Biotransformations: Microbial Degradation of Health Risk Compounds
Ved Pal Singh, editor

Degradation of hazardous organic compounds by rhizosphere microbial communities

Todd A. Anderson[a], David C. White[b] and Barbara T. Walton[c]

[a]Pesticide Toxicology Laboratory, Department of Entomology, Iowa State University, Ames, IA, U.S.A.

[b]Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN, U.S.A.

[c]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, U.S.A.

INTRODUCTION

The rhizosphere, or root zone of plants, contains a diverse microbial community that contributes to plant health and to soil homeostasis. In addition, recent studies indicate that microorganisms in the rhizosphere can degrade toxicants of concern to human health and the environment. The application of these findings to remediation of chemically contaminated soils will be facilitated by a better understanding of the variables that control biotransformation in the rhizosphere. For example, the increased density and diversity of microorganisms in the rhizosphere, as compared to nonvegetated soils, may be a critical factor for microbial degradation of xenobiotic compounds. In addition, the secretion of readily degradable substrates by roots may facilitate cometabolic transformation of hazardous organic compounds [1]. Other mechanisms can also be invoked to explain how *microbial transformations* occur in the rhizosphere. Collectively, these factors have important implications for the successful use of vegetation to increase the participation of microorganisms in biotransformation of toxicants at hazardous waste sites.

The existing evidence for microbial degradation of xenobiotics in the rhizosphere is examined with special attention to whether a community or single species of microorganism is responsible for biotransformations in the root zone. In addition, previously unpublished data resulting from biochemical analyses of trichloroethylene-degrading microorganisms are presented and discussed with respect to microbial biomass, metabolic activity, nutritional status, and community structure in the rhizosphere.

RHIZOSPHERE MICROBIOLOGY

Plant roots affect the soil in which they grow in a multitude of ways, making the soil more conducive to microbial growth and activity. For

example, roots affect soil carbon dioxide and oxygen concentrations, osmotic and redox potentials, pH, and moisture content. The rhizosphere is a zone of increased microbial activity and biomass at the root-soil interface [2]. The large microbial populations in the rhizosphere are sustained by exudation of substances such as carbohydrates and amino acids from the root as well as sloughing of root epidermis. All roots are protected from abrasion by root cap cells which are sloughed off during root growth; sometimes as many as 10,000 cells per plant per day [3]. As the root grows downward, cells in the root cap produce a gel that helps lubricate the root, allowing the root to force its way through the soil. This mucigel, along with other root excretions is classified as root exudate [4]. Both root cap cells and root exudates, are useful sources of nutrients for microorganisms in the root zone or rhizosphere. In addition, microorganisms can stimulate exudation, whereas the absence of bacteria and fungi can lead to less exudate production by the plant [2]. These rhizosphere characteristics typically result in microbial populations an order of magnitude or more above microbial populations in nonvegetated soil. This *rhizosphere effect* is often expressed as the ratio of microorganisms in rhizosphere soil to the number of microorganisms in non-rhizosphere soil, the R/S ratio [5]. R/S ratios commonly range from 5 to 20, but occasionally are as high as 100 and above [6].

The actual composition of the microbial community in the root zone is dependent on root type, plant species, plant age, and soil type [3] as well as other selection pressures, such as foreign chemicals [7-10]. Typically, the rhizosphere is colonized by a predominantly Gram-negative microbial community [6]. In addition, leguminous plants exhibit a greater rhizosphere effect than non-leguminous plants, presumably because of increased N levels in soil where legumes grow. The ability of the plants to select for different rhizosphere microbial communities in both composition as well as size is intriguing from the standpoint of exploring whether this selection translates into differences in the rates of microbial degradation of organic compounds in the rhizosphere.

The rhizosphere, which was first described by Hiltner [11], has been the focus of agricultural research for several years, primarily because of its influence on crop productivity. Several excellent comprehensive reviews on the rhizosphere are available [2,3], and due to their extensive nature, the current review will be limited to discussing findings on rhizosphere microbiology within the context of degradation of organic compounds by rhizosphere microbial communities.

The interaction between plants and microbial communities in the rhizosphere is a complex relationship, that has evolved to the mutual benefit of both groups. In addition to the accepted relationships described thoroughly in the aforementioned reviews, other connections between plants and the microorganisms in their root zones undoubtedly exist. One possible additional relationship is the rhizosphere microbial

community's role in protecting the plant from chemical injury. Previous research has shown that plants increase root exudation in the presence of xenobiotic chemicals [12,13]. In hydroponic cultures of corn, the presence of simazine (2-chloro-4,6-bis ethylamino-S-triazine), a preemergence herbicide used for controlling weeds in corn, caused a two-fold increase in exudation of organic acids [14]. In addition, simazine increased the length and weight of roots, but only if microorganisms were also present in the medium. It is not clear whether the increase in exudation is an evolved response by the plant to attract more microorganisms (and possibly degrade the chemical faster) or simply the physiological effect of the chemical on the plant. The tolerance of corn to the herbicidal effects of simazine may be the result of rapid metabolism of the compound by the plant. The tolerance of corn to the herbicidal effects of simazine may be the result of rapid metabolism of the compound by the plant. Most herbicides used to control weeds are readily metabolized by non-target plants [15]. Nonetheless, rhizosphere microbial communities may also play a role in protecting the plant from chemical injury. This idea is further supported by the work of Herring and Bering [16]. They found that the toxic effect of phthalate esters on spinach and pea seedlings could be abated or reversed by the presence of microorganisms in the soil.

MICROBIAL DEGRADATION IN THE ROOT ZONE

Studies of microbial degradation of toxicants in the root zone of plants have included a variety of plant types from diverse taxonomic families (Table 1). These studies have been generated from examination of agrochemicals, aquatic systems, and industrial chemicals and are summarized below. Recently, two reviews on microbial degradation of organic compounds in the rhizosphere and the beneficial effects of vegetation at contaminated sites have been published [17,18].

