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Phytoremediation for the treatment of energetic material releases on testing and training ranges at Eglin Air Force Base

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PHYTOREMEDIATION FOR THE TREATMENT OF ENERGETIC MATERIAL RELEASES ON TESTING AND TRAINING RANGES AT EGLIN AIR FORCE BASE

by

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

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CHAPTER 1
INTRODUCTION AND OBJECTIVES

1.1 Background

Eglin Air Force Base (EAFB) is located on the panhandle of Florida adjacent to the towns of Niceville and Valparaiso as shown in Figure 1-1. The base occupies 724 square miles of land as well as nearly 98,000 square miles of air space over the Gulf of Mexico making it one of the largest military installations in the world. The base also houses the headquarters of the Air Armament Center, responsible for the development, acquisition, testing, and fielding of all air-delivered non-nuclear weapons for the United States and allies. In addition, most of the undeveloped land on the base is home to endangered plants and animals such as the long leaf pine, red-cockaded woodpecker, bald eagle, piping plover, Okaloosa darter, Gulf sturgeon, flatwoods salamander, Eastern indigo snake, loggerhead sea turtle, green sea turtle, leatherback sea turtle, and the Florida perforate lichen (Jacobson & Marynowski, 2002). Many of these areas are also open to the public for recreational uses such as hunting, fishing, camping, hiking, and wildlife observation.

In order to protect natural resources and ecosystems at EAFB, a strategy must be developed for the containment and/or treatment of explosive contaminants on testing and training ranges. The firing of munitions, the detonation of ordnance, and the disposal of unexploded ordnance (UXO) result in contamination of soil with explosive compounds (Jenkins et al., 1999; Thiboutot et al., 1998). The energetic compounds most commonly found to contaminate soil on military testing and training ranges include 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-

1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). These compounds are persistent environmental contaminants and pose risks to the health of humans and ecosystems (Flokstra, Van Aken, & Schnoor, 2008; Jenkins, Bartolini, & Ranney, 2003). However, plants and microbes have been shown to degrade these explosive compounds (Hawari, Beaudet, Halasz, Thiboutot, & Ampleman, 2000; Hawari et al., 2001; Jenkins et al., 2006; Van Aken & Agathos, 2001).

Phytoremediation is the direct use of living plants for *in situ* (in place) remediation of contaminated soil, sludges, sediments, and groundwater through contaminant removal, degradation, or containment (USEPA, 1999). Due to its ability to continuously treat large areas at low cost with low impact to the site, phytoremediation will be implemented through a field study at EAFB in order to increase the sustainability of range operations.

1.2 Properties of TNT, RDX, and HMX

As shown in Table 1-1, TNT is a nitroaromatic compound, which biodegrades readily under both aerobic and anaerobic soil conditions (Hawari et al., 2000). The mineralization of TNT is rare because its nitroaromatic ring is resistant to attack (Spain, 1995). However, the nitro groups are easily reducible, resulting in metabolites that are sometimes rather persistent. TNT has also been shown to be toxic. TNT has been shown to cause neurological disorders in workers in large-scale manufacturing operations and can be highly toxic and mutagenic to aquatic species (McCormick, Feeherry, & Levinson, 1976; Won, Disalvo, & Ng, 1976). Table 1-1 shows the physical and chemical constants of TNT. Losses through volatilization from soil or groundwater to the atmosphere are negligible due to TNT's low vapor pressure and moderately low Henry's

law constant. TNT is moderately water soluble and has low partition coefficients which would favor the movement of the compound with little absorption to the soil. However, the products of aerobic and anaerobic biotransformation can irreversibly bind to organic material in the soil (Hawari et al., 2000). TNT is subject to photolysis in an aqueous state (Talmage et al., 1999).

RDX is a cyclic nitramine as shown in Table 1-1. It is a major component of most military explosives. RDX represents 90% of Composition 4 (C4) and 60% of Composition B (Comp B) (Hewitt et al., 2007). Also from Table 1-1, it can be seen that RDX does not readily volatilize because of the low vapor pressure and Henry's constant. The solubility and low partition coefficients would suggest RDX exhibits a high degree of mobility in the environment.

