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### Plant-Soil-Contaminant Specificity Affects Phytoremediation of Organic Contaminants

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## Plant-Soil-Contaminant Specificity Affects Phytoremediation of Organic Contaminants

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### ABSTRACT

The objective of this study was the evaluation of seven forage and conservation crop species for phytoremediation of trinitrotoluene (TNT) and pyrene-contaminated soils. TNT and pyrene were added to soil at 100 mg kg<sup>-1</sup>. Crop species screening studies were conducted in a greenhouse and growth chambers on two soil types with different organic matter contents. Under high soil organic matter conditions, adsorption or covalent binding to the soil organic matter appeared to be a dominant force of removal limiting TNT and pyrene availability. In both soil types, pyrene dissipation could not be attributed to the presence of plants. However, in soils with lower organic matter content, all of the plant species treatments showed a significantly higher degree of TNT transformation compared with the unplanted control. Statistically significant differences in TNT transformation were observed among crop species grown in the low OM soil. Reed canarygrass (*Phalaris arundinacea* L.) and switchgrass (*Panicum virgatum* L.) were the most effective species in enhancing TNT transformation. Our data indicated that use of plants was effective for phytoremediation of TNT-contaminated low OM content soils, but did not have any significant effect on pyrene dissipation. Based on these observations, it appears that plant-soil-contaminant interactions are very specific, and this specificity determines the effectiveness of phytoremediation schemes.

**KEY WORDS:** TNT, PAH, pyrene, soil contamination, forage crops, bioremediation.

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## INTRODUCTION

Trinitrotoluene (TNT,  $C_7H_5N_3O_6$ ) has been one of the most widely used explosives in the world. Some of the reported harmful health effects of TNT are anemia and abnormal liver function, cataract development, and skin irritation (Agency for Toxic Substances and Disease Registry (ATSDR), 1995 and 2000). Soils around military bases and explosives manufacturing facilities are often contaminated with munitions waste. TNT is the most prevalent contaminant present in these soils and remedial action has often been limited to incineration (Pennington, 1999). However, this technology is very expensive and usually involves excavation and the transportation of contaminated soils to a site where an incinerator is available. Transportation costs and liabilities associated with it require additional expenses and precautions. As a result, there is a growing interest in developing *in situ* remediation strategies that can reduce the above-mentioned risks and cost (Reiger and Knackmuss, 1995). One such strategy is phytoremediation.

Plant uptake and transformation of TNT and its metabolites from soil and water has been documented in numerous studies (Palazzo and Leggett, 1986; Schnoor *et al.*, 1995; Carreira and Wolfe, 1996; Peterson *et al.*, 1996; Medina *et al.*, 1997; Bhadra *et al.*, 1999; Gong *et al.*, 1999; Hughes *et al.*, 1999; Larson *et al.*, 1999). Most of the earlier work dealing with TNT-plant interactions was mainly concerned with its possible effect on human health via the food chain (Palazzo and Leggett, 1986). Limited research has been done to determine the phytotoxicity of TNT to plants (Peterson *et al.*, 1996; Gong *et al.*, 1999). Peterson *et al.* (1996) reported the possibility of using tall fescue in phytoremediation of soils that are marginally contaminated with TNT. According to Bhadra *et al.* (1999), plant-assisted transformation of TNT is usually accompanied by the appearance of monoamino derivatives such as 2-amino-4, 6-dinitrotoluene, and 4-amino-2, 6-dinitrotoluene. These researchers also reported that plants are able to sequester or integrate TNT metabolites into their biomass (conjugation of the amino products). Heckman (1999) reported that smooth bromegrass (*Bromus inermis* L.) grown in a sterilized environment was able to physically remove and/or break down TNT into less toxic byproducts. Thompson *et al.* (1998) reported that TNT was strongly bound to the roots of poplar trees (*Populus* spp.). According to this study, the bulk of the TNT was transformed within the root system, and only a small portion was translocated to above-ground parts.