Agrochemicals

Research on microbial transformations in the rhizosphere has been concerned mainly with agricultural chemicals, such as pesticides and fertilizers. Several researchers [1,7,19-21] have described an increased capacity for mineralization of various pesticides by rhizosphere microbial communities as compared with microbial communities in nonvegetated soil. Occasionally, this increased mineralization capacity is correlated with increased numbers of pesticide-degrading microorganisms. Sandmann and Loos [7] found an increase in the number of 2,4-D (2,4-dichlorophenoxyacetate)-degrading bacteria in the rhizospheres of previously untreated African clover and sugarcane. Similarly, work by Abdel-Nasser and coworkers [8] showed higher microbial counts in the

Table 1
Studies relevant to organic chemical degradation in the rhizosphere

Plant	Family	Chemical	Comments	Refs
Wheat	Gramineae	Mecoprop[A] 2,4-D[B] MCPA[C]	Mixed culture capable of using compounds as a carbon source.	[21]
Sugarcane African clover	Gramineae Fabaceae	2,4-D[B]	Higher population of 2,4-D-degrading microorganisms in the rhizosphere of sugarcane, a plant nonsensitive to 2,4-D, compared with African clover, a plant sensitive to the herbicidal effects of 2,4-D.	[7]
Bush bean	Fabaceae	Diazinon[D] Parathion[E]	Increased mineralization of both compounds in the rhizosphere.	[1]
Rice	Gramineae	Parathion[E]	Increased mineralization in the rhizosphere especially under flooded conditions.	[20]
Tobacco	Solanaceae	MH[F]	MH caused enhanced nitrification and mineralization of organic substances in the rhizosphere.	[50]
Rice	Gramineae	Benthiocarb[G]	Eight-fold increase in heterotrophic bacteria in the rhizosphere of treated rice plants.	[22]
Corn Bean Cotton	Gramineae Fabaceae Malvaceae	Temik[H]	Higher counts of microorganisms in treated vs untreated rhizosphere.	[8]

Wheat Corn	Gramineae Gramineae	Diazinon[D]	Rhizosphere microbial counts increased by 2 orders of magnitude.	[10]
Flax	Linaceae	2,4-D[B]	Ammonifying, nitrifying and cellulose-decomposing bacteria in the rhizosphere increased by 1 to 2 orders of magnitude.	[9]
Corn	Gramineae	Atrazine[I]	Increase in production of atrazine degradation metabolites by rhizosphere microorganisms in the presence of decomposing roots.	[19]
Legumes	Fabaceae	Petroleum	Describes the importance of leguminous plants in reclamating petroleum-contaminated sites.	[51]
Rice	Gramineae	Oil residues	*Bacillus* sp. isolated from rice rhizosphere was capable of growth on oil residues, but only in the presence of root exudates.	[28]
Prairie grasses	Gramineae	PAHs[J]	Increased disappearance of PAHs in vegetated vs. nonvegetated soil columns.	[27]
Lespedeza Loblolly pine Bahia grass Goldenrod Soybean	Fabaceae Pinaceae Gramineae Compositae Fabaceae	TCE[K]	Increased degradation of TCE in rhizosphere soil and increased mineralization of ^{14}C-TCE in soils containing lespedeza, loblolly pine, and soybean.	[29,30]
Reeds	Gramineae	VOCs[L]	Vegetated microbial filters increased removal of both aromatics and aliphatics.	[26]

Table 1 continued

Plant	Family	Chemical	Comments	Refs
Corn Soybean	Gramineae Fabaceae	Surfactants[M]	Rhizosphere treatments significantly increased initial rates of mineralization by a factor of 1.1-1.9.	[52]
Cattails	Typhaceae	Surfactants[M]	Mineralization of surfactants was more rapid in the rhizosphere than in root-free sediments.	[24]
--	--	Organo-chlorines[N]	A rhizosphere-competent fungus was able to degrade a variety of organochlorine compounds.	[53]
--	--	PCBs[O]	Compounds produced by photosynthetic plants were shown to support the growth of PCB-degrading bacteria. The organisms retained their ability to metabolize PCBs.	[54]
Wheatgrass	Gramineae	PCP[P]	Mineralization of pentachlorophenol was enhanced in soils containing wheatgrass.	[55]

[A]2-(2-Methyl-4-chlorophenoxy)propionic acid.
[B]2,4-Dichlorophenoxyacetic acid.
[C]2-Methyl-4-chlorophenoxyacetic acid.
[D]O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate.
[E]O,O-diethyl-O-*p*-nitrophenyl phosphorothioate.
[F]Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione).

[G]S-*p*-chlorobenzyl diethylthiocarbamate.
[H]2-Methyl-2-)methylthio) propionaldehyde O-(methylcarbamoyl)oxime.
[I]2-Chloro-4-ethylamino-6-isopropylamino-S-triazine.
[J]Polycyclic aromatic hydrocarbons (benz[a]anthracene, chrysene, benzo[a]pyrene, dibenz[a,h]anthracene.
[K]1,1,2-Trichloroethylene.
[L]Volatile organic compounds (benzene, biphenyl, chlorobenzene, dimethylphthalate, ethylbenzene, naphthalene, *p*-nitrotoluene, toluene, *p*-xylene, bromoform, chloroform, 1,2-dichloroethane, tetrachloroethylene, 1,1,1-trichloroethane).
[M]Dodecyl linear alkylbenzene sulphonate, dodecyl linear alcohol ethoxylate, dodecyltrimethylammonium chloride.
[N]Pentachlorophenol, endosulphan, DDT.
[O]Polychlorinated biphenyls.
[P]Pentachlorophenol.

rhizospheres of corn, beans, and cotton, treated with temik [2-methyl-2(methylthio) propionaldehyde O-(methylcarbamoyl) oxime]. More recently, Sato [22] found an 8-fold increase in heterotrophic bacteria in rice rhizosphere after benthiocarb (S-*p*-chlorobenzyl diethylthiocarbamate) addition as compared with plate counts before addition.

