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# **Fate of Explosives in Plant Tissues Contaminated During Phytoremediation**

Philip G.Thorne

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Abstract: Kenaf plants were grown in uncontaminated soil using irrigation water contaminated with TNT, RDX, and HMX, and in soil contaminated with TNT, RDX, and HMX using uncontaminated irrigation water. Plant growth was minimal in contaminated soil but unaffected by contaminated irrigation water. TNT and its free amino transformation products were recovered from root tissues by solvent extraction. The conjugated amino transformation products were recovered from root tissue by base/acid hydrolysis. RDX and HMX were recovered by solvent from the roots and foliar tissues. At harvest, the kenaf was chopped and mixed in with soil, where it was

allowed to humify. Very little TNT or its transformation products were recoverable either by solvent extraction, base/acid hydrolysis, or aqueous leaching. Both RDX and HMX were recovered in significant amounts by both solvent extraction and aqueous leaching; furthermore, the nitroso transformation products of RDX were detected in the leachates. Kenaf plants could be used to remediate contaminated groundwater by removing TNT from irrigation water and sequestering the residues as conjugated transformation products. These plants were ineffective for the remediation of groundwater contaminated with RDX and HMX.

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Philip G. Thorne

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## PREFACE

This report was prepared by Philip G. Thorne, Research Physical Scientist, Geological Sciences Division, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL), Engineer Research and Development Center (ERDC), Hanover, New Hampshire.

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# Fate of Explosives in Plant Tissues Contaminated During Phytoremediation

PHILIP G. THORNE

## INTRODUCTION

The remediation of soils and groundwater contaminated with explosives is an enormous task facing many governments worldwide. In recent years, ex-situ bioremediation techniques such as composting and slurry digestion have gained favor as rapid and cost-effective solutions to the soil contamination problem (Griest et al. 1993, Pennington et al. 1995, Breitung et al. 1996, Lenke et al. 1998, Shen et al. 1998). The toxicity of the finished compost and its release of toxic compounds during long-term weathering is acceptably low (Gundersen et al. 1997). Part of the favorable cost benefit of ex-situ treatments results from the high degree of contamination that is found in waste streams from manufacturing, load and pack, and washout activities. Often, deposits of explosives are found in relatively small disposal areas (a few acres) (Walsh et al. 1993, Jenkins et al. 1996). Such soils can be efficiently excavated and remediated in a nearby facility. The finished remediation mixture can then be placed in a controlled landfill.

The contamination at open burning/open detonation (OB/OD) areas and firing ranges presents a very different problem that affects the cost and benefits of excavation and ex-situ remediation and disposal of remediated mixtures. The typical concentration of explosives residues in these areas tends to range from hundreds to thousands of mg/kg, and the contamination is predominantly within the top 15 cm of the soil (Jenkins et al. 1996, 1997). Unfortunately, the distribution of contaminants is extremely heterogeneous. There are "hot" spots and "non-detect" spots in close proximity (i.e., a few meters). On an operational scale, this means that every bucketload excavated

could contain widely different levels of contamination. It would be inefficient to waste resources on remediating truckloads of excavated soil that may be, on average, below the target remediation goals. Without extensive sampling and analysis, it is unlikely that the total mass of explosives can be estimated accurately enough to provide design data for remediations such as composting or slurry digestions. In these systems care must be taken to protect the microbial consortia that perform the remediation from toxic overloads, while attempting to maximize the throughput of soil.

A different approach to the cleanup of moderately and heterogeneously contaminated soils is in-situ phytoremediation. In this approach, the cost of treatment must be low enough so that large areas can be treated without the necessity of detailed spatial quantification of contamination. Sampling should be sufficient to define the boundaries of the suspected contamination and proceed with a first-year treatment. It is expected that several years might be required to remediate an entire area. Previous research has focused on the effects of the explosives on plant growth and transport of explosives in crop tissues (Simini et al. 1995, Thompson et al. 1998).

