HAZARDOUS SUBSTANCE RESEARCH CENTER • SOUTH & SOUTHWEST •

Research Brief #11

1996

Biochemical Transformations and Pathways From Phytoremediation of TNT-Contaminated Soils

Summary of the Problem

Researchers at Georgia Institute of Technology, Louisiana State University, and Rice University are investigating the effectiveness of aquatic plants as a means of cleaning soils contaminated with trinitrotoluene (TNT). An explosive compound used in munitions, TNT is found in soil at many ammunition plants and military bases in the United States. The ability of aquatic-plant enzymes to reduce and assimilate TNT and convert it into nonhazardous plant mass has been investigated in bench-scale and pilot-scale studies with aquatic plants and natural organic matter in soils and sediments. Researchers have developed and investigated a working hypothesis in which TNT is initially transformed to aminated intermediates which are then conjugated and assimilated into plant mass.

Rice University is conducting fundamental studies of plant transformations and pathways. Georgia Tech is studying whole-plant biochemical processes, as well as the physicochemical processes that affect the fate of biochemical intermediates produced by the remediative plant/soil system. Georgia Tech researchers also conducted pilot-scale studies aimed at placing (and testing) the plant remediation system in the field. Louisiana State University characterized the transport of TNT in contaminated soils from soil to water, and are developing models of TNT transport for use in investigating an enhanced-flow TNT-treatment system.

The three-university project on phytoremediation of TNT was managed by HSRC/S&SW Co-Director F.M. Saunders at Georgia Tech. This fact sheet focuses on Rice University's phase of the project.

Introduction

Military bases and ammunition manufacturing plants have site-contamination problems that can benefit from innovative remediation technology. Soils and groundwaters at many of these military installations have become contaminated over a period of decades with trinitrotoluene (TNT) and other explosives used in munitions manufacturing. TNT not only may be toxic to animals, plants, and humans, but it also remains potentially explosive for years after it is produced.

A critical concern at TNT-contaminated sites is determining what happens to TNT during and after remediation. In fact, the use of bioremediation processes at TNT-contaminated sites has been deterred because of the formation of recalcitrant or unknown end products. One promising cleanup method under consideration by the HSRC/South & Southwest, the Environmental Protection Agency, and the Department of Defense is phytoremediation, using vegetation to assimilate and detoxify hazardous substances. While the ability of plants to take up TNT has been established, still unknown is what happens to the TNT after the plant has assimilated it. Rice University researchers are studying the pathways and end products that occur when aquatic plants transform TNT.

Studies

The ability of plants to assimilate and transform TNT into less hazardous chemical substances has been established in bench-scale studies. In past studies, mass balances were not performed; therefore, it was impossible to evaluate the final distribution of TNT and transformation products.

The HSRC/S&SW research team is using aquatic plants from local ponds, greenhouses, and the Alabama Army Ammunition Plant site, and plant tissue cultures from Rice laboratories

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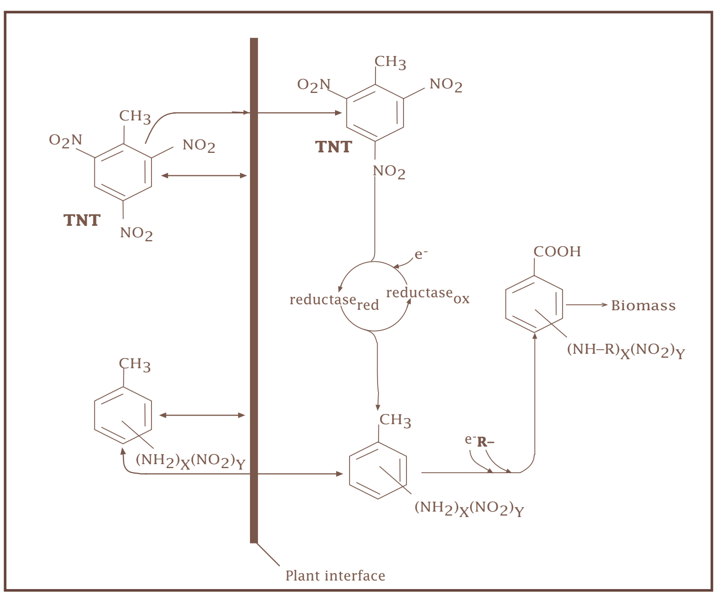

Working hypothesis for biochemical pathways in plant systems to assess the ability of plants to transform TNT. Hairy-root cultures serve as a microbe-free surrogate for plant-root activity, allowing researchers to determine whether microbes in or surrounding the plant play a role in absorbing the TNT.

Plants and tissue cultures suspended in a medium containing dissolved TNT, tagged with carbon 14, were used to help the researchers follow the progress of TNT. These studies have shown that plants can transform TNT rapidly, without accumulating aminonitrotoluenes. Researchers evaluating a variety of aquatic plants have determined the rate of TNT disappearance, the extent of TNT mineralization, and the production of recalcitrant end products.

Transformation Studies

In the experimental setup, media are equilibrated with TNT and added to flasks containing a known amount of plant material, and then are maintained in the dark. Aqueous samples are taken directly from the flasks and analyzed for TNT and metabolites, products that occur as a result of transformation. Researchers have seen plants and hairy-root cultures rapidly transform TNT; however, they have not observed mineralization of TNT. The studies of plant tissue culture, hairy-root culture, and aquatic plant systems have revealed that a significant percent of the C-14 is retained in the plant cell and is not TNT or its identifiable byproducts. Researchers are using solvent extraction and combustion techniques to quantify and characterize this fraction of C-14.

The disappearance of TNT alone is not sufficient for remediation. The resulting production of mono- and diamino compounds or unidentified products may create a bigger problem than TNT itself. A catalyst that can mineralize, or completely degrade, TNT would be most desirable. Understanding pathways and how physical conditions may influence pathways is critical for success at the field scale.

Rate Studies

Experiments have been carried out in batch studies for all plants at temperatures ranging from 20˚C to 25˚C with no sunlight, and hairy-root cultures studies were conducted in the dark at 25˚C. Known masses of plants or tissue cultures were added to flasks containing TNT media. The researchers determined the concentrations of TNT and aminated nitrotoluene concentrations by sampling and analyzing the media over time.

They then evaluated the rates of TNT transformation by comparing the apparent rate of TNT disappearance in experiments with different plants at varying plant density-to-TNT ratios.

Results and Discussion

These results indicate that TNT does not simply bioaccumulate in plants. Instead transformation processes occur, and the main products of transformation are different from those in microbial processes. TNT enters the plant, is transformed, and “disappears” into plant tissue as new growth. TNT transformation occurred in all systems, and traces of TNT reduction products were identified. In these studies, mineralization did not occur and mass balances accounted for all TNT labeled.

Field Tests

Based on laboratory studies, the research team built an experimental aquatic plant lagoon to look at these processes in real-world settings. More detailed information on the field studies are included in Research Brief #14.

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