International Journal of Phytoremediation, 5(3):225–234 (2003)

ISSN: 1522-6514
DOI: 10.1080/16226510390255670

Degradation of Crude Oil in the Rhizosphere of *Sorghum bicolor*

M. K. Banks,[1] Peter Kulakow,[2] A. P. Schwab,[3] Zeke Chen,[4] and Karrie Rathbone[2]

[1] *School of Civil Engineering, Purdue University;* [2] *Department of Agronomy, Kansas State University;* [3] *Department of Agronomy, Purdue University;* [4] *Department of Civil Engineering, Kansas State University*

* Corresponding author: M. K. Banks, Civil Engineering, CIVL, Purdue University, West Lafayette, IN 47907.

ABSTRACT

Dissipation of petroleum contaminants in the rhizosphere is likely the result of enhanced microbial degradation. Plant roots may encourage rhizosphere microbial activity through exudation of nutrients and by providing channels for increased water flow and gas diffusion. Phytoremediation of crude oil in soil was examined in this study using carefully selected plant species monitored over specific plant growth stages. Four sorghum (*Sorghum bicolor* L.) genotypes with differing root characteristics and levels of exudation were established in a sandy loam soil contaminated with 2700 mg crude oil/kg soil. Soils were sampled at three stages of plant growth: five leaf, flowering, and maturity. All vegetated treatments were associated with higher remediation efficiency, resulting in significantly lower total petroleum hydrocarbon concentrations than unvegetated controls. A relationship between root exudation and bioremediation efficiency was not apparent for these genotypes, although the presence of all sorghum genotypes resulted in significant removal of crude oil from the impacted soil.

KEY WORDS: phytoremediation, petroleum, crude oil, sorghum, rhizosphere.

I. INTRODUCTION

In one specialized type of phytoremediation, the presence of plants has been shown to accelerate the process of hydrocarbon bioremediation through enhancing degradation by soil microorganisms. The area adjacent to a plant root, referred to as the rhizosphere, is a continuum extending from the root surface with maximum activity as compared to the bulk soil, which has far less activity. The rhizosphere has nutrients and water exuded from the plant roots, resulting in enhanced microbial activity (Walton and Anderson, 1990; Hou *et al*., 2001). The root surface and soil surrounding

healthy plant roots are ideal habitats for many soil microorganisms (Gerhardson and Clarhom, 1986; Whipps and Lynch, 1990; Hutchinson *et al*., 2001). The organic substrate produced from the decay of dead root hairs serves as an important carbon source for rhizosphere microorganisms that have the potential to degrade organic pollutants (Heinonsalo *et al*., 2000). Due to the recalcitrant nature of petroleum contaminants, a healthy and metabolically diverse community in the rhizosphere would be more capable of contaminant degradation (Cunningham *et al*., 1996).

The impact of the rhizosphere on microbial communities and contaminant degradation varies among plant species and varieties. Some genotypes produce amino acids and other critical microbial growth factors, whereas other genotypes may lack this capability. These root exudates enhance the growth of specific microbes, resulting in a net benefit to the rhizosphere community. For example, there is selective stimulation of gram negative rods by plant roots, promoting their colonization in the rhizosphere. Gram negative bacteria have been identified as comprising the majority of petroleum degraders (Sarand *et al*., 1998).

Allelopathy is the chemical modification of an environment to encourage growth of specific organisms or the exclusion of others. Allelopathy can be exhibited by plants or microbes, producing chemicals that may be toxic to other organisms or may encourage an association between a plant and microbes. This association can be used *in situ* through phytoremediation to promote maximum contaminant degradation. The limiting factor would be the volume and extent of the rhizosphere. An extensive root system could increase the plant–microbe association and encourage contaminant degradation (Aprill and Sims, 1990). Many plants establish a synergistic relationship between their roots and specialized soil fungi (mycorrhizae) for the exchange of nutrients and water. Sometimes this relationship is essential for plant growth, but it may also promote degradation of contaminants. Root debris and sloughed hyphae will increase soil organic matter and distribute microorganisms for maximum contact with contaminants (Sarand *et al*., 1998; Heinonsalo *et al*., 2000).