Treatment of groundwater contamination has relied on pumping water through granular activated charcoal (GAC) beds where the explosives are retained, and then reinjecting the cleaned water into the aquifer. The irrigation of plants with contaminated water is an alternative treatment to GAC removal (Harvey et al. 1991, Rivera et al. 1998, Larson et al. 1999). Although the costs of pumping remain the same, savings could be realized without the capital investments in GAC

systems and the operational costs thereof. If the irrigated plants could be sold, there would be an added benefit.

In previous research we studied the conjugation of TNT transformation products in bioremediation matrices resulting from composting and slurry digestion (Thorn 1997; Thorne and Leggett 1997, 1999). We demonstrated that there is a time-course of conjugation strength. Amino transformation products are initially conjugated through bonds that are reversible under strong hydrolytic conditions. As humification continues, either the bonds change to forms that are resistant to hydrolysis or additional bonds form at different sites, resulting in unhydrolyzable residues. These results apply to uncontaminated plant tissues that were mixed with contaminated soil at the beginning of remediation.

Researchers have shown that extractable TNT and some bound amino transformation products remain in root tissue (Palazzo and Leggett 1986, Rivera et al. 1998, Larson et al. 1999). We and others have observed that the degradation of RDX in composting and slurry remediations takes more time (Griest et al. 1993, Shen et al. 1998) and shows no evidence of proceeding through a reversible binding of transformation products (Thorne and Leggett 1999). RDX is known to accumulate in leaf and shoot tissues of plants grown in contaminated soils or irrigated with contaminated water (Harvey et al. 1991, Rivera et al. 1998), and a recent study (Larson et al. 1999) has shown that the majority of products of RDX transformation are incorporated into plant compounds that have molecular weights in the range of 350 to 1000 daltons. A different time-course of conjugation may result when humification begins with tissues that were contaminated with explosives by plant uptake during phytoremediation projects.

The objective of this project was to test the hypothesis that plant-accumulated HMX, RDX, TNT, and their transformation products would be rapidly remediated following in-situ humification when soil conditions alternate between anaerobic and aerobic. At high soil moistures, oxygen transport into the tissues would be limited, yet available carbon and microbial activity would be high. The resulting anaerobic environment would encourage the reductive transformation of HMX, RDX, and TNT. When the soil dried to below saturation, the return to an aerobic environment would support the oxidative conjugation of amino transformation products to the developing humic

material and the further degradation of reduced HMX and RDX intermediates.

In this study, plants were allowed to accumulate explosives through two routes of exposure: from contaminated soil using clean irrigation water, and from clean soil irrigated with contaminated water. Kenaf (*Hibiscus cannabinus*, var. Tainung 2) was chosen as the test plant because it uses large quantities of water and produces a considerable amount of biomass with a high value both as fiber from the stem cortex and biodegradable sorbent from the pith. It was hoped that this plant could remediate enough contaminated groundwater to be a useful replacement for GAC treatment. If the kenaf grew in moderately contaminated soil, it could be used to remediate large areas with heterogeneous distributions of explosives. Furthermore, areas of inhibited plant growth could serve as indicators of "hot spots" within OB/OD areas. These small spots could then be targeted for excavation and ex-situ remediation. The accumulated biomass from the irrigation and soil remediations could either be turned under in place for humification or the biomass could be processed into commercial products, e.g., pulping for "treeless" paper, provided that the treatments destroyed residual explosives.

## MATERIALS AND METHODS

### Experimental methods

For the irrigation experiment, kenaf seedlings that were germinated in peat pots were transplanted into 2-gal. plastic pots (three plants per pot) containing 4 kg of clean, sandy soil. Plants were irrigated with water contaminated with 12.4 mg/L of TNT, 12.4 mg/L of RDX, and 2.43 mg/L of HMX. Irrigation rates were maximized to keep soil moisture close to field capacity. The total quantity of contaminated water applied per pot was 12 L. After two months' growth, the kenaf was harvested, divided into root, stem, and leaf samples, and diced with scissors to approximately 5-mm-sized pieces. Some diced pieces were air-dried for analysis. The remaining moist tissue was turned into its own growth soil for in-situ humification studies. The soil moisture was cycled three times between saturated (anaerobic) and unsaturated (aerobic) over a three-week period. No leaching was allowed from these pots. Samples of soil and humifying tissue were collected during the aerobic phase when the contents of the pots were stirred and homogenized with a spatula. A second set of soils with turned-in contaminated

plant tissue was watered daily so that field capacity was repeatedly exceeded. The leachate was collected and analyzed. The sum of all analytes from the leachates was compared to the total loading of explosives that was applied to the plants.