HMX is also a cyclic nitramine commonly found in military explosives. The production of HMX also produces small quantities of RDX as a production contaminant. Therefore, both compounds are commonly found together in the environment (Thiboutot, 1998). Octol consists of 70% HMX and 30% TNT (Ampleman, Marois, & Thiboutot, 1999) and is used in rockets, causing extensive contamination on anti-tank ranges (Jenkins et al., 1999). As seen in Table 1-1, HMX has relatively low water solubility at 6.6 mg/L, but once solubilized the partition coefficients suggest that it will be readily transported through the subsurface.

1.3 Remediation Technologies for Explosives

Treatment technologies for the remediation of explosive compounds include biodegradation, bioaugmentation, permeable reactive barriers, pump and treat, soil slurry reactor, excavation, landfilling, incineration, composting, adsorption to activated carbon,

and advanced photooxidation processes (Van Aken et al. 2004). Many of these techniques involve invasive work requiring excavation. Since most explosive contamination occurs over a large area, any excavation work will be extremely expensive and ecologically damaging. There are also hazards associated with excavation due to the risk of striking underlying UXO on military testing and training ranges. Phytoremediation usually involves the *in situ* treatment of contaminants meaning it will be comparatively less disruptive to the environment and can be implanted at lower cost (Hannink, Rosser, & Bruce, 2002).

1.4 Phytoremediation of Explosives

Phytoremediation is a general term for many processes which plants utilize to transport, transform, or store environmental pollutants. The specific processes include phytoextraction, rhizofiltration, phytostabilization, phytodegradation, rhizodegradation, and phytovolatilization. Phytoextraction refers to the uptake and translocation of contaminants by plant roots. Rhizofiltration is the adsorption or precipitation of contaminants onto the plant roots or absorption into the roots. Phytostabilization is the use of plants to immobilize contaminants by adsorption, absorption, or precipitation of contaminants in the root zone. Phytodegradation is the breakdown of contaminants through metabolic processes within the plant or through interaction with plant exudates in the soil. Rhizodegradation is the breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the plants. Phytovolatilization is the uptake and transpiration of a contaminant into the atmosphere (USEPA, 1999).

1.4.1 Phytoremediation of TNT

TNT biodegrades readily under both aerobic and anaerobic soil conditions (Hawari et al., 2000). TNT has also been shown to degrade in plant tissues, but very few plants can translocate the TNT to leaves (Schneider, Oltmanns, Radenberg, Schneider, & Mundegar, 1996). As shown by phosphor imager autoradiography, TNT remains in roots with little to no translocation to leaves and stems (Brentner, Mukherji, Walsh, & Schnoor, 2009). This is due to TNT's high biochemical reactivity of the aromatic nitro group which forms oxidative couplings on roots (Thompson, Ramer, & Schnoor, 1998). Common metabolites of TNT include 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT). 2-ADNT and 4-ADNT are formed by aerobic reduction of TNT (Hannink, et al., 2002). 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoleune (2,6-DNT) have also been shown as TNT metabolites in plants. 2,4-DNT and 2,6-DNT have been shown to be potent animal carcinogens (Rickert, Butterworth, Popp, 1984).

1.4.2 Phytoremediation of RDX

RDX is fairly soluble and does not bond well to organic or soil fractions, therefore it is readily translocated by plants. Phosphor imager autoradiography showed that RDX is translocated and stored or transformed in the plant leaves (Brentner et al., 2009). Several studies have shown different degradation pathways once translocated to the leaves. The transformation of RDX to polar metabolites and bound residues containing RDX metabolites has been observed (Hannink et al., 2002; Just & Schnoor, 2004). A pathway resulting in the mineralization of RDX has also been observed (Van Aken, et al., 2004).

1.4.3 Phytoremediation of HMX

HMX exhibits poor solubility. However, when HMX does solubilize it is readily taken up by plants due to its low affinity to bond to organic and sediment particles. This results in the translocation of HMX into the leaves. No degradation or transformation is known to occur before, during, or after translocation. Over half of the HMX contained in leaf tissues was observed to leach out of fallen leaves (Yoon, Oh, Just, & Schnoor, 2002). This is of serious concern if phytoremediation is to be applied for the treatment of HMX.