Seibert et al. [19] observed an overall increase in atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine) degradation by rhizosphere microorganisms in the presence of decomposing roots. Also, the concentration of unchanged atrazine was lower in the rhizosphere soil, and the concentration of hydroxyatrazine and two other hydroxylated metabolites were 3-fold higher than concentrations outside the rhizosphere. Studies on $^{14}CO_2$ evolution from ^{14}C-parathion (O,O-diethyl-O-*p*-nitrophenyl phosphorothioate) in rice rhizospheres indicated similar results [20]. Only 5.5% of the ^{14}C-parathion was evolved as $^{14}CO_2$ in unplanted soils while 9.2% was evolved from rice rhizospheres under non-flooded conditions. The rice variety used in this experiment grew better in flooded soil, thus when flooded conditions prevailed, 22.6% of the radiocarbon was evolved as $^{14}CO_2$. Reddy and Sethunathan [20] argued that the close proximity of the aerobic-anaerobic interface in rice rhizosphere under flooded conditions favoured the ring cleavage of parathion.

Parathion and diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] appear to be degraded in soil initially by cometabolic attack [23], a process that requires the presence of a growth substrate other than the compound being degraded. As indicated earlier, root exudates provide microorganisms with a wide range of organic substrates for use in growth and reproduction, and as energy sources. These factors lead Hsu and Bartha [1] to hypothesize that the rhizosphere would be especially favourable for cometabolic transformations of pesticides. Using radiolabelled diazinon and parathion, they were able to show accelerated mineralization of these compounds in bean rhizospheres. Beans were chosen because of their reported inability to metabolize diazinon [23]. Approximately 18% of the parathion and 13% of the diazinon were mineralized in the bean rhizospheres compared with 7.8% and 5.0% in the root-free soil for parathion and diazinon, respectively. Similar results with diazinon were previously found by Gunner and coworkers [23], although they did not observe an increase in microbial biomass in the rhizosphere after diazinon application. Rather, the diazinon (and probably the root exudates) exerted a selective effect, which resulted in the enrichment of a particular isolate capable of diazinon metabolism.

Lappin et al. [21] found that a microbial community isolated from wheat roots was capable of growth on the herbicide, mecoprop [2-(2-methyl 4-chlorophenoxy) propionic acid] as the sole carbon source. The

authors isolated five species, none of which was capable of growth on mecoprop individually. This microbial community was also shown to degrade 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxyacetic acid).

Aquatic Systems

The increased degradative capability of rhizosphere microbial communities is not limited to terrestrial plants. Federle and Schwab [24] and Federle and Ventullo [25] have made similar observations of the increased microbial degradation of surfactants in the rhizospheres of aquatic plants. Mineralization of linear alkylbenzene sulphonate (LAS) and linear alcohol ethoxylate (LAE) was more rapid in the rhizosphere of cattails (*Typha latifolia*) than in root-free sediments [24]. Surprisingly, the source of the cattails (plants were obtained from a pristine pond and a pond receiving laundromat wastewater) had no significant influence on the rates of LAS and LAE degradation. Additionally, microbial communities associated with duckweed (*Lemna minor*) readily mineralized LAE, but not LAS. Similar results on microbial degradation of LAS and LAE by the microbiota of submerged plant detritus were obtained by Federle and Ventullo [25].

To assess the possible additional benefits of microbial filters (*biofilms*), containing aquatic vegetation in biotransformation of hazardous organic compounds, Wolverton and McDonald-McCaleb [26] compared removal of a variety of EPA priority pollutants in nonvegetated filters and filters planted with the common reed, *Phragmites communis*. In 24 hours, the nonvegetated microbial filter removed 61-99% and 39-81% of the aromatics (benzene, biphenyl, chlorobenzene, dimethylphthalate, ethylbenzene, naphthalene, *p*-nitrotoluene, toluene, *p*-xylene) and aliphatics (bromoform, chloroform, 1,2-dichloroethane, tetrachloroethylene, 1,1,1-trichloroethane) tested, respectively. The vegetated filter system increased the removal of both the aromatics (81->99%) and aliphatics (49-93%). Although sterile controls for elucidating volatilization rates as well as possible abiotic degradation and adsorption mechanisms were not performed, losses due to volatilization appeared to be minor in these systems.

Industrial Chemicals

Although most of the studies described previously have dealt with agricultural chemicals, they provided evidence for the accelerated microbial degradation of organic compounds in the rhizosphere and also gave an incentive for exploring the possibility of similar results with other hazardous organic compounds. Two recent studies have detailed the accelerated disappearance of nonagricultural chemicals in the root zone; a series of polycyclic aromatic hydrocarbons (PAHs) in prairie grass rhizospheres [27], and the increased degradation of oil residues by microorganisms isolated from oil-polluted rice rhizospheres [28].

Rasolomanana and Balandreau [28] appear to be the first to show enhanced microbial degradation of nonagricultural chemicals by rhizosphere microorganisms. This serendipitous discovery came during studies of improved growth of rice in soil to which oil residues had been applied. The authors hypothesized that the increased growth was brought about by the initial removal of the oil residues from the rhizosphere by microorganisms, utilizing the oil, and isolated a *Bacillus* sp. with the ability to grow on the oil residues, but only in the presence of rice root exudates.

The use of eight prairie grasses for stimulating microbial degradation of four PAHs, benz[a]anthracene, chrysene, benzo[a]pyrene, and dibenz[a,h]anthracene, in soil columns was evaluated by Aprill and Sims [27]. Based on residue analysis of the soil columns, PAH disappearance was consistently greater in the vegetated columns compared with nonvegetated controls. Although sterile soil controls were not included in the experiments, the authors speculated that microbial degradation may account for the increased disappearance of the PAHs in the vegetated columns. However, the rhizosphere effect may have been obfuscated by addition of manure to all soil columns during PAH addition. Root uptake of the PAHs may have also obscured the contribution of microorganisms to the disappearance of PAH from the soil columns. Nonetheless, this research does provide evidence for the accelerated disappearance of hazardous organic compounds in the rhizosphere, and also presents a germane discussion on plant and root biology in relation to stimulating soil microbial activity and enhancing microbial degradation of organic compounds in the root zone.