For the contaminated soil experiments, kenaf seedlings were transplanted into pots with two levels of explosives contamination and watered with tap water at intervals sufficient to maintain the soil at close to field capacity. One set of soil had TNT, RDX, and HMX at 1920, 6600, and 619 mg/kg, respectively, and a second set had TNT, RDX, and HMX at 471, 1420, and 142 mg/kg, respectively.

Controls were kenaf plants grown in clean sand and irrigated with tap water.

#### Analytical methods

Solvents used for extractions and analysis were HPLC grade from Alltech (Deerfield, Illinois, USA). Acids, bases, and buffers were reagent grade from Baker (Phillipsburg, New Jersey, USA). Standards for HPLC analysis were made from standard analytical reference material (SARM) obtained from the U.S. Army Environmental Center (Aberdeen Proving Ground, Maryland, USA). The diaminoNTs, azoxys, and nitroso-RDXs were supplied by Dr. Ronald Spanggord, SRI International (Menlo Park, California, USA). Solid-phase extraction cartridges were Sep-Pak<sub>RDX</sub> from Waters (Milford, Massachusetts, USA) and Alumina-A from Supelco (Bellefonte, Pennsylvania, USA). Contaminated soils from several military sites were pooled to create a mixture that had sufficient TNT, RDX, and HMX for the experiments. A portion of this soil was extracted with acetone and was then diluted 1:1000 with reagent-grade water to create the contaminated irrigation water.

HPLC analysis was performed using a Waters system (717 autosampler, 616 pump, 600S controller, 996 photodiode array detector, Millennium workstation). A Phenomenex (Torrance, California, USA) Ultracarb 5 ODS(20) (4.6 mm × 250 mm, 5 μm) reverse-phase column was used for the analytical separations. The aqueous/ methanol (volume %/volume %) gradient elution time steps were as follows: start at 85/15, ramp to 65/35 at 8 minutes, ramp to 42/58 at 10 minutes and hold for 13 minutes, ramp to 0/100 at 28 minutes and hold for 7 minutes, ramp down to 85/15 at 40 minutes and hold for 10 minutes before the next injection. The flow rate was 0.8 mL/minute.

Quantification was performed at 254 nm, while peak identities and purities were assessed by comparing sample and standard peak spectra and retention times. The detection limits for the analytes were approximately 0.1 mg/kg.

Soil samples were taken by compositing the contents of three 3/4-inch-diameter cores taken through the entire depth of soil in the pots. Root hairs were removed before analysis. Samples of plant material were taken by sacrificing individual plants, washing soil from the roots and cutting up the tissues with scissors, then air-drying. Samples of soil weighing 2.00 g were added to 22-mL glass vials; 10 mL of acetonitrile was added. Samples of tissue were of variable weights. The quantities of roots were small and most of each harvest could be extracted in one vial. As many stem and leaf tissues as could be stuffed into a vial were extracted with enough acetonitrile to cover them. After adding the acetonitrile, sample vials were sealed with Teflon-lined caps and vortexed for one minute. The vials were then placed in a cooled, sonic bath and sonicated for 18 hours. Following sonication, the extract was centrifuged and the supernatant decanted. A portion was mixed 1:1 with reagent-grade water containing 3.5 g/L of CaCl<sub>2</sub>, centrifuged again, and analyzed by HPLC. The acetonitrile extracts of stems and leaves were dark green. Most of the pigments were removed by passing the extract through an Alumina-A solid-phase extraction cartridge prior to mixing with water.