Plants are generally incapable of assimilating highly adsorbed contaminants such as polycyclic aromatic hydrocarbons (PAHs) (Anderson and Coats, 1994; Pichtel and Liskanen, 2001). As a result, the greatest research emphasis for phytoremediation of petroleum contaminants has been placed on microbial degradation because of environmental limitations of contaminant transport and the physiological diversity of the relevant rhizosphere microorganisms.

The obvious advantages of remediating contaminated soils with vegetation are: 1) the process is solar-energy driven, requiring little or no inputs; 2) a high potential for public acceptance, having minimum disturbance of the soil surface; and 3) avoidance of the need to transfer contaminants from one phase to another (Cunningham *et al*., 1996). Investigations of the influence of different plant varieties on phytoremediation are rare. A limited number of studies have directly compared different plant species for their potential to enhance bioremediation (Shann and Boyle, 1994; Schwab and Banks, 1994; Adam and Duncan, 1999). The use of plants was found to improve bioremediation efficiency for both herbicides (Coleman *et al*., 2002; Anhalt *et al*., 2000) and PAHs (Banks *et al*., 1999. Olson *et al*., 2001); differences existed between plant species.

Although many organic contaminants have been studied, the role of vegetation for bioremediation enhancement of total petroleum hydrocarbons (TPHs) in soils is not well known. Most phytoremediation studies have examined the potential of different plant species, but the traits within each plant species that enhance phytoremediation have not been thoroughly assessed. A better understanding of the role of root structure (root morphology), nitrogen fixation, root exudation, and vegetative growth patterns in contaminated soil may allow for better prediction of phytoremediation performance.

Plant traits need to be evaluated within the same species, if possible, to determine those traits that are important in phytoremediation. Variability of phytoremediation efficiency within a plant species is unknown. Just as enhancement of bioremediation is variable between plant species, it is likely that genetic variability exists within a plant species.

The objective of this research was to test four sorghum varieties for their phytoremediation potential. Based on a field phytoremediation project, sorghum (*Sorghum bicolor* L.) grew well in petroleum-contaminated soil and had an elevated TPH degradation rate. Sorghum varieties having different genotypic properties—high exudates, low exudates, N-efficient, and high root density—were chosen for this greenhouse study to determine if specific root characteristics were associated with enhanced phytoremediation efficiency. Results from this research may allow for more meaningful choices of plant species for phytoremediation.

II. MATERIALS AND METHODS

A. Soil Characteristics and Contamination

Uncontaminated agricultural soil (fine sandy loam) from an agricultural farm of Kansas State University (Manhattan, KS) was used in this project. Table 1 lists soil characteristics for the soil, which was dried at room temperature and passed through a 2-mm sieve before use.

Crude oil (R1350) was provided by a Texaco Refinery (El Dorado, KS). The oil was aged by placing it in an aluminum pan in an open area for seven days. By the end of the aging cycle, the volatile organic compounds that are toxic to plants had been removed.

TABLE 1. Selected properties of the soil used in this study. All determinations, according to NCR 221 (1998).

pH[a]	7.7
Cation exchange capacity ($cmol_+/kg$)[b]	9
Organic matter (%)[c]	0.7
Sand (%)[d]	55
Silt (%)[d]	42
Clay (%)[d]	3

[a] Measured in 1:1 in water.
[b] 1 *M* ammonium acetate method.
[c] Determined by chromic acid oxidation.
[d] Hydrometer method.

The aged crude oil was incorporated into the soil by adding 12.5 g of oil to 4.5 kg of sieved soil and mixing in a rotary V-mixer for 45 cycles. To ensure that this mixing method was effective at evenly distributing oil into the soil, four subsamples were taken at different locations and the TPH was measured. This method was found to be effective and the resulting initial contaminant concentration was found to be 2710 ± 70 mg TPH/kg.