1.5 Phytoremediation of Explosives in Field Studies

There were no examples of field-scale *in situ* phytoremediation to treat explosives contaminated soils in published literature. There have been field studies demonstrating the use of phytoremediation in wetland systems to treat explosives. Phytoremediation was implemented in a wetland system for the treatment of explosives at the Iowa Army Ammunition Plant in Middletown, Iowa producing positive results (McCutcheon & Schnoor, 2003). Following construction of two treatment wetlands, monitoring results conducted for 2 years found no TNT and RDX concentrations above the EPA human health advisory level of 0.002 mg/L when the wetlands were discharging to adjacent surface waters.

1.6 Previous Work Completed Under ER-1499

Work completed under the Strategic Environmental Research and Development Program (SERDP) grant ER-1499, titled "Phytoremediation for the Containment and Treatment of Energetic and Propellant Material Releases on Testing and Training Ranges," has been ongoing since 2006. Two additional investigators, Travis J. Anderson and Laura B. Brentner, completed project tasks under the grant which contributed both

directly to the field study as well as to further the understanding of physical and chemical processes associated with the phytoremediation of explosive compounds.

Laura Brentner's work included the expression of glutathione-*S*-trasferase in poplar trees exposed to TNT, the expression of transferase enzymes in poplar and soybeans exposed to TNT, the localization of RDX and TNT in poplar and switchgrass plants using phosphor imager autoradiography, and completed the initial feasibility study for phytoremediation of TNT at EAFB (Brentner, 2008a). This work resulted in two publications (Brenter et al., 2008b; Brentner et al., 2009). Travis Anderson's work included research into the biodegradation of explosives in unplanted soils from EAFB, the characterization of the microbial community in soils from EAFB, and completed the first two samplings of the field study (Anderson, 2010).

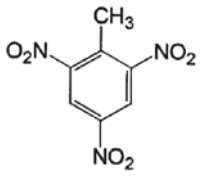
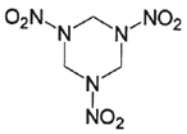
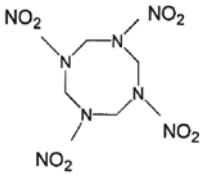
1.7 Objectives

The main objective of the field study at EAFB was to determine whether phytoremediation through the use of Bahiagrass Pensacola (*Pensacola notatum)* is a viable alternative for the treatment of energetic compounds, and to prevent the compounds from migrating offsite through surface runoff or by migration through the soil and into the groundwater. Additional laboratory studies will be performed to understand the processes in which the energetic compounds are degraded or transported using actual soils from the site with Bahiagrass and Hybrid Poplar (*Populus deltoides x nigra, DN34*) as model plants. The field study at EAFB is unique by virtue of being the only field scale phytoremediation demonstration on a military range to date. The specific objectives of this research are to:

- Determine if Bahiagrass Pensacola significantly improves the biodegradation of explosives in soil at EAFB through comparisons of the planted and unplanted regions of three plots located within Range C-62.
- Determine whether plants can significantly uptake and degrade explosives in the field.
- Compare fate and transport processes in laboratory studies using actual soils from the site of the field study with the field demonstration results.

In order to meet these objectives, previous research accomplished for this study will be investigated as well as an investigation presented in Chapter 2 to determine the rate at which RDX concentrations are reduced in the soil in the presence of Bahiagrass Pensacola and hybrid poplar. Detailed results of the field study conducted at EAFB are detailed in Chapter 3.