In order to determine the potential role of rhizosphere microorganisms in biodegradation of trichloroethylene (TCE), we tested rhizosphere soils and nonvegetated soils from a former solvent disposal site [29]. Initial experiments with soil slurries monitored disappearance of TCE from the headspace, utilizing gas chromatography techniques [30]. These initial experiments provided the incentive for more rigorous tests with soil samples and soil-plant systems using ^{14}C-TCE. Mineralization of ^{14}C-TCE to $^{14}CO_2$ was monitored in specially designed Erlenmeyer flasks incubated under vegetated, nonvegetated, and sterile conditions (Anderson and Walton, in preparation). Vegetation tested included four plant species from the contaminated site (*Lespedeza cuneata*, *Solidago* sp., *Paspalum notatum* and *Pinus taeda*), as well as soybean, *Glycine max* germinated from commercially available seeds. In soils containing *L. cuneata*, *P. taeda*, and *G. max*, the levels of $^{14}CO_2$ produced were significantly greater ($p \leq 0.05$, t-test) than $^{14}CO_2$ production in both nonvegetated and sterile control soils. Radiolabelled CO_2 production in soil containing *Solidago* sp. and *P. notatum* was elevated, however, there was no statistically significant difference ($p \leq 0.05$, t-test) from $^{14}CO_2$ produced in the respective nonvegetated soils.

PHOSPHOLIPID FATTY ACID ANALYSIS

The observed variations in the TCE biodegradation activity of the different rhizosphere microbial communities provided the impetus for further exploration of their composition, activity, and nutritional status. Specific biochemical methods have been developed to assay for indicators of microorganisms in soil and sediment samples. Membrane phospholipids are present in all cells, have a rapid turnover, and are easily extracted from environmental samples and quantified, making them ideal for determining viable microbial biomass [31]. Essentially identical estimates of microbial biomass were found by the membrane phospholipid and direct count methods [32]. The phospholipid fatty acid (PLFA) assay has been used to describe microbial communities from such environmental samples as plant rhizospheres [33] and creosote-contaminated soils and sediments [34]. Detection of specific phospholipid fatty acids can indicate the presence and abundance of certain groups of microorganisms. For example, Nichols and coworkers [35] found relatively high proportions of 18-carbon fatty acids relative to 16-carbon fatty acids in a natural gas-exposed soil column capable of TCE degradation. This indicated the presence of a large population of type II methanotrophic bacteria. Marker fatty acids have also been discovered for, among others, sulphate-reducing bacteria, aerobes, anaerobes, and actinomycetes [31].

Phospholipid fatty acid assays can also indicate metabolic changes in a microbial community. During nutrient deprivation and other environmental stresses, fatty acid ratios can shift. Guckert et al. [36] found increases in the *trans:cis* ratio of monoenoic 16-carbon fatty acids in *Vibrio cholerae* during nutrient starvation. The protocol used to extract the lipids from environmental samples simultaneously extracts other biochemical indicators of nutritional status in microbial communities, for example, poly-β-hydroxyalkanoates [31].

Incorporation of ^{14}C-acetate into microbial lipids is a simple, yet useful technique for measuring heterotrophic microbial activity. The rate of acetate incorporation into microbial phospholipids has been shown to be an accurate and sensitive measure of growth in sediment samples [37]. The technique has been used to measure activity in sewage sludge [38], marine sediments [39], antarctic rock microbiota [40], and soils [41]. Acetate incorporation has also been used to assess the effects of toxicants, both inorganic and organic, on microbial metabolic activity [42].

Methods

The extraction procedure, a modification of Bligh and Dyer [43], was performed on lyophilized soil samples from the rhizosphere as well as from nonvegetated areas. Extracts were fractionated on silicic acid columns into neutral lipids, glycolipids, and phospholipids. Phospholipid

fatty acids were derivatized and quantified as described previously [29,44]. Total picomoles of phospholipid was converted to active microbial biomass. A cluster analysis of the phospholipid fatty acid profiles of different rhizosphere and nonvegetated soil samples was performed to help in analyzing the different sample types. The glycolipid fraction from the total lipid extraction, described above, was used to determine the endogenous lipid storage products poly-β-hydroxyalkanoates (PHAs) using the techniques of Findlay and White [45].

In order to determine heterotrophic microbial activity in rhizosphere and nonvegetated soils, incorporation of ^{14}C-labelled acetate into total lipids was measured. Soil samples (2 g) from rhizosphere and nonvegetated areas at the MCB were placed in 15-ml polypropylene tubes together with 0.5 ml of sterile, distilled, deionized water and incubated in the dark. Samples were extracted and fractionated as described above. Liquid scintillation spectrometry was used to quantify the radioactivity in total lipids as well as the radioactivity in the 3 lipid fractions after separation.

Results

Microbial biomass estimates, calculated from PLFA analysis, were fairly consistent with the estimates based on CO_2 efflux [30], and also illustrated the increased microbial biomass associated with the rhizosphere soils. Microbial biomass estimates (mean ± standard deviation) of rhizosphere soils of *Lespedeza cuneata, Paspalum notatum, Pinus taeda* and *Solidago* sp. were 1449 ± 423 μg/g, 3624 ± 522 μg/g, 1252 ± 175 μg/g, and 5825 ± 1176 μg/g, respectively. Microbial biomass estimates in the nonvegetated soil were 680 ± 423 μg/g. Although biomass estimates were useful for describing the microbiological properties of the study soils, they were not good predictors of TCE degradation. Rhizosphere soils from *P. notatum* and *Solidago* sp. had comparatively high levels of microbial biomass, yet both rhizosphere types did not degrade TCE as well as other samples with less microbial biomass.

