# The Role of Plants and Plant/Microbial Systems in the Reduction of Exposure

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The activities of plants and plant/microbial associations may offer a viable means of accomplishing the in situ remediation of contaminated soils. Two uses of plants for phytoremediation are reported here. In one set of studies, the ability of plants to foster degradative microorganisms was investigated. Results indicated that the degradation of several chlorinated pesticides increased in rhizosphere soil and that this same increase occurred when unplanted soils were given materials released from plant roots. In current investigations, the potential for plants to remove and accumulate metals from their environment is being considered. This work employs a unique testing system, the target-neighbor method, that allows evaluation of how planting density influences metal uptake. Results of these studies could provide the information needed to manipulate plant density for optimization of metal removal (remediation of metal-contaminated soil) or minimization of the amount of toxic metals in important crops (reduction of human exposure). - Environ Health Perspect 103(Suppl 5):13-15 (1995)

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#### Introduction

A majority of the work currently being conducted in the areas of biodegradation and bioremediation focuses on the role of microbial systems. While microorganisms are certainly significant, there is another form of biota that also poses promise in terms of hazardous material degradation. That biota is vegetation—the vegetation that we find growing not only on pristine lands but also in some of our most impacted, contaminated soils. This report will discuss two ways in which plants may act to minimize the risk of human exposure by biodegradation and phytoremediation-by supporting degradative microorganism in <sup>a</sup> soil region known as the rhizosphere and by removing toxic metals from contaminated soils and accumulating/concentrating them in above-ground tissues. Although it will be the premise of this report that plants offer a viable means of accomplishing in situ biodegradation and bioremediation, it should be noted that plants also offer, perhaps, the greatest threat to human health. Plants are the primary source of carbon for the remainder of life forms on earth and, as such, can act as vectors for contaminant introduction into the food chain. Thus, it is essential that we begin to understand the complex interactions between plants and the range of compounds now found in the environment that may be hazardous to human or other populations.

### The Rhizosphere and Xenobiotic Degradation

The rhizosphere is defined as the region in the soil influenced by the presence of plant roots. This influence may be associated with physical changes in the area such as compaction or with the deposition of rootderived material into the rhizosphere. Rhizodepositions take several forms: inorganics; low-molecular-weight organics (sugars, fatty acids, organic acids, and phenolics); or high-weight organic polymers such as polysaccharides and polygalactic acids. Although the benefit of root deposition into the rhizosphere (for the plant) has not been fully elucidated, studies indicate that these materials can be used by microorganisms as carbon sources. The rhizosphere thus is capable of supporting a greater number of microorganisms than bulk, nonvegetated soil. The increased number of organisms in this region may (or may not) result in higher rhizosphere microbial activity.

Given the above, recent studies have begun to investigate the potential for degradation of xenobiotics in the rhizosphere. The postulated scenario is depicted in Figure 1; microorganisms living in the rhizosphere on plant-derived substrates are better able to degrade or transform xenobiotics than are those in the bulk soil. This increased ability may be associated with the greater number of microorganisms or with the availability of growth-supporting substrates for co-metabolizing ones. Since plants release compounds that are similar to aromatic xenobiotics, it is also possible that these compounds act as structural analogs and select (over time) for adapted, degrading microbial populations.

Increased degradation has been reported in rhizosphere systems  $(1-8)$ . One of the specific interests of my laboratory is the investigation of organic xenobiotic degradation in the rhizosphere as well as the mechanisms and soil/plant factors associated with it. Before investigation of the potential mechanisms that might be responsible for any increased degradation, it was necessary to demonstrate that microorganisms isolated from natural system rhizospheres actually show greater rates of xenobiotic degradation. This was accomplished by collecting rhizosphere soil



Figure 1. The potential for xenobiotic degradation or transformation by microorganisms living in the plant rhizosphere.

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from eight species growing in two soil types (9). Preliminary studies had been conducted to optimize the collection (brief shaking of roots, followed by light brushing) and handling (kept at field waterholding capacity and used immediately) of rhizosphere soil. After collection, soils were analyzed for their physical/chemical characteristics and microbial activities. Serum bottle respirometer studies were then conducted to determine the degradation of phenol, 2,4-dichlorophenol (2,4-DCP), 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4,5-trichlorophenoxyacetic acid  $(2,4,5-T)$ . In the respirometer studies, 5 g of soil was placed in a 50-ml serum bottle fitted with an airtight stopper. A uniformly (ring) radiolabeled compound was then added to the soil, and the evolved  $^{14}CO_2$ was trapped on a base-saturated wick suspended in the bottle. Measurement, therefore, was of full xenobiotic mineralization over time. Without differentiating between species, the results of the 2,4-D and 2,4,5-T mineralization studies are shown in Figure 2. Across species and soils, rhizosphere microorganisms showed greater initial rates and cumulative extents of mineralization for these two compounds. Privilent mineral Calle Neither rates nor extents differed between **CONTAMINATEG SOIIS** rhizosphere and nonrhizosphere soils for mineralization of phenol and 2,4-DCP. Both phenol and 2,4-DCP are readily degraded compounds; the lack of a rhizosphere effect is probably due to the rapidity of their use by microorganisms. With the increasing structural complexity of 2,4-D and 2,4,5-T, the rhizosphere effect becomes more significant in terms of mineralization.