B. Sorghum

Four varieties of sorghum, P1 (China 17), P2 (Shanqui Red), P3 (SC279), and P4 (SRN39), were used. P1 and P2 were selected because they are nitrogen efficient and tend to have reduced root mass. Shanqui Red (P2) is resistant to root rot, whereas China 17 (P1) is not. P3 is striga susceptible, possessing a root exudate that serves as the germination stimulant for striga. P4 is striga resistant and has reduced root exudation. Striga are destructive root parasites of many important cereals and legumes (Hess *et al.*, 1992; Siame *et al.*, 1993; Weerasuriya *et al.*, 1993).

C. Experimental Design

The experimental design was a split-plot lattice that accounted for variation due to greenhouse effects. Four sorghum varieties were planted, three plants of the same variety per pot, with four replications. The pots were physically arranged in strips to account for differences primarily in temperature with each treatment represented in every strip. Daytime temperature was set to 30°C and night temperatures were set to 25°C.

The four varieties of sorghum were germinated in the growth chamber. The seedlings were transplanted into 4.5 kg of soil contaminated with aged crude oil at a concentration level 2710 ± 70 mg TPH/kg. The pots were planted with three seedlings per pot and plastic liners were placed underneath for leachate collection. All pots were watered as needed. Fifteen grams of a slow-release fertilizer, Sierrablend 19-7-10, was added to each pot.

A contaminated non-vegetated control and an abiotic control also were prepared. Abiotic controls were bagged and placed in a controlled temperature room (4°C). At each stage of the experiment, the abiotic samples were analyzed for TPH. The TPH concentrations did not vary significantly from the starting concentration at any time. Because different sorghum varieties were used, growth characteristics could not be predicted. Therefore, harvest dates were based on plant development stage instead of time. Three harvest dates were chosen: five leaf stage, prior to flowering (boot stage), and maturity (seed set) stage. The five-leaf stage occurs approximately three weeks after germination, when the plant has five leaves fully expanded. At this stage, the root system is developing rapidly. At boot stage, all leaves are fully expanded, providing maximum leaf area and light interception. The head is developed to nearly full size and is enclosed in the flag-leaf sheath. At maturity stage, maximum total dry weight of the plant has occurred.

The pots were destructively sampled at the three different plant stages. Plant height (cm) was measured from the soil surface to the top point of stalk at each growth stage. Plant yield (above-ground biomass) was measured at the same time and based on

dry weight (g). The above-ground biomass was clipped, dried (48 h at 65°C), and weighed. The roots were separated from the soil, dried (65°C for 48 h), and weighed. The soil was mixed and allowed to dry at room temperature. The soil samples were ground and subsampled for TPH analysis.

D. Contaminant Analysis

Chemical extraction of soils was performed either by the soxhlet extraction (Method 3540, U.S. EPA, 2000) or by shaking for analysis of total petroleum hydrocarbons (Schwab *et al*., 1999).

The shaking method was a sequential methylene chloride extraction. One gram of plant material or soil was added to a 50-mL centrifuge tube; 10 ml of methylene chloride was added and the tube was capped. The solution was shaken for 30 min and then centrifuged for 10 min. The supernatant was decanted into a glass bottle. This process was repeated twice. After extraction was complete, the methylene chloride was evaporated at ambient temperature, the residue dissolved in freon, and the concentration of total petroleum hydrocarbons was determined by infrared spectrophotometry.

The Buck HC-404 Total Hydrocarbon Analyzer (Buck Scientific, East Norwalk, CT) was used to measure the infrared absorbance of the hydrocarbons dissolved in the freon (Method 418.1, U.S. EPA, 1983). A standard curve of absorbance versus concentration of TPH was created by analyzing standard solutions of known TPH concentrations created from the same crude oil.

E. Quality Assurance/Quality Control

At least one blank and one duplicate for every 10 soil samples were analyzed. For the IR analysis, 10% of total extraction samples were analyzed in duplicate.