Table 1-1. Physical and Chemical Constants for TNT, RDX, and HMX

	TNT	RDX	HMX
Vapor Pressure (mm Hg)	1.99×10^{-4}	4.0×10^{-9}	3.3×10^{-14}
Henry's Law Constants ($atm\text{-}m^3\text{-}mol^{-1}$)	4.57×10^{-7}	1.2×10^{-5}	2.6×10^{-15}
Solubility in H_2O (mg/L, 20 ºC)	130	38	6.6
Partition Coefficients			
Log K_{ow}	1.84	0.86	0.13, 0.06
Log K_p	4 - 53	0.83 – 4.13	< 8.7
Log K_{oc}	3.2	0.8 – 4.2	2.8
Structure (Hannink et al., 2002)			

Source: Groom, C. A., Halasz, A., Paquet, L., Morris, N., Olivier, L. Dubois, C., & Hawari, J. (2002). Accumulation of HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in Indigenous Plants Grown in HMX-Contaminated Anti-Tank Soil. *Environmental Science & Technology, 36*(1), 112-118.

Figure 1-1. Location of field study within Elgin Air Force Base which is adjacent to Niceville, FL. Map composed on Google Earth®.

Source: Anderson, T. J. (2010). Phytoremediation of energetic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

CHAPTER 2
PHYTOREMEDIATION OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) IN SOIL FROM EGLIN AIR FORCE BASE USING BAHIAGRASS AND POPLAR PLANTS

2.1 Introduction

Through previous work completed in the W.M. Keck Phytotechnology Lab, the degradation of TNT was shown using grasses and poplar in native EAFB soil (Brentner, 2008a). The biodegradation (or lack thereof) of TNT, RDX, and HMX in unplanted native EAFB soil was also explored (Anderson, 2010). In order to ensure the validity of applying phytoremediation for the treatment of RDX contamination at EAFB, a microcosm study was performed using the same plant species and soil utilized in the field study.

2.2 Materials and Methods

2.2.1 Experimental Setup

The laboratory study consisted of five microcosms using native Lakeland soil from EAFB planted with either Bahiagrass Pensacola (*Paspalum notatum*), the excised roots of Bahiagrass Pensacola, hybrid poplar (*Populus deltoides x nigra, DN34*), the excised roots of poplar, or remained unplanted as a control. The study included triplicates of each microcosm. Figure 2-1 shows the experimental setup of the Bahiagrass Pensacola, excised roots of the grass, and the control. The experiment was conducted for 56 days with soil samplings at days 0, 14, 28, and 56. On day 56, the plants were sacrificed. The leaves and roots of the poplars and the blades and roots of the Bahiagrass

were excised for analysis. The roots excised at the start of the experiment were extracted and analyzed. All roots were rinsed thoroughly of soil prior to processing.

2.2.2 Experimental Conditions

The triplicates of each microcosm were planted in individual 4-inch diameter by 3 ¾-inch high Panterra® plastic planting pots (Greenhouse Megastore, Danville, IL). The planting pots had holes in the bottom to allow excess water to drain in order to keep the soil aerobic. Tin foil was placed over each pot in order to prevent phototrophic organisms from growing on the surface of the soil. The experiment was conducted in an environmental growth chamber at 30 ºC under a 16:8 hour photoperiod (150 µmol s^{-1} m^{-2}). The soil was watered periodically with one-tenth strength Hoagland solution to maintain moisture and to provide nutrients for plants. Special care was given to not water to the point where water drained from the pots.

2.2.3 Soil Collection

Lakeland soil is the predominant soil type at EAFB and was therefore the soil used in conducting the laboratory study. The soil was collected during a site visit to EAFB on March 2, 2007 and was shipped to The University of Iowa in 28-quart coolers at 4-8 ºC. The soils were stored at 4 ºC until used for the study.

2.2.4 Soil Analysis

The soils sampled at EAFB were analyzed at A&L Analytical Laboratories (Memphis, TN) for nutrient, pH, bulk density, and texture analyses. The findings are shown in Table 2-1 and Table 2-2. Lakeland soil has low silt content, organic content, and has very low nutrient levels. It is considered a poor, sandy soil for plant growth.

2.2.5 Chemicals and Materials

Analytical standards were purchased through AccuStandard® (New Haven, CT) at a concentration of 1 mg/mL. RDX was synthesized in-house according to (Ampleman et al., 1995) and purified by recrystallization. Synthesized RDX was 99% pure or higher according to analysis by High Performance Liquid Chromatography (HPLC).