Some investigators have been able to isolate the enzyme nitroreductase of plant origin from sediments (Carriera and Wolfe, 1996). As has been reported elsewhere (Fiorella and Spain, 1997; Jackson *et al.*, 1999), this enzyme is capable of sequentially reducing TNT to triaminotoluene (TAT). Moreover, many plant species are capable of producing laccase enzymes, some of which can catalyze TAT mineralization (Medina *et al.*, 1997). In addition, TAT is a very unstable compound that is prone to autooxidation under aerobic conditions.

The greatest deficiency of past TNT phytoremediation/phytotoxicity studies is that these experiments were not done in soil and/or the plants were not allowed to finish their full growth cycles. As a result, the effects of different plant rhizospheres on TNT transformation were not fully investigated.

## Plant-Soil-Contaminant Specificity Affects Phytoremediation

Polycyclic aromatic hydrocarbons (PAHs) are generated during combustion, burning, smoking, and cooking of such everyday organic materials as petroleum, coal, tobacco, and hamburger. Their production is also associated with incomplete combustion during coal gasification and petroleum refining (Sigman *et al.*, 1998). These compounds have been implicated as a source of carcinogenic risk (ATSDR, 2000).

PAHs have been the subject of a number of phytoremediation studies (April and Sims, 1990; Schwab and Banks, 1994; Banks *et al.*, 1997; Ferro *et al.*, 1997; Qiu *et al.*, 1997; Pradhan *et al.*, 1998). Most of the results from these studies indicated that use of plants-enhanced biodegradation of PAHs.

The results reported by Qiu *et al.* (1997) stand out as an exception to the widely accepted norm. Contrary to most of the above-cited studies, Qiu *et al.* reported that concentrations of PAHs were lower in the control unplanted plots compared with those in the vegetated plots. According to the authors, these differences could be attributable to sampling or analytical errors or could be due to differences in the complex physicochemical and biological process determining the fate of PAHs in the soil. Other researchers have also reported that bioremediation of PAHs is extremely difficult due to their resistance to biodegradation, low water solubility (e.g., pyrene = 0.135 mg/L) and high ability to sorb on to organic materials (Sigman *et al.*, 1998).

Understanding the biological, chemical, and physical processes that affect the fate of contaminants in soil is critical for selecting suitable remediation technology (Luthy *et al.*, 1997). Transport, bioavailability, and biodegradation of organic compounds in soil are highly dependent on the phenomenon known as sorption (Pignatello, 1989; Pignatello and Xing, 1996). The most widely accepted current view on adsorption of organic pollutants in the soil is that it occurs through physical and chemical binding on natural surfaces or by partitioning into the soil organic matter (Chiou, 1989; Pignatello and Xing, 1996).

This study was initiated to examine the phytoremediation potential of several forage and conservation crops grown on TNT and pyrene-contaminated soils with different organic matter content.

## MATERIALS AND METHODS

Three legume and four grass species were used in these experiments. Legumes included alfalfa (*Medicago sativa* L.), flatpea (Wagner pea) (*Lathyrus sylvestris* L.), and sericea lespedeza (*Lespedeza cuneata* Dum.-Cours.). The grass species were deertongue (*Panicum clandestinum* L.), reed canarygrass (*Phalaris arundinacea* L.), switchgrass (*Panicum virgatum* L.), and tall fescue (*Festuca arundinacea* Schreb.). TNT and pyrene were added to soil at 100 mg kg<sup>-1</sup>.

Plant species screening experiments on soil with high OM (6.3%) content were conducted in the greenhouse. Temperatures in the greenhouse were maintained at 29°C and 24°C day/night, respectively. Experiments with the low OM (2.6%) soil were conducted in four Model BDR8 (Conviron, Winnipeg, Canada) growth chambers. Growth chamber temperatures were maintained at 25/16°C, and the light regime was 16/8-h day/night cycle, with photosynthetic photon flux rate of 400 to 500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  from metal halide bulbs. The relative humidity in the chambers was set at 65  $\pm$  5%.

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The high organic matter content soil used for the greenhouse study was Adelphia silt loam (fine-loamy, glauconitic (mixed), nonacid, mesic, Aquic Hapludult), with pH (1:1 water) 5.1; organic matter (OM) 6.3%; cation exchange capacity (CEC) 8.82 cmol/kg, low phosphorus, and high potassium levels. The low organic matter soil used for the growth chamber experiments was Hatboro (coarse-loamy, mixed, nonacid, mesic, Typic Fluvaquent). Soil test results showed pH (1:1 water) 6.1; OM 2.6% and CEC 5.72 cmol/kg.