III. RESULTS AND DISCUSSION

The heights of plants grown in uncontaminated and contaminated soils at the five-leaf stage are listed in Table 2. In both cases, P1 had the greatest average plant height, whereas P4 had the least. Contamination significantly reduced plant heights for the P3 and P4 species at this stage of growth.

TABLE 2. Average height of the sorghum plants at the five-leaf stage.

Variety[a]	Contaminated	Uncontaminated
	Height (cm)	
P1	19.7	18.5
P2	16.0	16.5
P3	11.7	13.7
P4	7.5	10.1
l.s.d.[b] ($P < 0.05$)	2.0	

[a] P1—nitrogen use efficient; P2—nitrogen use inefficient; P3—high root exudates; P4—low root exudates.
[b] Least significant difference comparing all means.

TABLE 3. Above-ground biomass in contaminated and uncontaminated soils at flowering and maturity stages.

	Flowering		Maturity	
Variety[a]	Contaminated	Uncontaminated	Contaminated	Uncontaminated
		Biomass (g)		
P1	27.3	33.9	80.1	95.7
P2	38.6	43.2	62.0	99.7
P3	32.7	32.6	51.5	64.2
P4	29.8	44.8	66.0	52.7
l.s.d.[b] ($P < 0.05$)	13.8		30.1	

[a]P1—nitrogen use efficient; P2—nitrogen use inefficient; P3—high root exudates; P4—low root exudates.
[b]Interaction least significant difference for all means within a given growth stage.

The above-ground dry biomass in contaminated and uncontaminated soils for flowering and maturity stages is listed in Table 3. At the flowering stage, the only significant difference in biomass resulting from contamination was for the P4 variety, in which the crude oil contamination supressed plant growth. At maturity, only the P2 variety was associated with a significant difference, again with crude oil contamination reducing biomass.

The average sorghum root weights at the three sampling times are listed in Table 4. At the five-leaf stage, no significant differences were observed, either between varieties or because of soil contamination. At the flowering stage, trends emerged. Although soil contamination did not result in differences in root biomass, the nitrogen-inefficient varieties, P3 and P4, had significantly higher biomass than the nitrogen-efficient varieties, P1 and P2, for both contaminated and uncontaminated soils (at $p < 0.10$, with some of the differences being significant at $p < 0.05$, Table 4). At maturity, the same trends existed for the contaminated soils, but no significant differences existed within the uncontaminated soils. Apparently, nitrogen efficiency provided no advantage to the root growth for P1 and P2 in the contaminated soils. Instead, the

TABLE 4. Average mass of roots recovered from the sorghum plants in this study as affected by maturity and soil.

	Five-leaf		Flowering		Maturity	
Variety[a]	Contam.	Uncontam.	Contam.	Uncontam.	Contam.	Uncontam.
			Root biomass (g)			
P1	0.32	0.35	4.5	4.4	8.6	9.3
P2	0.26	0.17	6.8	4.5	8.7	10.0
P3	0.21	0.24	8.7	7.5	13.3	9.5
P4	0.42	0.27	7.5	8.9	11.5	11.9
l.s.d.[b] ($P < 0.05$)	0.18		3.6		5.1	

[a]P1—nitrogen use efficient; P2—nitrogen use inefficient; P3—high root exudates; P4—low root exudates.
[b]Interaction least significant difference for all means.

TABLE 5. Average TPH concentration as impacted by sorghum variety and plant maturity. Concentrations of TPH in abiotic controls (contaminated soils stored at 4°C until analysis) did not vary significantly from the original 2710 ± 70 mg/kg.