Residues from the acetonitrile extractions of soil and root tissues were repeatedly rinsed with acetonitrile/water mixtures, centrifuged, and decanted until no more solvent-extractable analytes were detected. The exhaustively rinsed residues of soils and tissues were then air-dried in the sample vials and 5 mL of 0.5 N NaOH added. The samples were mixed for three minutes and sonicated overnight at 30°C. Then, a 5-mL aliquot of ice-cold, 50% H<sub>2</sub>SO<sub>4</sub> was added, and the vials were returned to the sonic bath for six hours. After sonication, the contents of the vials were transferred to 125-mL flasks and neutralized to pH 6.5 with approximately 75 mL of 1.0 M dibasic phosphate (pH = 8.6). The neutralized base/acid digest was centrifuged and the supernatant passed through a Sep-Pak<sub>RDX</sub> solid-phase extraction cartridge, which retained the explosives and their transformation products. The cartridge was eluted with 5 mL of acetonitrile, which was then diluted 1:1 with reagent-grade water for HPLC analysis.

## RESULTS AND DISCUSSION

Kenaf plants were germinated in peat pots and allowed to grow to 15 cm in height, when they had two sets of mature leaves. The plants in their peat pots were transplanted into either clean or contaminated soil. The control plants in clean soil watered with clean tap water grew to a height of about 2 m. A few of the control plants were transplanted to moderately contaminated soil when they reached 50 cm in height. The plants grown in clean soil and irrigated with contaminated water grew as high or higher than the controls and actually appeared to be healthier. They were greener than the controls and more tolerant to water stress. Their roots filled the 2-gal. pots completely, as did the control plants. In the moderately contaminated soil, the plants grew very little after transplanting, and the roots extended from the peat pots only a few cm. The 15-cm-tall transplants in the highly contaminated soil grew only a few cm, with few additional leaves. Their roots did not extend beyond the peat pots. After the exposure and sampling parts of the experiment were completed, a few of the plants in each treatment were irrigated with clean water and allowed to grow until flowering after six months. For all treatments, the plants survived and produced flowers. However, the plants in the highly contaminated soil were only 20 cm

high compared to the control plants, which were 200 cm high.

Table 1 shows the concentrations of explosives and transformation products found in the various plant tissue components after exposure to contaminated soil and water for two months. As reported elsewhere (Palazzo and Leggett 1986, Harvey et al. 1991, Rivera et al. 1998, Thompson et al. 1998, Larson et al. 1999), solvent extraction recovered TNT and its aminoDNT transformation products only from root tissues. The solvent-extracted nitramines RDX and HMX were recovered at moderate concentrations from the roots and stems but at much higher concentrations from leaf tissues. No nitroso transformation products of RDX were detected anywhere in the tissues.

The base/acid hydrolyses of solvent-extracted root tissue released an additional quantity of the monoaminodinitrotoluene (4ADNT and 2ADNT) transformation products of TNT. The diamino-nitrotoluenes (2,4DANT and 2,6DANT) were not detected in the solvent extract but were recovered from the base/acid hydrolysate. Spike-recovery studies done earlier (Thorne and Leggett 1999) indicated that the base/acid hydrolyses procedure did not produce artifactual transformations or increase the recovery of unbound analytes. Therefore, it appears that in root tissue, the sec-

**Table 1. Concentrations (mg/kg) of explosives and transformation products in plant tissues after two months' exposure to contaminated soil or water. Acetonitrile solvent extractions of stems, leaves, and roots, and base/acid hydrolysates of roots.**

	TNT	4ADNT	2ADNT	2,4DANT	2,6DANT	RDX	HMX
<b>Clean soil with contaminated water</b>							
Soil	1.4	1.2	1	nd	nd	10.3	3.6
Stems	nd	nd	nd	nd	nd	144	8
Leaves	nd	nd	nd	nd	nd	3130	107
Roots	53.2	79	21	nd	nd	205	161
Roots b/a	nd	156	44.5	65.1	4.9	nd	nd
<b>Clean water with moderately contaminated soil*</b>							
Stems	nd	nd	nd	nd	nd	169	10
Leaves	nd	nd	nd	nd	nd	1350	46.8
Roots	297	454	724	nd	nd	726	167
Roots b/a	nd	456	280	214	147	nd	nd
<b>Clean water with highly contaminated soil†</b>							
Stems	nd	nd	nd	nd	nd	326	21.4
Leaves	nd	nd	nd	nd	nd	787	12.1
Roots	385	191	358	nd	nd	389	92
Roots b/a	nd	313	325	146	86.1	nd	nd

\* TNT, RDX, and HMX at 471, 1420, and 142 mg/kg.

