Phytoremediation of Organic and Nutrient Contaminants

Pilot and full-scale studies are demonstrating the promise and limitations of using vegetation for remediating hazardous wastes in soils and sediments.

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Phytoremediation, the use of vegetation for the in situ treatment of contaminated soils and sediments, is an emerging technology that promises effective and inexpensive cleanup of certain hazardous waste sites. The technology has already been shown to be effective in a number of full-scale and pilot studies. Phytoremediation is most suited for sites with shallow contamination (< 5 m depth); moderately hydrophobic pollutants such as BTEX compounds (benzene, toluene, ethylbenzene, and xylenes), chlorinated solvents, or nitrotoluene ammunition wastes; or excess nutrients (nitrate, ammonium, and phosphate).

Because phytoremediation is still in development, the techology is not yet widely accepted by regulatory agencies and therefore not commonly used. In addition, phytoremediation may take longer than traditional approaches to reach cleanup goals or may be limited by soil toxicity. However, as a rule, plants will survive higher concentrations of hazardous wastes than will most microorganisms used for bioremediation.

A potential application of phytoremediation would be bioremediation of petrochemical spills and contaminated storage areas, ammunition wastes, fuel spills, chlorinated solvents, landfill leachates, and agricultural nonpoint source runoff (i.e., pesticides and fertilizers). Generally, phytoremediation is used in conjunction with other cleanup approaches.

Plants remediate organic pollutants via three mechanisms: direct uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites into plant tissue; release of exudates and enzymes that stimulate microbial activity and biochemical transformations; and enhancement of mineralization in the rhizosphere (the root–soil interface), which is attributable to mycorrhizal fungi and the microbial consortia. It is also possible to concentrate metals in higher plants, and phytoremediation includes the use of plants to remediate sites contaminated by metals. However, in this article we focus on organic and nutrient pollutants.

Vegetation offers other benefits at contaminated sites; phytoremediation increases the amount of organic carbon in the soil which, in turn, stimulates microbial activity. In addition, the establishment of deep-rooted vegetation helps to stabilize soil. When windblown dust is controlled, it reduces an important pathway for human exposure via inhalation of soil and ingestion of contaminated food. Plants also transpire considerable amounts of water. This loss of water can reverse the downward migration of chemicals by percolation and can lead to absorption of surface leachate.

Figure 1 shows a schematic of mass flow through a woody, flood-tolerant tree species. (Oxygen, water, and carbon transport mechanisms vary among plant species.) Plants supply oxygen to the soil rhizosphere; for example, seedlings in the laboratory can transport considerable quantities of oxygen to roots in the rhizosphere (0.5 mol O_2 per m^2 of soil surface per day) (*1*). However, roots also demand oxygen for respiration and, therefore, the total effect of dense root systems needs to be considered in the engineering design. The figure also demonstrates how plants are able to take up contaminants directly from the soil water and release exudates that help degrade organic pollutants via co-metabolism.

Direct uptake of organic pollutants

Direct uptake of organics by plants is a surprisingly efficient removal mechanism for moderately hydrophobic organic chemicals (octanol–water partition

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Researcher measures one season of growth of hybrid poplar tree being used as a riparian zone buffer at Amana, IA.

coefficients, log K_{ow} = 0.5–3) in shallow contaminated sites. These include most BTEX chemicals, chlorinated solvents, and short-chain aliphatic chemicals. Hydrophobic chemicals (log K_{ow} > 3.0) are bound so strongly to the surface of roots that they cannot easily be translocated within the plant, and chemicals that are quite water soluble (log K_{ow} < 0.5) are not sufficiently sorbed to roots or actively transported through plant membranes (*2*).

Once an organic chemical is taken up, a plant can store the chemical and its fragments in new plant structures via lignification; or it can volatilize, metabolize, or mineralize the chemical all the way to carbon dioxide and water. Detoxification mechanisms may transform the parent chemical to nonphytotoxic metabolites, including lignin, that are stored in various places in plant cells.

The direct uptake of a chemical through the roots depends on the plant's uptake efficiency and transpiration rate as well as the concentration of the chemical in soil water. Uptake efficiency, in turn, depends on physical–chemical properties of the contaminant, chemical speciation, and the plant itself (plants vary in the transporting agents they use to take up organic contaminants). Transpiration is a key variable that determines the rate of chemical uptake for a given phytoremediation scheme—it depends on the plant type, leaf area, nutrients, soil moisture, wind conditions, and relative humidity.