Variety[a]	Five-leaf	Flowering	Maturity
		TPH concentration (mg/kg)	
P1	2430	1230	839
P2	2110	1060	850
P3	1740	1100	932
P4	2190	1080	992
Unplanted	2370	1690	1770
l.s.d.[b] ($P < 0.05$)		452	

[a]P1—nitrogen use efficient; P2—nitrogen use inefficient; P3—high root exudates; P4—low root exudates.
[b]Interaction least significant difference for all means.

finer root structure of P3 and P4 yielded greater root biomass at the flowering stage in uncontaminated soils and at both flowering and maturity stages for contaminated soils.

Residual soil TPH concentrations for the five-leaf stage were not significantly different among the different genotypes, with the exception that concentrations for P3 were less than P1 and the unplanted control (Table 5). At the flowering stage, unvegetated soils had significantly higher TPH concentrations than soil with all vegetated treatments (Table 5, Figure 1). This trend continued until the end of the experiment.

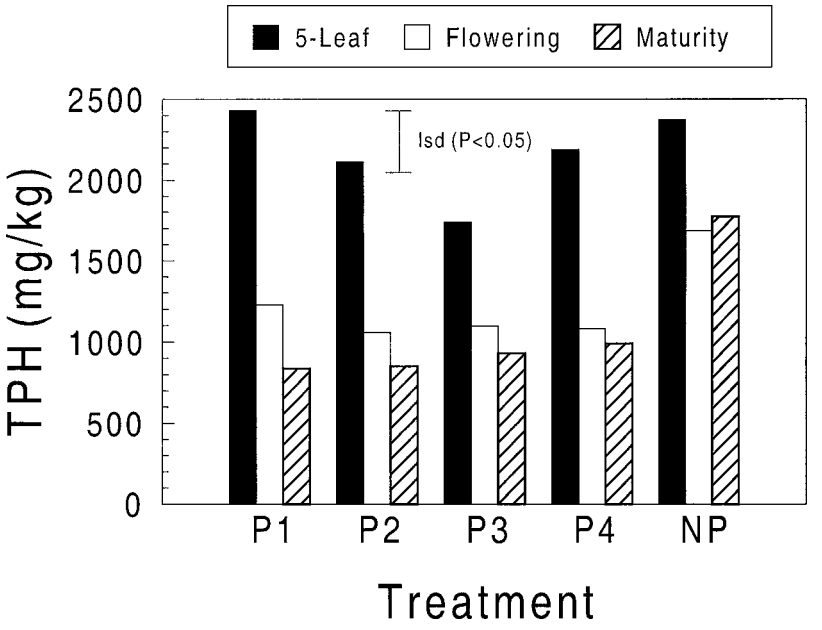

FIGURE 1. Mean concentrations of TPH in soil as affected by growth stage and sorghum cultivar. P1—nitrogen use efficient; P2—nitrogen use inefficient; P3—high root exudates; P4—low root exudates.

At the flowering stage, among the planted soils, the TPH concentration for P1 was the highest and P2 the lowest. However, these differences were not significant. The TPH concentration for the unvegetated control was 1690 mg/kg.

At maturity, the vegetated soils had equivalent concentrations of TPH and the unplanted soil had the highest average TPH concentration (1770 mg/kg). The differences between the unvegetated control and all planted soils were significant.

The magnitude of degradation observed in this experiment was similar to previous dissipation rates during phytoremediation of petroleum-contaminated soil (Pichtel and Liskanen, 2001; Nedunuri *et al.*, 2000; Hutchinson *et al.*, 2001). Published changes in concentration ranged from approximately 35% to 80%, depending on the conditions of the experiment. In this study, the TPH in the unplanted soils decreased 35%, whereas an average decrease of 69% was observed in the four sorghum species.

As was anticipated prior to the initiation of the experiment, the rate of petroleum degradation paralleled the growth of plant roots. The greatest decrease in TPH concentrations occurred between the five-leaf and flowering stages, the period with the greatest root growth. Concentrations continued to decline in the planted soils between the flowering and maturity stages, but at a slower rate. During that same time period, a decline was observed in the rate of root biomass accumulation. In the unplanted soils, the TPH concentrations remained unchanged between the flowering and maturity stages.