In addition to microbial biomass, PLFA analysis was also used to determine the microbial community structure of the soil samples from the contaminated site. Although taxonomic characterization of the microorganisms from the soil samples was not undertaken, it was possible to compare qualitative differences between the groups of microorganisms present in the different samples. A dendogram of the cluster analysis results from the PLFA analyses (Figure 1) illustrates the primary clustering of nonvegetated soil samples with *Lespedeza cuneata* rhizosphere soil samples, and *Solidago* sp. rhizosphere soil with *Pinus taeda* rhizosphere soil and *Paspalum notatum* rhizosphere soil samples. Secondary clustering occurred between *Pinus taeda* soil samples and *Paspalum notatum* soil samples. A principal components analysis indicated that the fatty acid which most determined the primary

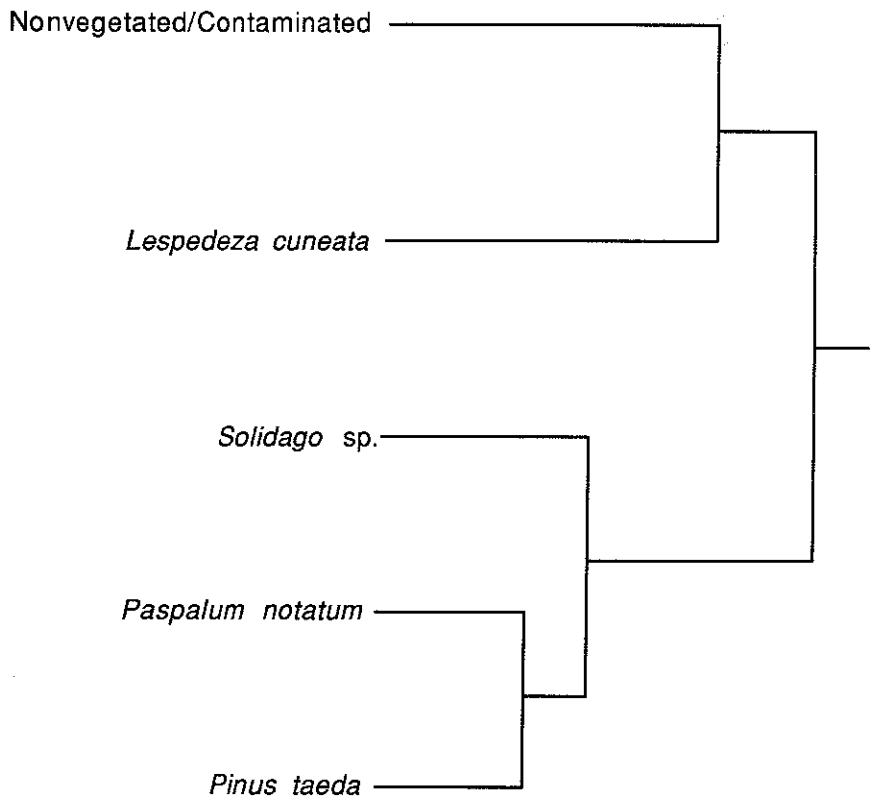

Figure 1. Dendogram of cluster analysis results from phospholipid fatty acid profiles of rhizosphere and nonvegetated soils from the contaminated site. Comparisons of qualitative differences between the groups of microorganisms present in the different samples illustrated the primary clustering of nonvegetated soil samples with *Lespedeza cuneata* rhizosphere soil samples, and *Solidago* sp. rhizosphere soil with *Pinus taeda* rhizosphere soil and *Paspalum notatum* rhizosphere soil samples. Secondary clustering occurred between *Pinus taeda* soil samples and *Paspalum notatum* soil samples.

clustering (separation of the nonvegetated soil and *L. cuneata* rhizosphere soil from the others) was cy19:0. The secondary clustering, which separates *P. notatum* and *P. taeda* from *Solidago* sp., was explained by the greater presence of 10Me16:0 and 10Me18:0 in samples of *P. taeda* and *P. notatum* rhizosphere soil. These fatty acids (10Me16:0 and 10Me18:0) are characteristic of actinomycetes [31].

Overall, the PLFA results were similar in both rhizosphere and nonvegetated soils (Table 2). The most abundant PLFA in both rhizosphere and nonvegetated soils was palmitate (16:0), a common saturated fatty acid. The strong presence of monounsaturated (16:1ω7c and 18:1ω7c), and branched phospholipid fatty acids (i15:0 and i16:0 for example) indicated that the communities of both sample types (rhizosphere and nonvegetated) were composed of Gram-negative and Gram-positive microorganisms in approximately equal abundance. Ratios of *cis* fatty acids to *trans* fatty acids (specifically 16:1ω7c to 16:1ω7t and 18:1ω7c to 18:1ω7t), which can be indicative of stress or unbalanced growth [31], were not significantly different among the rhizosphere and nonvegetated soils. Cyclopropyl fatty acids such as cy17:0 and cy19:0, which can be indicative of unbalanced growth and/or anaerobic microorganisms [46], were present in all sample types. These results are probably best explained by the soil conditions at the contaminated site. Namely that the soil is low in organic carbon, and fluctuates between aerobic and anaerobic conditions. Recent research has also shown that solvent-degrading microorganisms convert *cis* unsaturated fatty acids in their membranes to *trans* fatty acids to avoid substrate toxicity [47]. Previous analyses of a TCE-degradading soil column, enriched with natural gas [48], revealed the strong presence of Type II methanotrophs, as indicated by the fatty acid 18:1ω8c [35]. Because methanotrophs are likely to be found in zones that fluctuate between aerobic and anaerobic conditions, such as surface soils that periodically flood and drain and subsurface soils at the capillary fringe, these bacteria are likely to be present in soils from the site. However, the characteristic fatty acid for Type II methanotrophs (18:1ω8c) was not detected in these soils. It is possible that because enrichments were not performed, the concentration of methanotrophs was below the detection limits for PLFA analysis. Nonetheless, these microorganisms are undoubtedly present, based on their ubiquity in nature and the favorable conditions for their proliferation at the study site.