### **Experimental Design**

The experimental design was a split-plot in a randomized complete block. Chemical treatments were the main plots and the crop species were the subplot treatments. Data were analyzed using Proc MIXED and multiple mean comparisons were done with Tukey-Kramer test (SAS Systems Release 7, 1999 Cary, NC).

### **Soil Sampling, Extraction, and Analysis**

At the end of each experiment, soil samples were placed in Qorpak<sup>®</sup> glass containers and stored in a freezer (−50°C) until extraction. For soil TNT analysis, 10 g oven-dried equivalent (ODE) of soil samples in 40 ml of acetonitrile were shaken for 18 h at 150 rpm at room temperature. Samples were allowed to settle for 30 min, after which 5 ml of the extract was pipetted into a graduated cylinder and 5 ml of CaCl<sub>2</sub> was added. After 30 min, samples were filtered into amber vials thru a 0.2 μm filter. The first 2 to 3 ml of filtrate was discarded, and the remainder was retained for analysis. Soil TNT levels were analyzed using a high-pressure liquid chromatograph (HPLC) system (Waters, Milford, MA). This system was equipped with an autosampler (Model 717), a fluid pump (Model 600E), and a diode array UV-visible detector (Model 996). An isocratic analysis run (10 min) was employed. The mobile phase was a pump-proportioned mixture of 50/50 (v/v) water/methanol at a flow rate of 1 ml/min. Sample injection volume was 100 μl and analytes were separated using Waters Nova-Pak- C18 column (3.9 × 150 mm) at room temperature. A standard analytical reference material, TNT 100 μg/ml in acetonitrile was purchased from Chem Service, West Chester, PA, and HPLC-grade water and methanol were obtained from Waters, Milford, MA. Quantitation was done via chromatogram extraction at 254 nm as the spectra were continuously acquired between 200 to 300 nm.

Soil pyrene extraction and analysis for the greenhouse study was done at the State Chemist Laboratory of the Maryland Department of Agriculture, Annapolis, MD. Soil pyrene extractions from the growth chambers experiments were done using a Microwave Extraction Unit (CEM, Mathews, NC). Ten grams (ODE) of soil were transferred quantitatively into Teflon<sup>®</sup>-lined extraction vessels and 40 ml of acetone: hexane (1:1) was added. The extraction was performed at 110°C for 10 min at 100% power (1000 W). The vessels were allowed to cool to room temperature before opening, after which samples were filtered into amber vials thru a 0.2-μm filter. The first 2 to 3 ml of filtrate was discarded, and the remainder was retained for analysis. Residual soil pyrene levels were determined using the same HPLC setup as TNT with the following exceptions; the mobile phase for pyrene analysis was a pump-propor-

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tioned mixture of 25/75 (v/v) water/acetonitrile at a flow rate of 1 ml/min, the standard analytical reference material, pyrene 1000 µg/ml in acetonitrile, was purchased from Chem Service and HPLC-grade water and acetonitrile were obtained from Waters, Milford, MA. Quantitation was also done using an area count calculation based on known standards of chromatograms extracted at 240 nm.

Quality assurance–quality control (QA-QC) checks were conducted with each analysis. These included material blank measurements, repeat analysis, and fresh spike recoveries.

### RESULTS AND DISCUSSION

For the high OM soil (6.3%), very low levels of TNT ( $\leq 0.4\%$ ) were recovered from all the spiked soils, including the unplanted control (data not presented). For the low OM soil (2.6%) all of the treatment pots had significantly higher levels of TNT recovered compared with the high OM soil. Moreover, planting had a significant effect on TNT transformation in the low OM soil; 85% of the initial TNT was recovered from the unplanted control soils, while as low as 23% was recovered from the planted pots (Table 1). This means that 77% of the initial TNT levels were

**TABLE 1. Residual TNT Levels (mg/kg) After 4 Months of Plant Growth in Low OM Soil**

Treatment	TNT (mg/kg)
<b>Legumes</b>	
Alfalfa	34.2ab <sup>a</sup>
Sericea lespedeza	63.7d
<b>Grasses</b>	
Deertongue	41.2bc
Reed canarygrass	26.8a
Switchgrass	23.4a
Tall fescue	36.6b
Control (no crop)	84.5e

<sup>a</sup> Means followed by the same letter are not significantly different as determined by Tukey-Kramer multiple comparison procedure ( $P < 0.05$ ) and the standard error of the diff. was 9.9.