Enzymes and exudates

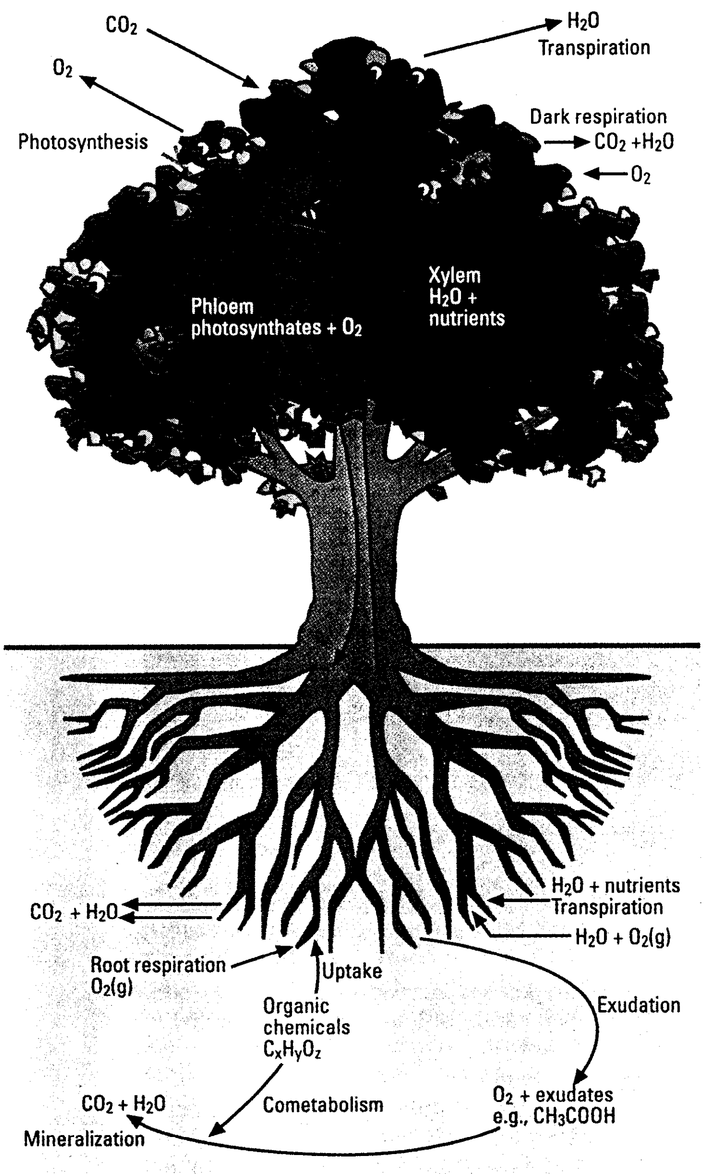
Plants may release to the soil environment exudates that help degrade toxic organic chemicals. Leakage of exudates (sugars, alcohols, and acids) from the plant can amount to 10–20% of plant photosynthesis on an annual basis (*3*).

For example, in work at the University of Iowa we characterized the molecular weight distribution of organic exudates from the root systems of hybrid poplar trees. Dissolved organic carbon concentrations were substantial: 10–120 mg L^{-1}, with a median molecular weight of 1100 daltons and 1–10 mg L^{-1} of acetic acid (acetic acid is a good substrate for soil microorganisms).

We also examined enzyme reactions in plant sediment, plant soil, and exudate systems. Wherever we have found significant natural activity in the transformation of contaminants mixed with sediment and soil, we have isolated plant enzymes as the causative agent. In studies at EPA's laboratory in Athens, GA, five enzyme systems—dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase—have been identified. Tracing natural indigenous processes exclusively to plants provides strong evidence of the po-

FIGURE 1

Oxygen, water, and chemical cycling through a tree



tential for phytoremediation and indicates that the future development of innovative phytoremediation must revolve around discovering which enzyme systems will degrade chemicals of concern. Table 1 specifies some plants and associated enzymes that degrade organic chemicals.