The microbial metabolic activity of soil samples was determined by measuring the incorporation of ^{14}C-acetate into cellular lipids. The metabolic activity (mean incorporation rate ± standard deviation) of the four rhizosphere samples was significantly greater ($p \leq 0.05$, t-test) than metabolic activity in the nonvegetated soil (1407 ± 10, 905 ± 35, 599 ± 33, 1727 ± 180 pMoles/g soil/h for *L. cuneata*, *P. taeda*, *P. notatum*, and *Solidago* sp., respectively vs. 445 ± 86 pMoles/g soil/h for the nonvegetated soil). In addition, there were significant differences in ^{14}C-acetate incorporation rates among the four rhizosphere samples ($p \leq 0.05$, t-test) from each other, with *Solidago* sp. rhizosphere soil having the highest rate. The greater metabolic activity of rhizosphere samples is consistent with the biomass estimates reported earlier in this study and also consistent with the findings of others that microbial

Table 2
Phospholipid fatty acid (PLFA) profiles of soil samples collected from the Miscellaneous Chemicals Basin at the Savannah River Site

PLFA[A]	Mole%±SD[B]				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
i14:0	0.7±0.6	0.7±0.1	0.9±0.1	0.9±0.1	1.0±0.2
14:0	1.4±0.9	1.4±0.2	1.4±0.1	1.3±0.1	1.8±0.3
i15:0	7.1±2.1	8.8±0.4	10.9±0.4	8.6±0.4	10.3±0.2
a15:0	2.7±0.9	3.8±0.3	4.5±0.1	3.8±0.1	5.3±0.4
15:0	1.0±0.7	0.9±0.2	0.9±0.1	1.1±0.0	1.2±0.1
i16:0	4.1±1.0	2.2±0.1	2.4±0.1	2.7±0.2	2.7±0.1
16:1ω9c	1.1±0.7	1.3±0.1	1.7±0.1	1.9±0.2	2.2±0.2
16:1ω7c	5.3±0.5	6.2±0.7	7.6±0.6	5.6±0.1	6.2±0.7
16:1ω7t	0.2±0.3	0.3±0.1	0.3±0.0	0.3±0.0	0.1±0.1
16:1ω5c	2.8±0.8	3.8±0.3	4.9±0.2	4.1±0.1	4.1±0.2
16:0	13.0±2.3	14.9±1.2	14.9±0.4	14.1±0.4	14.8±0.4
i17:1	2.1±0.9	2.3±0.1	2.7±0.1	2.6±0.2	2.7±0.6
10Me16:0	3.8±1.0	4.6±0.8	3.5±0.2	5.6±0.4	5.4±0.4
i17:0	2.4±0.6	1.6±0.1	1.3±0.0	1.6±0.1	1.7±0.1
a17:0	2.4±0.9	1.6±0.1	1.6±0.1	1.7±0.1	1.6±0.3
cy17:0	2.8±1.1	3.8±0.3	3.2±0.2	3.5±0.2	3.5±0.2
18:2ω6	4.5±2.4	3.9±1.1	5.4±1.1	6.6±1.3	5.3±1.0
18:3ω3	2.4±1.5	0.0±0.0	0.9±0.1	1.0±0.2	0.6±0.4
18:1ω9c	5.9±1.8	4.0±0.5	3.9±0.3	3.1±0.1	3.2±0.0
18:1ω7c	6.7±1.2	8.5±0.8	8.4±0.6	7.9±0.1	6.4±0.4
18:1ω5c	0.7±0.6	1.0±0.2	0.7±0.1	0.9±0.0	0.7±0.1
18:0	2.7±0.7	2.9±0.2	2.1±0.6	1.7±0.0	1.6±0.1

Table 2 continued

PLFA[A]	Mole%±SD[B]				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
br19:1	0.4±0.4	1.3±0.5	0.8±0.0	0.7±0.0	0.7±0.1
10Me18:0	1.8±0.6	0.8±0.1	0.7±0.0	1.1±0.0	1.2±0.4
cy19:0	8.0±2.2	9.7±1.2	4.2±0.2	4.6±0.2	5.2±0.5
20:0	2.5±1.6	2.0±0.2	0.7±0.1	0.5±0.4	0.7±0.5

[A]The shorthand nomenclature used to describe fatty acids is as follows: The number before the colon indicates the number of carbon atoms in the fatty acids. The number after the colon indicates the number of double bonds in the fatty acid chain. The position of the initial double bond is indicated by the number of carbon atoms from the methyl, or ω, end of the molecule. The configuration of the double bond is shown by the 'c' for *cis* and 't' for *trans*. Because almost all unsaturations are *cis*, 'c' is often omitted. Methyl branching can be indicated as iso ('i'; the second carbon from the methyl end), anteiso ('a'; the third carbon) or 'br' (branch) if the position is unknown. When a branch is known but not in the 'i' or 'a' position, it is indicated by the position from the carboxyl end followed by 'Me' before the carbon chain length. Cyclopropane fatty acids are indicated as 'cy' (Adopted from [31]).

[B]For the following fatty acids, mole % values were < 1 for all sample types: a13:0, i15:1, a15:1, 15:1, 16:3, 16:1, 16:1ω7t, 16:1ω13t, 17:1ω6, 17:1, 17:0, 18:3ω6, 18:1ω7t, 18:1, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω3, 20:1ω9c, 20:0, 23:0, 24:0.

biomass and activity is greater in the rhizosphere [6]. In addition, acetate incorporation appeared to be a good predictor for the ability to degrade TCE in whole-plant experiments. Rhizosphere samples from *L. cuneata* and *P. taeda* had relatively high rates of acetate incorporation and also had the highest TCE degradation rates, while samples of *P. notatum* rhizosphere and nonvegetated soils had lower acetate incorporation rates and lower TCE degradation rates.

Differences in the incorporation of ^{14}C-acetate into the three lipid classes between rhizosphere and nonvegetated soil samples may indicate unbalanced growth in the nonvegetated samples. Soil from nonvegetated areas incorporated ^{14}C-acetate predominantly into neutral and glycolipids (storage lipids) during the first three hours of incubation, possibly indicating unbalanced growth of the microbial community in this soil. Only 20% of the radioactivity was found in the phospholipid (membranes) fraction after 3 hours. In contrast, soils from *L. cuneata, P. taeda* and *Solidago* sp. incorporated the acetate into membrane lipids (phospholipids). Radioactivity in the phospholipid fraction for *P. taeda* and *Solidago* sp. rhizosphere soil was greater than 50% of the total radioactivity in all lipid fractions after 1 hour, and greater than 80% for *L. cuneata* rhizosphere soil after 3 hours. Soils collected from *P. notatum* rhizosphere soil incorporated most of the ^{14}C-acetate into the glycolipid fraction after 1 hour, similar to the results of the nonvegetated soil.

