 19:324–333

Chen W, Peter RM, McArdle S, Thummel KE, Sigle RO, Nelson SD (1996) Baculovirus expression and purification of human and rat cytochrome P450 2E1. Arch Biochem Biophys 335:123–130

Cole DJ (1983) Oxidation of xenobiotics in plants. In: Hutson DH, Roberts TR (eds) Progress in pesticide chemistry and toxicology. Wiley, New York, pp 199–254

Comai L, Moran P, Maslyar D (1990) Novel and useful properties of a chimeric plant promoter combining CaMV 35S and MAS elements. Plant Mol Biol 15:373–381

Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719

Cunningham SD, Berti WR, Hung JW (1995) Phytoremediation of contaminated soils. Trends Biotechnol 13:393–397

Curl EA, Truclove B (1986) The rhizosphere. Springer, Berlin Heidelberg New York

Daane LL, Harjono I, Zylstra GJ, Maggblom MM (2001) Isolation and characterization of polycyclic aromatic hydrocarbondegrading bacteria associated with the rhizobia of salt marsh plants. Appl Environ Microbiol 67:2683–2691

Davis LC, Banks ML, Schwab AP, Muralidharan N, Erickson LE, Tracy JC (1996) Plant based bioremediation. In: Sikdar I (ed) Bioremediation. Technomics, Basel, Switzerland

Doty SL, Shang TQ, Wilson AM, Tangen J, Westergreen AD, Newman LA, Strand SE, Gordon MP (2000) Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. Proc Natl Acad Sci USA 97:6287–6291

Doty SL, Shang TQ, Wilson AM, Moore AL, Newman LA, Strand SE, Gordon MP (2003) Metabolism of the soil and groundwater contaminants, ethylene dibromide and trichloroethylene, by the tropical leguminous tree, *Leuceana leucocephala*. Water Res 37:441–449

French CE, Rosser SJ, Davis GJ, Nicklin S, Bruce NC (1999) Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase. Nat Biotechnol 17:491–494

Gorden M, Choe N, Duffy J, Ekuan G, Hilman P, Muiznieks I, Marty R, Shurtleff BB, Strand S, Wilmoth J, Newman L (1998) Phytoremediation of trichloroethylene with hybrid poplars. Environ Health Perspect 106:1001–1004

Groom CA, Halasz A, Paquet L, Morris N, Olivier L, Dubois C, Hawari J (2002) Accumulation of HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in indigenous and agricultural plants grown in HMX-contaminated anti-tank firing range soil. Environ Sci Technol 36:112–118

Hatzios KK, Penner D (1982) Metabolism of herbicides in higher plants. CRC Press, Boca Raton, Fla.

Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoremediation. Environ Sci Technol 31:800–805

Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R (1997) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31:266–271

Komoßa D, Langerbartels C, Sanderman H (1995) Metabolic processes for organic chemicals in plants. In: Trapp S, McFarland JC (eds) Plant contamination-modeling and simulation of organic chemical process. CRC Press, Boca Raton, Fla., pp 69–103

Krämer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl Microbiol Biotechnol 55:661–672

Lalande TL, Skipper HD, Wolf DC, Reynolds CM, Freedman DL, Pinkerton BW, Hartel PG, Grimes LW (2003) Phytoremediation of pyrene in a Cecil soil under field conditions. Int J Phytoremed 5:1–12

Larson SL, Jones RP, Escalon BL, Parker D (1999a) Classification of explosives transformation products in plant tissues. Environ Toxicol Chem 18:1270–1276

Larson SL, Weiss CA, Escalon BL, Parker D (1999b) Increased extraction efficiency of acetonitrile/water mixtures for explosives determination in plant tissues. Chemosphere 38:2153–2162

Liste HH, Alexander M (2000) Accumulation of phenanthrene and pyrene in rhizosphere soil. Chemosphere 40:11–14

Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 18:23–34

Mackova M, Macek T, Kucerova P, Burkhard J, Trisk J, Demnerova K (1998) Plant tissue cultures in model studies of transformation of polychlorinated biphenyls. Chem Papers 52:599–600