† TNT, RDX, and HMX at 1920, 6600, and 619 mg/kg.

nd = <0.1 mg/kg.

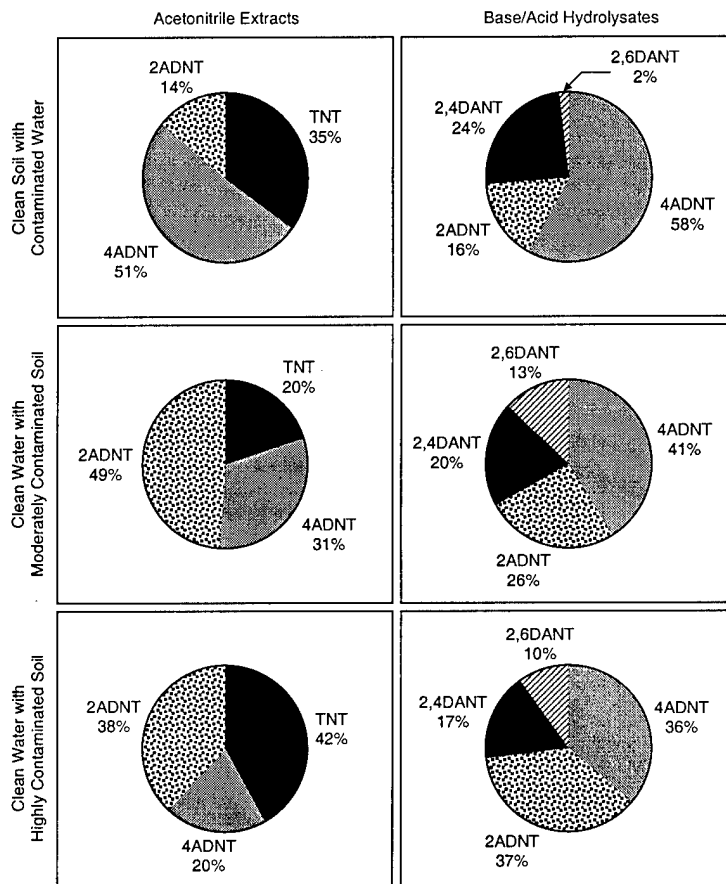


Figure 1. Relative quantities of ADNT and DANT isomers in root tissues.

ond nitro group on the TNT molecule is reduced to create the DANT only after the ADNT is bound.

The relative quantities of the two ADNT isomers, 2ADNT and 4ADNT, are shown in Figure 1. In the plants irrigated with contaminated water, where the concentrations of explosives were moderate and the growth not adversely affected, more 4ADNT than 2ADNT was recovered. The soil in these pots contained TNT and its transformation products (Table 1). The plants grown in the contaminated soil were stunted and no TNT transformation products were detected in the soil. In these plants, more 2ADNT than 4ADNT was recovered. This difference may reflect a lack of microbial activity in the highly contaminated soil as opposed to some active microbial activity in the soils that received only moderate amounts of explosives in small increments at each irrigation event. Hughes et al. (1997), in experiments with hairy-root cultures grown in the presence of TNT, also found that the quantity of 4ADNT exceeded 2ADNT in cultures grown with associated rhizo-

sphere microbes, whereas the quantity of 2ADNT exceeded 4ADNT for axenic cultures. Thus, it could be hypothesized that the greater quantity of 4ADNT found in root tissue grown in conditions that favor microbial activity is due to the uptake of 4ADNT that was already produced outside the roots. The predominance of 4ADNT over 2ADNT also accounts for the greater amount of 2,4DANT than 2,6DANT in the hydrolysates of the plants grown in contaminated water compared to the plants grown in contaminated soils. The 2,4DANT isomer can come from a secondary reduction of either 2ADNT or 4ADNT; 2,6DANT, however, can come only from 2ADNT.