Through the use of rigorous mass balances and pathway analyses we have shown that nitroreductase and laccase enzymes break down ammunition wastes (2,4,6-trinitrotoluene or TNT) and incorporate the broken ring structures into new plant material or organic detritus that becomes a part of sediment organic matter. Another plant-derived enzyme, dehalogenase, helps reduce chlorinated solvents such as trichloroethylene (TCE) to chloride ion, carbon dioxide, and water. Determination of each of the metabolites, pathways, and reaction kinetics through dynamic mass balances and radiolabeled studies provides vital information for ecological engineering that we hope will replace a trial-and-error selection of plants. A thorough understanding of pathways and end products of enzymatic processes also simplifies toxicity investigations of in situ phytoremediation.

Although isolated enzymes such as nitroreductase rapidly transform substrates such as TNT, our experience indicates that remediation should involve whole plants. Isolated enzymes are destroyed and inactivated by low pH, high concentrations of metals, and bacterial toxins. When plants are grown in soil or sediment slurries, pH is neutralized, metals are biosorbed or chelated, and enzymes remain protected inside the plant or sorbed to plant surfaces.

In our studies of TNT breakdown, plants such as hornwort increased pH from 3 to 7, sorbed high concentrations of metals that would usually inhibit bacteria, and remained healthy and viable. Overall, plants can accommodate mixed wastes (organic and metals) and other harsh conditions.

Rhizosphere biodegradation

Anderson et al. (*4*) have demonstrated the importance of biodegradation in the rhizosphere. Plants help with microbial transformations in the rhizosphere in many ways.

Roots harbor mycorrhizae fungi, which metabolize organic pollutants. These fungi, growing in symbiotic association with the plant, have unique enzymatic pathways that help to degrade organics that could not be transformed solely by bacteria.

Plants supply exudates, which stimulate bacterial transformations and build up the organic carbon in the rhizosphere. In addition, the rapid decay of fine-root biomass can become an important addition of organic carbon to soils. The additional organic carbon, in turn, increases microbial mineralization rates. The increase in carbon also serves to retard organic chemical transport into groundwater. Moreover, we have found that microbial mineralization of atrazine is directly related to the fraction of organic carbon in the soil (*5*).

Finally, plants provide habitat for increased microbial populations and pump oxygen to the roots, a process that ensures aerobic transformations near the root that otherwise may not occur in the bulk soil. Microbial assemblages are abundant in the rhizosphere. Typical communities comprise 5×10^6 bacteria, 9×10^5 actinomycetes, and 2×10^3 fungi per gram of air-dried soil; bacteria live in colonies that cover as much as 4–10% of the plant root surface area (*1, 6*).

Applications of phytoremediation

Each cleanup situation requires a different plant or a number of plants in tandem. Alfalfa has been used for its nitrogen-fixing ability and deep rooting. Rye grass and fescue offer dense cover crops, often below a woody species. Trees of the *Salicaceae* family (willow and poplar) have been planted at several locations because of their flood tolerance and fast growth. Parrot feather and Eurasian water milfoil have been applied in aquatic mesocosms to break down ammunition wastes.

For example, in a cooperative pilot test with Au-

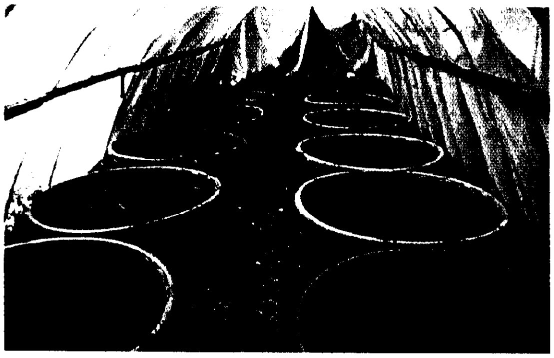


Photo 1. Mesocosm studies of TNT-contaminated soil: Studies conducted at the Army Ammunition Plant (Childersburg, AL) with 1 in. of TNT-contaminated soil (5000 ppm). Red color in the left front container (control) was the result of photolysis of TNT; most TNT remained in the control, which was still toxic to snails and tadpoles. The right front container was treated with parrot feather aquatic plants. The dark brown indicates degradation of the TNT; more than 90% removal occurred after 7 days, and toxicity was lowered.