Maier RM, Neilson JW, Artiola JF, Jordan FL, Glenn EP, Descher SM (2001) Remediation of metal-contaminated soil and sludge using biosurfactant technology. Int J Occup Med Environ Health 14:241–248

Meagher RB (2000) Phytoremediation of toxic elements and organic pollutants. Curr Opin Plant Biol 3:153–162

Newman LA, Doty SL, Gery KL, Heilman PE, Muiiznieks I, Shang TQ, Siemieniec ST, Strand SE, Wang X, Wilson AM, Gordon MP (1998) Phytoremediation of organic contaminants: a review of phytoremediation research at the University of Washington. J Soil Commun 7:531–542

Paul EA, Clark FE (1989) Soil microbiology and biochemistry. Academic Press, New York

Pena L, Seguin A (2001) Recent advances in the genetic transformation of trees. Trends Biotechnol 19:500–506

Pletsch M, Araujo BSD, Charlwood BV (1999) Novel biotechnological approaches in environmental remediation research. Biotechnol Adv 17:679–687

Pradhan SP, Conrad JR, Paterek JR, Srivastava VJ (1998) Potential of phytoremediation for treatment of PAHs in soil at MGP sites. J Soil Contam 7:467–480

Rugh CL, Senecoff JF, Meagher RB, Merkle SA (1998) Development of transgenic yellow poplar for mercury phytoremediation. Nat Biotechnol 16:925–928

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668

Sandermann H (1994) Higher plant metabolism of xenobiotics: the green liver concept. Pharmacogenetics 4:225–241

Schneider KJ, Oltmanns J, Radenberg T, Schneider T, Pauly-Mundegar D (1996) Uptake of nitroaromatic compounds in plants. Implications for risk assessment of ammunition sites. Environ Sci Pollut Res Int 3:135–138

Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318–323

Schwab AP, Banks MK (1994) Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In: Anderson TA, Coats JR (eds) Bioremediation through rhizosphere technology. American Chemical Society, Washington, D.C.

Shang TQ, Doty SL, Wilson AM, Howald WN, Gordon MP (2001) Trichloroethylene oxidative metabolism in plants: the trichloroethanol pathway. Phytochemistry 58:1055–1065

Shanks JV, Morgan J (1999) Plant 'hairy root' culture. Curr Opin Biotechnol 10:151–155

Shimp JF, Tracy JC, Davis LC, Lee E, Huang W, Ericknon LE, Schnoor JL (1993) Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. Crit Rev Environ Sci Technol 23:41–77

Shiota N, Nagasawa A, Sakaki T, Yabusaki Y, Ohkawa H (1994) Herbicide-resistant tobacco plant expressing the fused enzyme between rat cytochrome P450 1A1 (CYP 1A1) and yeast NADPH-cytochrome P450 oxidoreductase. Plant Physiol 106:17–23

Siciliano SD, Goldie H, Germida JJ (1998) Enzymatic activity in root exudates of dohurian wild rye (*Elymus dauricus*) that degrades 2-chlorobenzoic acid. J Agric Food Chem 46:5–7

Sung K, Munster CL, Rhykerd R, Drew MC, Yavuz Corapcioglu M (2002) The use of box lysimeters with freshly contaminated soils to study the phytoremediation of recalcitrant organic contaminants. Environ Sci Technol 36:2249–2255

Thompson PL, Ramer L, Schnoor JL (1998) Uptake and transformation of TNT by hybrid poplar trees. Environ Sci Technol 32:975–980

Thompson PL, Ramer L, Schnoor JL (1999) Hexahydro-1,3,5-trinitro-1,3,5-triazine translocation in poplar trees. Environ Toxicol Chem 18:279–284

Trapp S (1995) Model for uptake of xenobiotics into plants. In: Trapp S, McFarlane JC (eds) Plant contamination: modeling and simulation of organic chemical process. CRC Press, Boca Raton, Fla., pp 107–152

Wilde HD, Meagher RB, Merkle SA (1992) Expression of foreign gene transgenic yellow-poplar plants. Plant Physiol 98:114–120

Yateem A, Balba MT, E1-Nawawy AS, A1-Awadhi N (1999) Experiments in phytoremediation of Gulf war contaminated soil. Soil Groundwater Cleanup 2:31–33