

PHYTOREMEDIATION: PAST PROMISES AND FUTURE PRACTICES

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ABSTRACT

Plant-based bioremediation technologies have received recent attention as strategies to clean-up contaminated soils and water. These strategies have collectively been termed phytoremediation and refer to the use of green plants and their associated microbiota for the *in situ* treatment of soil, sediment, and ground water. Biologically based remediation strategies, including phytoremediation, have been estimated to be four to 1000 times cheaper, on a per volume basis, than current non-biological technologies. Compounds targeted for phytoremediation strategies include heavy metals, chlorinated solvents, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides, munitions and radionuclides. While some of these contaminants are more readily degraded or detoxified than others, plants or their attendant rhizosphere microbes have been shown in several instances to transform these compounds to some degree. The main types of phytoremediation strategies used include the stimulation of non-specific and specific autochthonous and zymogenous rhizosphere microorganisms (both bacteria and fungi) for the accelerated biodegradation of herbicide and solvent contaminants, the use of "hyperaccumulating" plants for remediation of soils contaminated with metals, and the use of plants to transform soluble contaminants to less soluble or less toxic forms. The ultimate goal of all phytoremediation technologies is to either remove the contaminant from the affected area, a process termed phytodecontamination, or to stabilize the contaminant to prevent movement or toxicological affects. Below ground phytodecontamination processes are thought to chiefly rely on rhizosphere degradation activity (either plant enzyme- or microbiologically-driven) to transform hazardous waste materials. Future biotechnological strategies for enhancing phytoremediation include enlarging root mass to increase adsorption area, using *Agrobacterium rhizogenes*, the direct genetic engineering of plants for altered biodegradation potential, and the genetic engineering of rhizosphere microorganisms. However, while phytoremediation processes hold great promise as means to remediate contaminated soils and water, there are advantages and disadvantages associated with these strategies that must be carefully considered. Whereas attractions of phytoremediation processes include cost effectiveness and non-invasiveness, they require relatively long periods of time, often require the disposal of toxic vegetation, are ineffective at remediating sites containing pollutants located deep into the soil profile, do not work on all contaminants, are sensitive to contaminant types and concentrations, may end up producing secondary metabolites which are more toxic than parent compounds, and in many instances don't remove environmentally significant quantities of pollutants.

Remediation of soils, water, and sediments contaminated with organic and inorganic pollutants is of major importance and concern. It has been estimated that it will require over \$20 billion annually to clean-up contaminated sites in the United States and Europe [5]. However, estimates of the costs of remediating contaminated soil and water vary widely, depending on: (1) the location of the contaminant; (2) the chemical, physical and biological properties of the contaminant; (3) whether the contaminated soil

contains more than one type of pollutant; (4) the degree of remediation desired; (5) subsequent disposal requirements; and (6) the techniques used. For example *in situ* remediation techniques have been estimated to cost \$10-100/m³, *ex-situ* processes \$30-\$300/m³, and *in situ* soil vitrification processes over \$1,000/m³ [15]. On the other hand, biologically based remediation technologies, including phytoremediation, have been estimated to be 4 to 1000 times cheaper, on a per volume basis, than current non-biological techniques [14]. Consequently, the lower cost of phytoremediation makes it an attractive alternative over other existing technologies, and in many instances, cost will be the driving force behind adoption of plant-based remediation on a large scale.

Biologically-based remediation strategies (bioremediation) have received much recent attention as means to clean-up contaminated soils and water. Phytoremediation, collectively referring to all plant-based remediation strategies, uses green plants to remediate contaminated sites. Several features make phytoremediation an attractive alternative to most currently practiced *in situ* and *ex situ* techniques. These include low capital cost, relatively minor on-going maintenance costs, non-invasiveness, easy start-up, high public acceptance, regulatory agency acceptance, and the techniques provide a pleasant appearance to the landscape [5].

In the last several decades, phytoremediation strategies have been examined as a means to clean-up a number of hazardous organic and inorganic pollutants, including: heavy metals [11,28,38], chlorinated solvents [22,43], agrochemicals [1,24,27]; polycyclic aromatic hydrocarbons [2,33], polychlorinated biphenyls [7,18], munitions [39] and radionuclides [20]. Those soluble organic and inorganic contaminants, which move into plant roots or the rhizosphere by the processes of mass flow or diffusion, appear to be the most amenable to phytoremediation technologies [14,15,39]. In several instances, plants and/or their attendant rhizosphere microbes have been shown to transform these compounds to some degree [13,41,43]. Diverse plant species show great promise as phytoremediation agents. These plants include: grasses, legumes, trees and several other monocots and dicots [9,11,14,19,38,39]. Several different species of aquatic plants also appear to be useful for phytoremediating contaminated surface water [34].