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transformed in the planted pots compared with only 15% in the unplanted control soil. There were also statistically significant differences among the plant species and switchgrass (77%) and reed canarygrass (73%) were the most effective species.

The very low level of TNT recovered from the high organic matter soil indicates that TNT removal primarily occurred via adsorption by the soil organic matter and therefore was not available for extraction to determine the compound's biological degradation. This was evidenced by the microbial assays performed on the two soils that showed that there were no significant differences among treatments in the bacterial and fungal counts of these soils (Chekol, 2000; Dzantor *et al.*, 2000). The observed unavailability of TNT in the high organic matter soil could be due to an irreversible binding (chemisorption) of TNT and its metabolites to the humic fraction of the soil organic matter (Daun *et al.*, 1998). On the other hand, Achtnich *et al.* (1999) reported covalent binding of partially reduced TNT metabolites to the soil organic matter to be an important immobilization mechanism in soils. Furthermore, Daun *et al.* (1998) indicated that the soil organic matter (humic acids) has a greater specific binding capacity for TNT and its metabolites than the clay fraction of the soil. The high and low organic matter content soils used in these experiments had similar clay content (8%). Given the fact that the role of clay in binding TNT and its metabolites is less than the soil organic matter and no significant differences were observed in the microbial counts among treatments, it appears that the very low residual TNT levels found in the high OM soil are a result of adsorption and/or covalent binding to the soil organic matter. Chekol and Vough (2001) reported similar results, where the use of plants for phytoremediation was more effective in soils with lower organic matter content. The results of this study support this conclusion.

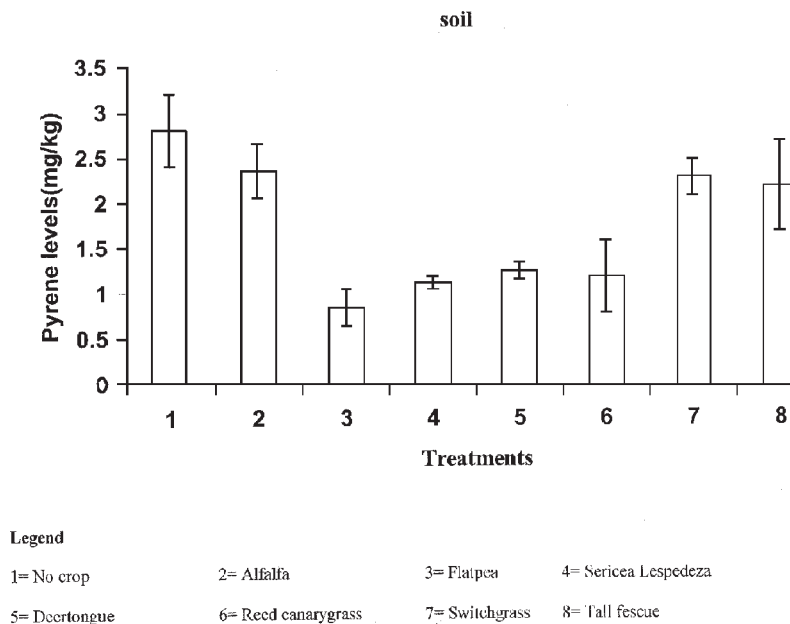
Recoveries of soil pyrene were also greater in the low organic matter content soil used in the growth chamber crop species screening experiment (Figure 2) compared with the high organic matter content soil used for the greenhouse experiment (Figure 1). Less than 4% of the initial dose of pyrene was recovered from all the treatments with the high OM soil. In contrast, 20% or more of the initial soil pyrene levels were recovered from treatments with the low OM soil (Figure 2).