The results of the in-situ humification and leaching experiments are listed in Figure 2 and Table 2. The mass balance calculations are approximations based on single sacrificed plants from one set of pots and leachates from a replicated set of pots. When the soil environment was cycled between anaerobic and aerobic, all of the TNT and nearly all of its aminoDNT transformation products eventually became undetectable follow-

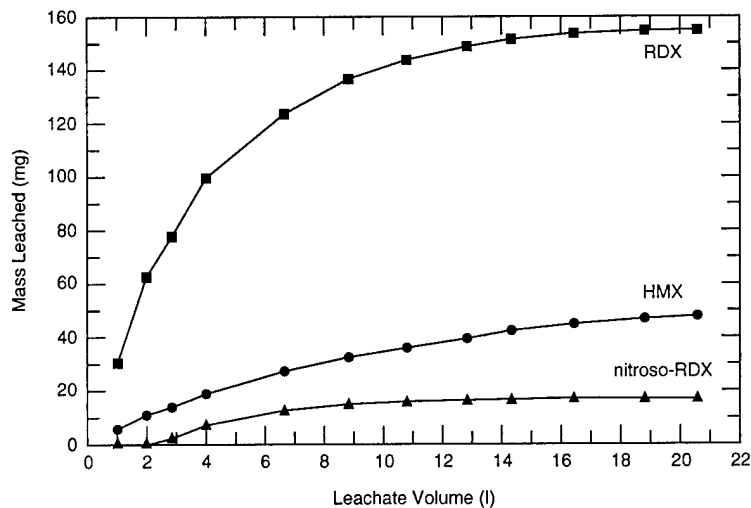


Figure 2. Cumulative mass of analytes leached from pots containing contaminated tissues.

ing both solvent extraction and base/acid hydrolysis. Approximately 14% of the RDX and a trace of its nitroso transformation product and 80% of the HMX remained. This result is similar to those reported for compost and digester sludges (Thorne and Leggett 1999). This would be a promising outcome for a limited remediation scheme; however, when field capacity was exceeded and leaching occurred, HMX, RDX, and

nitroso-RDX were recovered in the leachate. Most of the TNT was taken up by the plants or transformed by soil microbes. Only 10% of the applied TNT was recovered from the soil as TNT and ADNTs, while only an additional 2% was recovered from the tissues by both solvent extraction and base/acid hydrolysis. For the nitramines, 27% of the applied RDX and 47% of the HMX was recovered from the soil, while 37% of the RDX

Table 2. Mass (mg) of explosives and transformation products in soils, plant tissues, in-situ humification pots, and leachates after two months' irrigation with contaminated water. Acetonitrile solvent extractions of soil, stems, leaves, and roots, and base/acid hydrolysates of in-situ pots and roots.

	TNT	4ADNT	2ADNT	2,4DANT	2,6DANT	RDX	HMX	nitroso-RDX
<b>Clean soil with contaminated water</b>								
mg/pot *	150	nd	nd	nd	nd	150	30	nd
Soil	5.5	4.9	3.7	nd	nd	41.2	14.2	nd
% of mass remaining	10% (sum of TNT and ADNTs)					27%	47%	
Stems	nd	nd	nd	nd	nd	7.8	0.4	nd
Leaves	nd	nd	nd	nd	nd	46.5	1.7	nd
Roots	0.5	0.8	trace	nd	nd	1.4	0.5	nd
Roots b/a	nd	1.6	0.4	0.7	0.1	nd	nd	
<b>In-situ humification pots</b>								
Tissue and soil	nd	1.2	1.2	nd	nd	20.4	24	trace
Tissue + soil b/a	nd	nd	nd	nd	nd	nd	nd	nd
% of mass remaining	2% (sum of ADNTs)					14%	80%	
<b>Leachates (two growth pots combined)</b>								
Tissue and soil	1.6	4	2	4.6	trace	155	48	16.8
% recovery	3% (sum of TNT and transformation products)					57%	80%	added to RDX

\* 12 L of water contaminated with 12.4 mg/L TNT, 12.4 mg/L RDX, and 2.43 mg/L HMX. nd = 0.4 mg.

and 9% of the HMX was recovered from plants. When two of the pots containing their contaminated tissues were mixed together and leached, 3% of the TNT and its transformation products were leached in the first few liters, while 57% of the RDX (including its nitroso transformation product) and 80% of the HMX that was applied were recovered after 20 liters (13 sequential leachings). Rivera et al. (1998) also reported a significant difference in the relative removals of RDX and HMX from contaminated water.