Differences in phytoremediation efficiency for petroleum-contaminated soils were speculated to exist among the four varieties of sorghum because of differences in root exudates, structure of the roots, and nitrogen-use efficiency. Our data did not support this hypothesis. Significant differences did not exist among these four varieties in terms of phytoremediation performance at the flowering and maturity stages.

At the maturity stage, the pots with plants were fully root-bound. Under these circumstances, the physiology of roots can change radically and, consequently, the role of plant root exudates is unclear.

IV. CONCLUSIONS

The presence of sorghum significantly enhanced bioremediation of TPH in the crude oil contaminated soil. This is likely because the stimulation of microorganisms in the rhizosphere increased microbial populations and activity. This conclusion is supported by the observation that the presence of vegetation was associated with higher TPH degradation rates as compared to unvegetated controls.

The response of the sorghum varieties to contaminants in soil differed with plant-growth stage. From germination until the five-leaf stage, there were no consistent differences in TPH degradation for plants grown in contaminated and uncontaminated soils. However, after this initial growth stage, differences among treatments developed. This probably is due to the fact that the root systems were more fully developed at later growth stages.

At the five-leaf stage, no differences were observed for root weight. However, at the flowering and maturity stages, root weight differences existed among the varieties. Phytoremediation efficiency seemed to be more strongly correlated to root weight than to shoot biomass. Plant height and shoot biomass are good indicators

of plant health; however, greater shoot biomass measurements are not necessarily indicative of enhanced bioremediation efficiency. Greater root biomass is likely to be associated with more extensive root exploration of the soil and, subsequently, higher microbiological numbers.

The establishment of vegetation may be a feasible remediation approach for surface soils contaminated with petroleum hydrocarbons. The use of vegetation is attractive because it is inexpensive and requires minimum maintenance and little management. With few inputs, a successful vegetation-remediation system could be superior to many alternative clean-up technologies.

REFERENCES

Adam, G., and Duncan, H. J. 1999. Effect of diesel fuel on growth of selected plant species. *Environ. Geochem. Health* **21,** 353–357.

Anderson, T., and Coats, J. 1994. Screening of rhizosphere soil samples for the ability to mineralize elevated concentration of atrazine and metolachlor. *J. Environ. Sci. Health. B* **30,** 473–484.

Anhalt, J. C., Arthur, E. L., Anderson, T. A., and Coats, J. R. 2000. Degradation of atrazine, metolachlor, and pendimethalin in pesticide-contaminated soils: effects of aged residues on soil respiration and plant survival. *J. Environ. Sci. Health Pt. B. Pest. Food Contam. Agric. Wastes* **35,** 417–438.

Aprill, W., and Sims, R. C. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbons treatments in soil. *Chemosphere* **20,** 253–265.

Banks, M. K., Lee, E., and Schwab, A. P. 1999. Evaluation of dissipation mechanisms for benzo[a]pyrene in the rhizosphere of tall fescue. *J. Environ. Qual*. **28,** 294–298.

Coleman, J. O. D., Frova, C., Schroder, P., and Tissut, M. 2002. Exploiting plant metabolism for the phytoremediation of persistent herbicides. *Environ. Sci. Poll. Res*. **9,** 18–28.

Cunningham, S. D., Anderson, T. A., Schwab, A. P., and Hsu, F. C. 1996. Phytoremediation of soils contaminated with organic pollutants. *Advances Agron*. **56,** 55–114.

Gerhardson, B., and Clarhom, S. 1986. Root and plant reactions to inoculation of roots with fungi and bacteria. *Phytopathol*. **114,** 108–117.

Heinonsalo, J., Jorgensen, K. S., Haahtela, K., and Sen, R. 2000. Effects of *Pinus sylvestris* root growth and mycorrhizosphere development on bacterial carbon source utilization and hydrocarbon oxidation in forest and petroleum-contaminated soils. *Can. J. Microbiol*. **46,** 451–464.