Phytoremediation technologies can be directed to above or below ground contaminants and either remove pollutants from the affected area (phytodecontamination) or stabilize them to prevent off-site movement (phytosequestration or phytostabilization). These latter techniques are useful for contaminants having low biodegradation potential or those which rapidly move into the soil profile. Below ground phytostabilization processes involve the sequestration of contaminants into soil particles, cell wall lignins, or into the soil humus fraction [14] and reduce the bioavailability of contaminants [38]. Below ground phytodecontamination processes, on the other hand, often rely on rhizosphere degradation activity (either plant enzyme- or microbiologically-driven) to transform hazardous waste materials (see below). In addition, several of these processes can occur *ex planta* or *in planta*. *Ex planta* phytoremediation processes refer to those driven by the action of plant- or microbially-derived soil enzymes [39] or by plant-associated microorganisms [1,4,12,22,26,35,37]. While not yet used on field scale levels, enzymes responsible for *ex planta* soil enzyme biodegradation (dehalogenases, nitroreductases, nitrilases, peroxidases, and laccases) have been investigated in some detail [5,39]. Cell free enzyme systems, whether added to the soil or excreted from plant roots, may hold particular promise in environments that are adverse for the growth and persistence of microorganisms [4]. *The in planta* phytoremediation processes require that the pollutant is taken up into the plant. Pollutants are taken up by roots, and either sequestered or translocated to shoots and leaves [17]. Plants usually uptake organic compounds in the aqueous phase, by diffusion or mass flow processes, although in some instances vapor phase transport can occur [14]. For many organic compounds, root uptake has been shown to be proportional to K_{ow} , the n-octanol/water partition coefficient [8,15]. Organic and inorganic compounds can be transported to other portions of the plant apoplastically or symplastically [38]. Ultimately, the compound is either metabolized within the plant by conjugation to glutathione [21], sequestered, or transpired from the plant.

Sequestration of pollutants within plants is the basis for phytoextraction of soils and water contaminated with heavy metals [28,32]. Metals targeted for this type of phytoremediation process include Cd, Pb, Zn, Cu, Cr, Ni, Se, and Hg [see 11,14]. Phytoextraction, using "hyperaccumulating" plants is proving to be one of the most effective phytoremediation methods to clean-up metal contaminated soils and water [3]. Several plant species, including *Thlaspi caerulescens* have been shown to accumulate very high levels of Zn and Cd from soils [3]. *Brassica juncea* has also been found to be an excellent accumulator plant for metals in soils, such as Cd, Cr, Ni, Zn, and Cu [28,38] and several plant species have been shown to accumulate Pb [15,19,25]. Plants, such as *Eichhornia crassipes*, *Hydrocotyle umbellata*, *Lemna minor*, *Scirpus lacustris*, *Phragmites karka*, *Bacopa monnieri*, and *Azolla pinnata* and are also effective at removing metals from aquatic systems [see 10,38]. Plant shoots and roots containing metals are subsequently harvested and treated as hazardous waste or the metals are recovered as ore.

Ex planta phytoremediation can also occur via the degradative activity of rhizosphere microorganisms. The rhizosphere is operationally defined as the "soil-root interfacial area" and relatively large numbers of diverse species of microorganisms live in association with plant roots [16]. The word "rhizosphere", first introduced by Hiltner in 1904 [23] to describe the interaction between bacteria and the roots of legumes, has been operationally defined to mean many things to many researchers [29]. The rhizosphere consists of the endorhizosphere (various cell layers of the root), ectorhizosphere (the immediate soil area surrounding the root) and the rhizoplane (the root surface) [29]. Microorganisms colonize and live within these areas and the degree of intimacy at which a microorganism interacts with a root varies in proportion to the distance from the root surface. The closer a microbe is to the root surface, the more its growth and behavior is influenced by plant-released materials [36]. The intimacy of the association between soil microorganisms and plant roots is determined, in part, by the types and concentrations of compounds exuded by roots [35]. Root exudations are thought to have a stimulatory affect on rhizosphere microbes, which in turn, are purported to accelerate biodegradation in the rhizosphere [1,2,13,22,30,33,41]. While there has been much discussion on the usefulness of this technology, the reader is cautioned to examine published studies carefully, since in many instances reported enhanced biodegradation is relatively small, environmentally insignificant, or occurs at rates not suitable for field use. Moreover, inconsistent results have been reported from several studies, using various plants and microorganisms and different substrates. It has been hypothesized that this may be due to specific soil-chemical-plant interactions [26,37,42] or other abiotic factors. A variety of compounds have now been evaluated for their ability to be degraded in planted soils, including: hydrocarbons, halogenated aromatic, aromatic hydrocarbons, halogenated aliphatics, polycyclic aromatic hydrocarbons, pesticides, and nonvolatile hydrocarbons [6,12,43]. While there is some information available concerning the ecology of rhizosphere populations in planted, contaminated soils [30], there is little information about how polluted soils change microbial community structure in the rhizosphere.

Rhizosphere-enhanced degradation has also been reported to occur with genetically engineered microorganisms. In 1995, Brazil and coworkers [7] described the genetic construction of rhizosphere-competent pseudomonads which were engineered to contain the bacterial *bph* genes for biodegradation of PCBs. These strains have the potential to degrade PCBs in the rhizosphere and could be useful for bioremediation purposes. In addition, Crowley and coworkers [13] reported that a rhizosphere competent *Pseudomonas fluorescens* strain containing genes for 2,5-dichlorobenzoate degradation (2,5-DCB) had higher degradation rates in planted soil than non-planted ones. The degree of bioremediation appears to be related to the plant species present [40] and most likely reflects differences in: (1) root architecture; (2) plant metabolism; (3) quantity and quality of root exudates; (4) root radius [12]; and (5) soil type [6].

In summary, phytoremediation processes hold great promise as means to clean-up polluted soils and water. Currently, the most advanced and effective phytoremediation technology is phytoextraction

of heavy metals from soils using hyperaccumulating plants. In addition, aquatic plants also hold great promise to rid contaminated water of heavy metal contaminants. It is hoped that in the future, phytoremediation technologies using rhizosphere microorganisms will become more efficacious. This will most likely occur by using genetic engineering and plant breeding techniques and having a much better understanding of the ecology of rhizosphere microorganisms growing in polluted soils and water.

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