Figure 2. Cumulative percent mineralization of 2,4-D and 2,4,5-T (100% 0.00107 mmole for 2,4,5-T).

In a series of separate investigations, the influence of root exudates on soil mineralization was also determined. Solutions were flowed over the roots of sand-cultured plants and then allowed to percolate through soil columns. This soil was then used in serum bottle experiments (as described above). Mineralization was compared between soils that followed planted sand cultures and those under similar, but unplanted, setups. These studies reflected the same mineralization patterns as did the field collected soils. The degradation of the easier compounds (phenol and 2,4-DCP) did not differ between the soils, but the mineralization of the 2,4-D and  $2,4,5$ -T was significantly higher and faster when the soil had received solutions that flowed past plant roots. Current studies are attempting to isolate the fraction of material coming from the roots that is responsible for this increased mineralization. These isolation systems employ selective resins and will eventually be used to identify environmental conditions and plant factors that influence the quantitative and qualitative release of root-derived materials.

Unlike animals, plants cannot migrate or move substantially from the location in which they grow. Their survival in natural systems depends on their ability to adapt or acclimate to their environment. Since the industrial revolution, plants have faced increasing numbers and amounts of contaminants-many of which are metallic. The evolution of metal resistance in plants has been well studied and, for the most part, understood. Resistance strategies tend to fall into two categories: avoidance (via exclusion or restricted transport) and toler-2,4-D ance (via accumulation). Some species of Nonrhizosphere **plants tolerate metals in their environment** by hyperaccumulating them at levels even above that in the substrate. Phytoremediation may be possible by capitalizing on the inherent abilities of these types of plants to mobilize metals from the soil and into their shoot tissues. Harvested plants could be combusted or composted for metal recovery or waste minimization.

In my laboratory, the emphasis is not on the specifics of accumulation of metals by plants, but rather on the optimization of <sup>20</sup> <sup>40</sup> <sup>60</sup> this accumulation. Several factors, including planting density, are known to influence plant acquisition of elements from the environment. A study is currently being conducted that utilizes the "target-neighbor" cocropping approach to determine the effect of planting density on the uptake of metal (in this case, selenium) by known accumulating (mustard) and nonaccumulating, sensitive species (tomato). This method (outlined in Figure 3) has previously been used by my group (10) to investigate the release from atrazine inhibition at increasing densities of an insensitive (corn) or sensitive (soybean) species. In that case, the number of neighbors around a soybean plant determined to what extent the atrazine was inhibitory to growth. At high densities of neighbors (especially if they were corn), the soybean growth was equivalent to that of a soybean grown in nonatrazine soil.

The method relies on the ecological concept that, as density increases, competition for essential resources intensifies. As competition intensifies, each plant receives a smaller portion of the resource. Although the total biomass of the community may go up with increased density, the size of the individual goes down due to the decrease in essential resources.

If the resource for which plants are competing is a toxin or a contaminant, density should determine the amount that each individual acquires from the substrate. The concentration of metal in plant tissue may be highest for the individual at low density, but total metal found in a stand of vegetation should occur at the density where biomass is maximal. If accumulating plants are to be managed in a manner that optimizes the amount of metal removed from the substrate, then this maximal biomass den-



A is an accumulator or insensitive plant species. **"**B is a nonaccumulator or sensitive plant species.



Figure 3. Target-neighbor planting design for optimization/minimization of metal accumulation by plants. Combinations AA and NN represent monocultures of the accumulator, A, and nonaccumulator species, N, for establishment of density-dependent biomass and metal uptake patterns. Combinations of AN and NA test the potential to decrease metal uptake or phytotoxicity by cocropping.

sity needs to be determined. Our current study allows us to do this. In addition, the cocropping of an accumulator with a sensitive species will provide information on the possibility of growing crop species with accumulators, thus minimizing the amount of metal that is taken up by an individual. This could decrease metal phytotoxicity (as in the atrazine example) or could limit the tissue concentration of metals to a level considered safe for consumption. In this way, metal-contaminated land might be used for crop or forage production.

#### **Conclusion**

The use of plant and plant/microbial systems in the remediation of contaminated soils is certainly promising. At this point, we have a fairly good understanding of the component parts, the plant and the

microorganism, as they function in isolation. It is the challenge of future investigations to elucidate how these organisms function together when placed in the environment. This information will allow manipulation of these systems to predictably accomplish soil bioremediation to the greatest extent and over the shortest time periods.

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