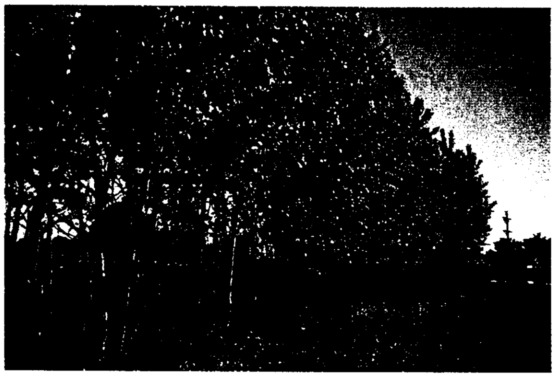


Photo 2. Four years of growth in a riparian zone buffer strip: Four rows of hybrid poplar trees make a 25-ft buffer along a stream in Amana, IA, that decreases nutrients, sediment, and pesticides.

TABLE 1

Plant-derived enzyme systems

Systems have been shown to remediate nitroaromatic compounds (e.g., TNT), halogenated hydrocarbons (e.g., chlorinated solvents and pesticides), and anilines.

	Half-life, h		
Plant	Nitro-reductase w/2,4,6,-TNT	Dehalo-genase w/ hexachloro-ethane	Laccase w/ 2,4,6,-triamino-toluene
Algae *Nitella* (stonewort)	10–50	90	70
Eleocharis sp.	20–110		
Anthrocerotae sp.	10–67	120	
Algae *Spirogyra*	4–100	95	
Potamogeton pusillus	8–57		
Myriophyllium spicatum (parrot feather)	20–240	120	70
Lemna minor (duckweed)	20		
Hydrilla verticillata	12		
Sagittaria sp. (arrowroot)	35		
Nostoc sp. (blue-green algae)	60		
Chara sp.	75		
Populus sp. (Hybrid poplars)	<10	50	

Half-lives are dependent on the initial concentration of contaminant and the plant:water ratios.

burn University, parrot feather was introduced into flooded mesocosms of TNT-contaminated soil. Rather than selecting plants by trial and error, we tested parrot feather from the site and detected the enzyme nitroreductase. At 5000 ppm of TNT, the contaminated soil was essentially sterile. In the initial sampling after one week, dissolved TNT concentrations decreased from 128 ppm (saturation) to 10 ppm. The disappearance of TNT attributable to parrot feather was rapid enough to support snails and tadpoles (Photo 1). However, new roots grew only along the edge of the contaminated soil, avoiding hot spots while breaking down the dissolved TNT in the water column.

Another plant system, hybrid poplar trees, offers some distinct advantages for treatment of contaminated soils with organic chemicals. These hybrid varieties are perennial, long-lived (25–50 years), fast growing, hardy, and tolerant of organics. Hybrid poplars grow easily from long cuttings planted deeply and can be harvested and regrown from the cut stump.

We have planted Imperial Carolina hybrid poplars (*Populus deltoides nigra*, DN34) from 2-m cuttings that have preformed root initials for rooting all along the buried depth (1.7 m). In dry years, roots will reach down toward the water table, establishing a dense root mass that will take up large quantities of water. This process increases soil suction and decreases downward migration of pollutants. In the dormant season, there may be some leakage of water through the system, but normally precipitation is not great during this period.

In good soils and temperate conditions, the trees can grow 2 m in the first growing season and reach a height of 5–8 m after three years. We plant at a density of 10,000 trees per hectare, but the trees naturally thin themselves to about 2000 trees per hectare after several years. Average carbon fixation in the early years is 2.5 kg m^{-2} yr^{-1}. In Amana, IA, hybrid poplar trees planted along a riparian zone for six seasons have produced an average of 12 tons of dry matter per acre per year.

To control agricultural runoff along a small creek in prime Iowa agricultural land (Photo 2), hybrid poplars were planted in four rows as a riparian zone buffer strip (8 m wide, 10,000 trees per hectare). The goal was to intercept and remove atrazine and nitrate pollutants before they were delivered to the creek and surficial groundwater. Nitrate in surficial groundwater dropped from 50–100 mg L^{-1} to < 5 mg L^{-1} as nitrate. Also, in a related small pilot study, we found that 10–20% of the applied atrazine was taken up by the trees (*7, 8*).