Although some of the differences among treatments in the high OM soil were statistically significant, due to the very low levels of pyrene recovered from all the treatment pots, the practical implications will be negligible.

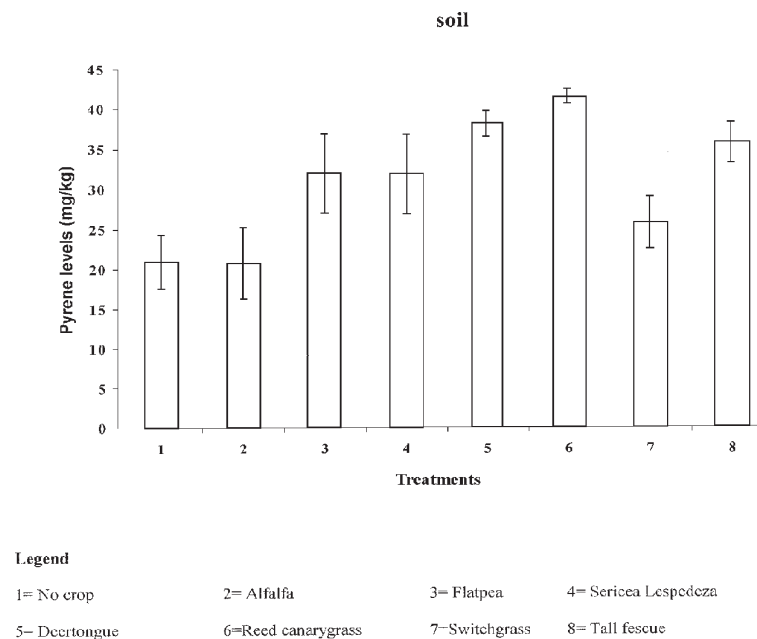
At the end of the 4-month experiment with the low OM soil, all of the treatments including the unplanted control pots had between 20 to 42% of the initial pyrene levels (Figure 2). It should be noted, however, that the unplanted control treatment had one of the lowest residual soil pyrene levels.

The absence of any significant effect of vegetation on pyrene loss is in complete agreement with the findings of Qui *et al.* (1997). Results from this study do not agree with most of the earlier studies on PAHs (April and Sims, 1990; Schwab and Banks, 1994; Banks *et al.*, 1997; Ferro *et al.*, 1997, Pradhan *et al.*, 1998) indicating increased levels of biotransformation of PAHs in vegetated treatments compared with those in unplanted controls. The discrepancies between the previously published results and this study could be indicative of the fact that plant-soil-contaminant

**Plant-Soil-Contaminant Specificity Affects Phytoremediation**



**FIGURE 1.** Mean soil pyrene levels (mg/kg), after 6 months of plant growth in the high OM soil.



**FIGURE 2.** Mean soil pyrene levels (mg/kg), after 4 months of plant growth in the low OM soil.

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interaction is not a simple system and complex physicochemical and biological factors can greatly impact the effectiveness of PAH phytoremediation. As a result, differences in soil type, plant species, microbial population and composition, and experimental and/or environmental conditions could be responsible for the observed differences in the phytoremediation potential of the studies.

## CONCLUSIONS

Recoveries of soil TNT and pyrene were highly dependent on the soil organic matter content: significantly, lower levels were recovered from all treatments in the higher organic matter soil (6.3%) compared with recoveries in the soil with lower organic matter (2.6%). Under high soil organic matter conditions, physical processes appeared to be the dominant forces of TNT and pyrene removal. In both soil types, pyrene dissipation could not be attributed to the presence of plants. Results from our experiments indicate that use of forage crops for phytoremediation of TNT-contaminated soils was effective under low OM conditions. The data for forage crops species screening experiment in the low OM soil showed that all the planted pots had significantly lower levels of TNT compared with unplanted control pots. Grass species with massive fibrous root system were more effective for TNT phytoremediation than legumes with a tap root system. For a TNT phytoremediation scheme to be effective, the use of plants with an extensive root system appears to be a good starting point. The results of the present study clearly showed that reed canarygrass and switchgrass are highly effective species for phytoremediation of TNT-contaminated low OM content soils.

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