Hess, D. E., Ejeta, G., and Butler, G. 1992. Selecting sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to striga. *Biochem.* **31,** 493–497.

Hou, F. S. L., Milke, M. W., Leung, D. W. M., and MacPherson, D. J. 2001. Variations in phytoremediation performance with diesel-contaminated soil. *Environ. Technol*. **22,** 215–222.

Hutchinson, S. L., Banks, M. K., and Schwab, A. P. 2001. Phytoremediation of aged petroleum sludge: effect of inorganic fertilizer. *J. Environ. Qual*. **30,** 395–403.

Nedunuri, K. V., Govindaraju, R. S., Banks, M. K., Schwab, A. P., and Chen, Z. 2000. Evaluation of phytoremediation for field-scale degradation of total petroleum hydrocarbons. *J. Environ. Eng.—ASCE*. **126,** 483–490.

North Central Regional Research Publication No. 221. 1998. *Recommended Chemical Soil Test Procedures for the North Central Region*. Missouri Ag. Expt. Sta. SB 1001, Columbia, MO.

Olson, P. E., Flechter, J. S., and Philp, P. R. 2001. Natural attenuation/phytoremediation in the vadose zone of a former industrial sludge basin. *Environ. Sci. Poll. Res*. **8,** 243–249.

Pichtel, J., and Liskanen, P. 2001. Degradation of diesel fuel in rhizosphere soil. *Environ. Eng. Sci*. **18,** 145–157.

Sarand, I., Timonen, S., Nurmiaho-Lassila, E. L., Koivula, T., Haahtela, K., Romantschuk, M., and Sen, R. 1998. Microbial biofilms and catabolic plasmid harbouring degradative fluorescent pseudomonads in Scots pine mycorrhizospheres developed on petroleum contaminated soil. *Fems. Microbiol. Ecol*. **27,** 115–126.

Schwab, A. P., Su, J., Wetzel, S., Pekarek, S., and Banks, M. K. 1999. Extraction of petroleum hydrocarbons from soil by mechanical shaking. *Environ. Sci. Technol*. **33,** 1940–1945.

Schwab, A. P., and Banks, M. K. 1994. Biologically mediated dissipation of polycyclic hydrocarbons in the root zone. In: *Bioremediation through Rhizosphere Technology*, pp. 70–81 (Anderson, T. A. and Coats, J., Eds.) ACS Symposium Series 563, York, PA, American Chemical Society, Maple Press.

Shann, J. R., and Boyle, J. J. 1994. Influence of plant species on in-situ rhizosphere degradation. In: *Bioremediation through Rhizosphere Technology*, pp. 70–81 (Anderson, T. A. and Coats, J., Eds.) ACS Symposium Series 563, York, PA, American Chemical Society, Maple Press.

Siame, B. A., Weerasuriya, Y., Wood, K., Ejeta, G., and Butler, L. G. 1993. Isolation of strigol, a germination stimulant for striga asiatica, from host plants. *J. Agric. Food Chem*. **41,** 1486–1491.

U.S. Environmental Protection Agency. 1983. Method 418.1, *Methods for Chemical Analysis of Water and Wastes*, EPA Publication EPA-600/4-79-020. NTIS # PB84-128677, Washington, D.C.

U.S. Environmental Protection Agency. 2000. SW-846. *Test Methods for Evaluating Solid Waste*. Office of Solid Waste and Emergency Response, Washington, D.C.

Walton, B. A., and Anderson, T. 1990. Microbial Degradation of trichloroethylene in the rhizosphere: potential application to biological remediation of waste sites. *Appl. Environ. Microl*. **56,** 1012–1016.

Weerasuriya, Y., Siame, B. A., and Hess, D. 1993. Influence of conditions and genotype on the amount of striga germination stimulants exuded by roots of several host crops. *J. Agric. Food Chem*. **41,** 1492–1496.

Whipps, J. M., and Lynch, J. M. 1990. Substrate flow in the rhizosphere. *Plant Soil* **129,** 1–10.