## CONCLUSIONS

The uptake and accumulation of explosives and transformation products in plants is possible and could provide a remediation method for both contaminated water and soil in limited circumstances. If the only contaminant in the water was TNT, then plants could be used to remove it. The above-ground tissues remain uncontaminated and could be sold. A plant such as kenaf that transpires a lot of water and has valuable biomass is a good candidate. It grew as well or better than controls when irrigated with water contaminated with very high levels of explosives. Such a contamination scenario is likely only at TNT production facilities. However, the majority of contaminated sites are load-and-pack and demilitarization activities, where the contamination is a combination of nitramines and TNT and other explosives not tested during this project. In these cases, the poor uptake of nitramines from contaminated-water irrigation would result in redeposition of HMX and RDX in surface soils. As accumulators of TNT and nitramines from contaminated soils, kenaf plants are effective, but growth is limited. If the plants were turned under and allowed to humify in situ, they may aggravate the situation by providing an environment that encourages the production of nitroso-RDX, an additional potentially toxic compound. If the nitramine-contaminated tissues could be removed and processed in a manner that degrades the explosives, then the use of plant-uptake of explosives from moderately contaminated soils might be a possible alternative to excavation and ex-situ treatment. In such a case, a plant that grows better than kenaf under these conditions would have to be chosen.

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## APPENDIX A: UPTAKE OF $^{15}\text{N}$ -LABELED TNT TRANSFORMATION PRODUCTS

### Incubation of kenaf

One kenaf plant was removed from a control pot and the soil completely washed from the roots. The root mass was teased apart into four sections; each section was placed in a 500-mL beaker. Each beaker was filled with water containing one of the  $^{15}\text{N}$ -labeled amino and diamino TNT transformation products. Water was replenished every day for one week. The roots were then cut from the plant, washed with water, extracted overnight with acetonitrile, finely cut up, and air-dried.

### NMR spectrometry

Solid state cross polarization/magic angle spinning (CP/MAS)  $^{15}\text{N}$  NMR spectra were recorded on a Chemagnetics CMX-200 NMR spectrometer at a nitrogen resonant frequency of 20.3 MHz, using a 7.5-mm ceramic probe (zirconium pencil rotors). Chemical shifts were referenced to glycine, taken as 32.6 ppm. Acquisition parameters included a 30,000-Hz spectral window, 17.051-ms acquisition time, and 5000-Hz spinning rate. Contact times and pulse delays were 2.0 ms and 1.0 s for the pure 2,4DANT, 5 ms and 0.5 s for the 2,4DANT-treated root, and 2.0 ms and 0.5 s for the unlabeled blank root sample. The line broadenings (LB) in hertz are shown in the figures.

### Results

Solid state CP/MAS  $^{15}\text{N}$  NMR spectra were recorded on the root sample from kenaf treated with  $^{15}\text{N}$ -labeled 2,4DANT, blank root, and pure  $^{15}\text{N}$ -labeled 2,4DANT (Fig. A1). The chemical shift position of