Consistently greater amounts of PHA were detected in rhizosphere soils compared with the nonvegetated soils, although the amounts were significantly greater ($p \leq 0.05$, t-test) only for soils from *Paspalum notatum* rhizosphere. All samples tested contained significant amounts of PHA, however, PHA levels varied among sample types. Mass spectroscopy revealed that almost all of the constituent beta-hydroxy acids from the PHAs was betahydroxybutyrate (PHB) although very small amounts of betahydroxy hexanoate were also detected. Samples of *L. cuneata* and *P. taeda* rhizosphere soils contained elevated levels of PHA compared to PLFA, and were also very capable of degrading TCE in whole-plant experiments. Correlations between TCE degradation rate and PHA production have been observed previously in bioreactors (Ringleberg and Phelps, Center for Environmental Biotechnology, The University of Tennessee, personal communication). Soil samples from *Solidago* sp. rhizosphere and nonvegetated areas at the site had the lowest levels of PHA and also degraded TCE more slowly in whole-plant experiments than rhizosphere soils of *L. cuneata* and *P. taeda*. Only soils from the rhizosphere of *P. notatum* failed to follow the correlation between degradation of TCE and production of PHA. Soils from *P. notatum* rhizosphere had comparatively high levels of PHA, but did not appear to degrade TCE as well as soils from the rhizosphere of *L. cuneata* and *P. taeda*.

APPLICATIONS

The highly versatile metabolic capabilities of fungi and bacteria can be applied to reclaim polluted ecosystems and minimize the potential adverse effects of hazardous chemicals released to the environment. However, a sufficient consortia of microorganisms, capable of degrading the contaminant(s), must be present, and environmental conditions conducive to degradation must be maintained. Environmental conditions onsite may significantly hinder microbial degradation of toxicants. In such cases, microbial degradation may be enhanced by altering conditions through nutrient additions, irrigation, or other interventions. The addition of external carbon sources may be especially important in such cases where the contaminant is degraded cometabolically.

The use of vegetation to enhance the microbial degradation of surface and near-surface soils contaminated with hazardous wastes, such as chlorinated solvents, polycyclic aromatic hydrocarbons (PAHs), or pesticide wastes under field conditions has not been demonstrated. Vegetation may prove to be an important variable, affecting microbial degradation of unwanted chemicals and provide a *cost-effective* remediation strategy for soils containing these compounds [49]. There is growing evidence that rhizosphere treatment systems may be used successfully in the field. Establishing or selectively cultivating vegetation on a contaminated site is a relatively simple site management technique, which could have substantial ramifications for reducing the adverse impact of contaminants on ecosystems. Continued exploration of critical environmental variables, affecting the soil-plant-microbe-chemical relationship, will help to identify situations, in which bioremediation using vegetation may be appropriate. The reviewed literature on rhizosphere microbiology, accelerated microbial degradation of agricultural chemicals in the root zone, and recent research on similar observations with hazardous organic compounds provides additional incentives to explore bioremediation of contaminated soils using vegetation.

CONCLUSIONS

The variety of plants and chemicals studied for evidence of microbial degradation in the rhizosphere strongly suggests that a diverse and synergistic microbial community, rather than a single species, is responsible for biotransformation of toxicants in the rhizosphere. Participation of a microbial community is implicated by (i) the extreme diversity and complexity of toxicants degraded, and (ii) the knowledge that many of these compounds are completely degraded only in the presence of interacting microbial populations (consortia). Moreover, data presented, herein, from phospholipid fatty acid analysis, ^{14}C-

acetate incorporation, and PHA analysis of microorganisms from the rhizosphere are consistent with participation of a microbial community in TCE degradation.

Moreover, the information presented, herein, illustrates the potential for rhizosphere microbial communities to remediate soil systems through biotransformation of hazardous organic compounds in the root zone. Future research in this area should include investigations of the possible role of mycorrhizae in degradation of toxicants and characterization of the microorganisms associated with different plant species and different histories of toxicant exposure. Closer examination of root exudation as a response to chemical challenge in the rhizosphere will also shed light on the complex relationship between plants, microorganisms, soil, and hazardous chemicals in the root zone.

ACKNOWLEDGMENTS

The authors thank A. M. Hoylman and David Ringleberg, The University of Tennessee, Knoxville, TN; A. V. Palumbo, T. J. Phelps, and N. T. Edwards, Oak Ridge National Laboratory, Oak Ridge, TN; T. C. Hazen of the Savannah River Site, Aiken, SC; and E. L. Kruger, Iowa State University, Ames, IA for helpful contributions to this work.

This research was sponsored by the Office of Technology Development, U.S. Department of Energy. Oak Ridge National Laboratory is managed by Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy. Environmental Sciences Division Publication No. 4035.

REFERENCES

1 Hsu TS, Bartha R. Appl Environ Microbiol 1979; 37: 36-41.
2 Curl EA, Truelove B. The Rhizosphere. Berlin, Heidelberg: Springer-Verlag, 1986; 288 pp.
3 Campbell R. Plant Microbiology. Baltimore: Edward Arnold, 1985; 191 pp.
4 Rovira AD, Foster RC, Martin JK. In: Harley JL, Russell RS, eds. The Soil-Root Interface. New York: Academic Press, 1979; 1-4.
5 Katznelson H. Soil Sci 1946; 62: 343-354.
6 Atlas RM, Bartha R. Microbial Ecology: Fundamentals and Applications. Menlo Park, CA: Benjamin/Cummings, 1993; pp.563.
7 Sandmann ERIC, Loos MA. Chemosphere 1984; 13: 1073-1084.
8 Abdel-Nasser M, Makawi AA, Abdel-Moneim AA. Egypt J Microbiol 1979; 14: 37-44.
9 Abueva AA, Bagaev VB. Izvestiya Timiiryazevsk Skh Akad 1975; 2: 127-130.