Poplar trees make an excellent cap and closure at municipal landfills. In collaboration with an engineering consulting firm, 10,000 trees per hectare were planted as the final cap on a landfill at Beaverton, OR. Photo 3 shows the side slope of the landfill before planting and after one year of growth. Treatment of organic wastes is not the main goal at this site; rather, it

TABLE 2

Applications of phytoremediation at contaminated sites

Location	Application	Contaminants	Site results	Reference
Amana, IA	Nonpoint source control, 1-mi stream with poplars	NO_3^-, atrazine, alachlor, soil erosion	NO_3^- and 0.10–20% atrazine were removed	1, 4, 7, 9, 10
Amana, IA	Municipal solid waste compost land application on poplars, corn, fescue	BEHP, B(a)P, PCB, chlordane	Small plot study, organics were immobilized	11
Beaverton, OR	Municipal landfill cap with hybrid poplars	Organics, metals, BOD	Landfill cap successful, full scale	12
Slovenia	Landfill cap, closure with hybrid poplars	Organics, metals, BOD	Two years of growth	13
Iowa City, IA	Landfill leachate abatement with poplars	Chlorinated solvents, metals, BOD, NH_3	Poplars survived in lab 1200 mg/L	14
Prince George's County, MD	Sewage sludge in trenches, poplars on degraded lands	Nitrogen in sludge	170 tons/acre of sludge treated full scale, 6-year plantation	15
Corvallis, OR	Organics in hydroponic system with poplars, Russian olive, soybean, green ash	Nitrobenzene and others	Essentially complete uptake in the lab	16
New Mexico	Contaminated soil with *Datura* sp. and *Lycopersicon* sp.	Trinitrotoluene (TNT)	Essentially complete treatment	17
Oak Ridge, TN	Organics-contaminated soils with pine, goldenrod, Bahia grass	Trichloroethylene and others	Enhanced biomineralization	6
Salt Lake City, UT	Contaminated soil by crested wheatgrass	Pentachlorophenol and phenanthrene	Enhanced lab mineralization	18
New Jersey, Illinois	Shallow groundwater and poplars	NO_3^-, NH_4^+	Decreased size of plume	19
McMinnville, OR	Landfill leachate irrigation on 14 acres of poplars	NH_3, salts	Zero discharge alternative to pumping to wastewater treatment plant	20
Childersburg, AL	Soil with parrot feather	TNT	Enhanced degradation, pilot scale	

is keeping the site natural and free from infiltration. Now in its third year, the project of evapotranspiration by the trees has kept the landfill free from leachate problems. Nearby residents accept the innovative solution, preferring the forest to a barren plastic or clay cap. A full-scale application (14 acres, 40,000 trees) using drip irrigation of landfill leachate on poplar trees has also proven effective at a McMinnville, OR, site. Although hybrid poplars seem to tolerate organic chemicals quite well, high concentrations of metals, salts, and ammonia are toxic.

Table 2 includes some recent applications of phytoremediation. Some are pilot or greenhouse studies, but most are full-scale operations. They span a range of pollutants from atrazine to TNT and several different plant species.

Limitations of phytoremediation

Researchers studying phytoremediation face some potential limitations. They still need to establish whether contaminants can collect in leaves and be released during litter fall or accumulate in fuelwood or mulch. It may be difficult to establish the vegetation because of soil toxicity or possible migration of contaminants off site by binding with soluble plant exudates. Possible migration of contaminants off site by binding with soluble plant exudates is a concern, but to date none of these problems has been observed. In some situations, regulatory restrictions will not allow contaminants to be left in place, even when a vegetative cover prevents erosional pathways of exposure.

Phytoremediation is most effective at sites with shallow contaminated soils, where nutrient and organic contaminants can be treated in the rhizosphere and by root uptake. Although deep-contaminated sites and those with deep pools of nonaqueous-phase liquids are not good applications, deep groundwater contaminants or leachate pond effluent may be treated by pumping and drip irrigation on plantations of trees.

Degradation of organics in conjunction with plant enzymes is so fast that desorption and mass transport of chemicals from the soil may become the rate-determining step. Therefore, phytoremediation may require more time to achieve cleanup standards than alternatives such as excavation or ex situ treatment, especially for hydrophobic pollutants that are tightly bound to soil particles.