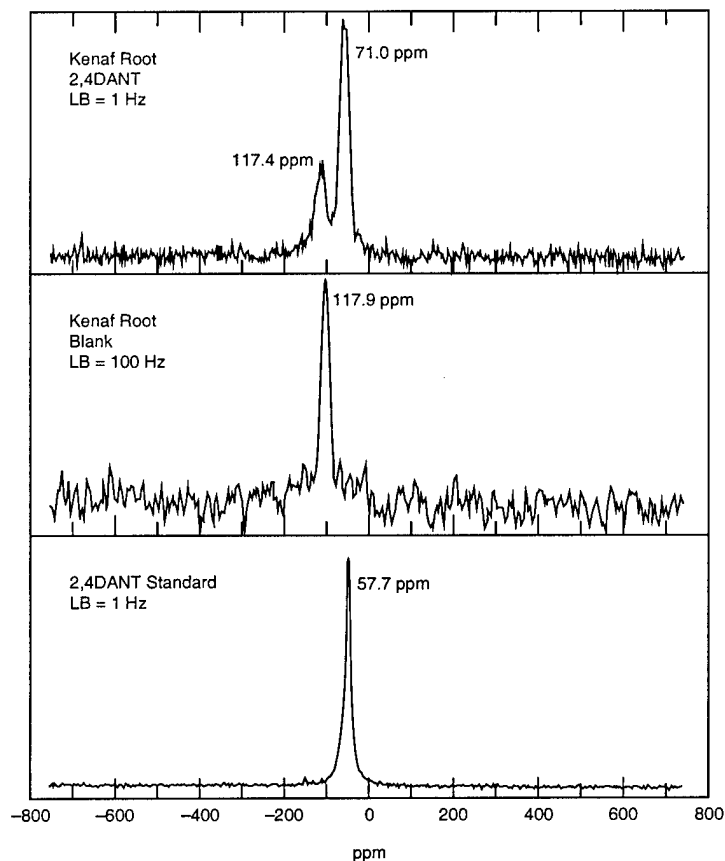
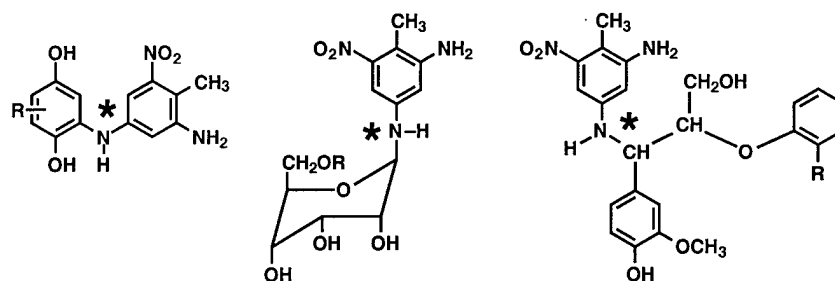
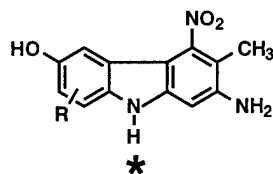


Figure A1.  $^{15}\text{N}$ -NMR spectra of kenaf root.

2,4DANT in the solid state is 57.7 ppm. The spectrum of the blank root sample consists of a broad peak at 117.9 ppm. This comprises the naturally abundant  $^{15}\text{N}$  nuclei in the root, and corresponds to the secondary amide nitrogens of amino acids in peptide structures. The spectrum of the root from kenaf treated with 2,4DANT exhibits this secondary amide peak as well as a major peak at 71 ppm. Direct, unequivocal evidence for incorporation of 2,4DANT into the biochemical constituents of the root tissue is absent. This is in contrast to spectra of the reduced TNT amines reacted with soil humic acid, in which case evidence for condensation of the amines with carbonyl groups was definitive (Thorn 1998). However, some subtle features of the spectrum may be interpreted as evidence for condensation of the 2,4DANT with biochemical constituents. Compared to the spectrum of the 2,4DANT standard, the major peak of the root spectrum is broader, and the maximum shifted downfield to 71 ppm. The downfield shift and broadening would be consistent with formation of aminohydroquinone, glycosidic, or arylamino linkages.



The latter structure is the 2,4DANT analog of the reported condensation product resulting from the peroxidase catalyzed reaction of aniline with conniferyl alcohol, a precursor to lignin (Lange et al. 1998). Vertical expansion of the spectrum also reveals that the downfield peak centered at 117.9 ppm extends to about 170 ppm. It is possible that 2,4DANT covalently bound in the form of heterocyclic structures such as indoles, pyrroles, or carbazoles overlaps with the naturally occurring amide nitrogens in the root tissue.



Spectra of roots from kenaf grown with the labeled 4ADNT and 2,6DANT (not shown) also exhibited broadening and downfield shifts from their respective 4ADNT and 2,6DANT standards, showing possible evidence for aminohydroquinone, glycosidic, or arylamino linkages.