10 Gavrilova EA, Kruglov YV, Garankina NG. Tr Vses Nauchno-Issled Instit Skh Mikrobiologii 1983; 52: 67-70.
11 Hiltner L. Arb Dtsch Landwirt Ges 1904; 98: 59-78. In: Curl EA, Truelove B. The Rhizosphere. Berlin, Heidelberg: Springer-Verlag, 1986; pp.288.
12 Hale MG, Foy CL, Shay FJ. Adv Agron 1971; 23: 89-109.
13 Hale MG, Moore LD. Adv Agron 1979; 31: 93-124.
14 Kaiser P, Reber H. Meded Fac Landouwwet Rijksuniv Gent 1970; 35: 689-705.
15 Ashton FM, Crafts AS. The Mode of Action of Herbicides. New York: John Wiley and Sons, 1981; pp.525.
16 Herring R, Bering CL. Bull Environ Contam Toxicol 1988; 40: 626-632.
17 Anderson TA, Guthrie EA, Walton BT. Environ Sci Technol 1993; 27: 2630-2636.
18 Shimp JF, Tracy JC, Davis LC, Lee E, Huang W, Erickson LE. Crit Rev Environ Sci Technol 1993; 23: 41-77.
19 Seibert K, Fuehr F, Cheng HH. Theory and Practical Use of Soil Applied Herbicides Symposium. European Weed Resource Society. Paris, France. 1981; 137-146.
20 Reddy BR, Sethunathan N. Appl Environ Microbiol 1983; 45: 826-829.
21 Lappin HM, Greaves MP, Slater JH. Appl Environ Microbiol 1985; 49: 429-433.
22 Sato K. In: Vancura V, Kunc F, eds. Interrelationships between Microorganisms and Plants in Soil. New York: Elsevier, 1989; 335-342.
23 Gunner HB, Zuckerman BM, Walker RW, Miller CW, Deubert KH, Longley RE. Plant Soil 1966; 25: 249-264.
24 Federle TW, Schwab BS. Appl Environ Microbiol 1989; 55: 2092-2094.
25 Federle TW, Ventullo RM. Appl Environ Microbiol 1990; 56: 333-339.
26 Wolverton BC, McDonald-McCaleb RC. J Miss Acad Sci 1986; 31: 79-89.
27 Aprill W, Sims RC. Chemosphere 1990; 20: 253-265.
28 Rasolomanana JL, Balandreau J. Rev Ecol Biol Sol 1987; 24: 443-457.
29 Anderson TA, Walton BT. Comparative plant uptake and microbial degradation of trichloroethylene in the rhizospheres of five plant species: Implications for bioremediation of contaminated surface soils. ORNL/TM-12017. Oak Ridge, TN. 1992; pp.186.
30 Walton BT, Anderson TA. Appl Environ Microbiol 1990; 56: 1012-1016.

31 Vestal JR, White DC. Bio Science. 1989; 39: 535-541.
32 Balkwill DL, Leach FR, Wilson JT, McNabb JF, White DC. Microbial Ecol 1988; 16: 73-84.
33 Tunlid A, Baird BH, Trexler MB, Olsson S, Findlay RH, Odham G, White DC. Can J Microbiol 1985; 31: 1113-1119.
34 Smith GS, Nickels JS, Kerger BD, Davis JD, Collins SP, Wilson JT, McNabb JF, White DC. Can J Microbiol 1986; 32: 104-111.
35 Nichols PD, Henson JM, Antworth CP, Parsons J, Wilson JT, White DC. Environ Toxicol Chem 1987; 6: 89-97.
36 Guckert JB, Hood MA, White DC. Appl Environ Microbiol 1986; 52: 794-801.
37 Moriarty DJW, White DC, Wassenberg TJ. J Microbiol Meth 1985; 3: 321-330.
38 McKinley VL, Vestal JR. Appl Environ Microbiol 1984; 47: 933-941.
39 White DC, Bobbie RJ, Morrison SJ, Oosterhof DK, Taylor CW, Meeter DA. Limnol Oceanogr 1977; 22: 1089-1099.
40 Vestal JR. Appl Environ Microbiol 1988; 54: 960-965.
41 White DC, Davis WM, Nickels JS, King JD, Bobbie RJ. Oecologia 1979; 40: 51-62.
42 Barnhart CL, Vestal JR. Appl Environ Microbiol 1983; 46: 970-977.
43 Bligh EG, Dyer WJ. Can J Biochem Phys 1959; 37: 911-917.
44 Findlay RH, White DC. J Microbiol Meth 1987; 6: 113-120.
45 Findlay RH, White DC. Appl Environ Microbiol 1983; 45: 71-78.
46 Guckert JB, Antworth CP, Nichols PD, White DC. FEMS Microbiol Ecol 1985; 31: 147-158.
47 Heipieper H-J, Diefenbach R, Keweloh H. Appl Environ Microbiol 1992; 58: 1847-1852.
48 Wilson JT, Wilson BH. Appl Environ Microbiol 1985; 49: 242-243.
49 Walton BT, Anderson TA. Curr Opin Biotechnol 1992; 3: 267-270.
50 Ampova G. Bulgarski Tyutyun 1971; 16: 39-43.
51 Gudin C. Proc International Symposium on Ground Water Pollution by Oil Hydrocarbons. Prague, Czechoslovakia, 1978; 411-417.
52 Knaebel DB, Vestal JR. Can J Microbiol 1992; 38: 643-653.
53 Katayama A, Matsumura F. Environ Toxicol Chem 1993; 12: 1059-1065.
54 Donnelly PK, Hegde RS, Fletcher JS. Chemosphere 1994; 28: 981-988.
55 Ferro AM, Sims RC, Bugbee B. J Environ Qual 1994; 23: 272-279.