EPA has not adopted phytoremediation as an approved technology, although we have been given special permission by the states to use hybrid poplar

Photo 3. Phytoremediation at a landfill: (above) Landfill slope in Beaverton, OR, before trees were planted; (below) Same site only one year after hybrid poplar trees were planted in 4 ft of soil as a cap and closure at the landfill. Trees keep the landfill dry by evapotranspiration. A dense, deep root system is shown by excavation of the roots to 6 ft.

trees as caps at several landfills as an alternative to a Subtitle D cap under the Resource, Conservation, and Recovery Act. However, the technology is still not widely used.

A comparison of costs with those for the standard practices of soil venting, soil washing, excavation, or bioremediation is not possible because phytoremediation is too new. Our experience indicates that it should be very competitive with other technologies. Planting costs are ~$10,000 per acre, and monitoring costs would be similar to those for other alternatives. In many cases, we view phytoremediation as a final "polishing step" to close sites after other cleanup technologies have been used to treat the hot spots.

Although phytoremediation is not a panacea for hazardous waste problems, it has proven effective in several applications for treatment of shallow contaminated sites. Before the technology can mature, we need a better understanding of the role of metabolites, enzymes, and the selection of plant systems for various wastes. Nevertheless, the technology holds great promise. In general, plants can withstand greater concentrations of organic pollutants than most microorganisms; they can take up the chemicals quickly and convert them to less toxic metabolites, and they are known to stimulate degradation of organics in the rhizosphere.

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References

(1) Shimp, J. F. et al. *Crit. Rev. Environ. Sci. Technol.* **1993**, *23*(1), 41–77.
(2) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. *Pestic. Sci.* **1982**, *13*, 495–503.
(3) Moser, M.; Haselwandter, K. In *Physiology Plant Ecology III—Responses to the Chemical and Plant Environment*; Lange, O. L. et al., Eds.; Springer-Verlag: Berlin, 1983; pp. 391–411.
(4) Anderson, T. A.; Guthrie, E. A.; Walton, B. T. *Environ. Sci. Technol.* **1993**, *27*(13), 2630–36.
(5) Nair, D. R.; Schnoor, J. L. *Water Res.* **1994**, *28*(5), 1199–1205.
(6) Foth, H. D. *Fundamentals of Soil Science*, 8th ed.; Wiley: New York, 1990.
(7) Paterson, K. G.; Schnoor, J. L. *Water Environ. Res.* **1992**, *64*(3), 274–83.
(8) Paterson, K. G.; Schnoor, J. L. *J. Environ. Eng.* **1993**, *119*(5), 986–93.
(9) Licht, L. A. Ph.D. Dissertation, University of Iowa, 1990.
(10) Nair, D. R. et al. *J. Environ. Eng.* **1993**, *119*(5), 842–54.
(11) Hsu, M. S. et al. *Compost Science & Utilization* **1993**, *1*(4), 36–48.
(12) Madison, M. F.; Licht, L. A. *Proceedings of the American Society of Agricultural Engineers*; ASAE: Albuquerque, NM, December 1991.
(13) Licht, L. A.; Marinko, A.; Macarol, B. *Proceedings of the Second International Conference on Energy and Environment: Transitions in East Central Europe, Prague, Czech Republic*; Energy & Environmental Research Center, University of North Dakota, Grand Forks, ND, November 1994.
(14) Kull, D. M.S. Thesis, University of Iowa, 1995.
(15) Gouin, F. R.; Flamino, P. "Sludge Tree Farm," University of Maryland, College of Agriculture, College Park, Maryland, 1988.
(16) McFarlane, C.; Pfleeger, T.; Fletcher, J. *Environ. Toxicol. Chem.* **1990**, *9*, 513–21.
(17) Bedell, G. W. *The World & I* **1992**, *12*, 260–63.
(18) Ferro, A. M.; Sims, R. C.; Bugbee, B. *J. Environ. Qual.* **1994**, *23*(2), 272–78.
(19) Gatliff, E. G. *Remediation* Summer 1994, 343–52.
(20) Madison, M.; Licht, L. A.; Wilson, D. *Proceedings of the 87th Meeting of the Air & Waste Management Association*; AWMA: Cincinnati, OH, June 1994.

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