2.2.6 Soil Contamination

The soil was freshly contaminated with RDX to a concentration of 25 mg/kg. Approximately 400 g of soil was used in each pot. In order to achieve homogenous soil contamination, 100 g of soil was contaminated with RDX dissolved in acetonitrile and was then well mixed. Additional mass was added and well mixed until the necessary mass of soil was achieved. The soil was then immediately used for the experiment.

2.2.7 Sampling and Explosives Extraction from Soil

For each sampling, eight samples from each pot were taken for the entire depth of soil. The samples were homogenized by mixing for approximately 2 minutes. The extraction of explosives from soil was performed according to a modified version of EPA Method 8330B (USEPA, 2006). The soil was left to dry at room temperature until a constant weight was achieved. A mortar and pestle were used to crush the soil into fine grains. Two grams of soil was placed in a 15 mL vial with 10 mL of acetonitrile for the extraction of the energetic compounds. The vials were then placed in an ultrasonic bath for 18 hours. Samples were filtered with 0.20 µm Durapore© membrane filters. The sample filtrate was then used for analysis.

2.2.8 Explosives Extraction from Plant Material

The extraction of explosives from plant tissue was performed according to a modified version of EPA Method 8330B (USEPA, 2006). Following the excision of the roots and leaves or blades, a sample of each tissue was crushed and homogenized using a mortar, pestle, and liquid nitrogen. Two grams of plant tissue was placed in a 15 mL vial with 10 mL of acetonitrile for the extraction of the energetic compounds. The vials were then placed in an ultrasonic bath for 18 hours. Samples were filtered with 0.20 µm Durapore© membrane filters. The sample filtrate was then used for analysis.

2.2.9 Chemical Analysis

The explosives extracted from soil were analyzed using HPLC (HP Series 1100; Hewlett-Packard, Palo Alto, CA) using an Acclaim® Explosives E1 column (Dionex Corporation). The samples were analyzed with a mobile phase of methanol:deionized water 43:57 v/v at a flow rate of 1.0 mL/min. Detections were measured at a UV absorbance of 230 nm and 254 nm using a UV visible photodiode array detector (HP Series 1100).

Explosives extracted from plant samples were analyzed using liquid chromatography-mass spectrometry (LC/MS). An Agilent 6140 Quadrupole LC/MS was used with an Acclamin 120 Å C_{18} column (2.1 x 150 mm, 3µm; Dionex Corporation). The mass spectrometer was operated in negative-ion electrospray mode. A mobile phase of acetonitrile:2mM ammonium acetate 50:50 v/v at a flow rate of 0.4 mL/min.

Calibration curves were constructed using standards before and after each sample run for quality control. Standards were also placed after every ten sample vials in order to verify retention times and elution order. The standards, EPA 8330-R Explosives Mix,

were ordered from AccuStandard® (New Haven, CT). The explosives mix included the following components: 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), nitrobenzene (NB), tetryl, TNT, RDX, and HMX. The standards were used to identify and quantify explosives and metabolites in samples.

2.3 Results

2.3.1 Reductions in RDX Concentrations in Soil

The study on the hybrid poplar microcosms was ended prematurely on day 40 due to a spider mite (*Tetranychus urticae*) infestation in the lab which caused the health of the poplars to decline rapidly. The other three microcosm studies were carried out for the entire 56 day period. As shown in Figure 2-2, substantial root density was achieved with the Bahiagrass. The results of the study are shown in Figure 2-3. The RDX concentration in soil was reduced by 77.7% after 14 days and 98.6% after 56 days in the planted Bahiagrass microcosm. The RDX concentration in soil was reduced by 96.3% after 14 days and 99.1% after 40 days in the planted poplar microcosm. The excised roots and unplanted control saw no significant reduction in concentration over the 56 day period.

2.3.2 RDX Uptake into Plants

There were no detectable concentrations of RDX found in the excised leaves, blades, or roots.

2.4 Discussion

The reduction of RDX concentrations in soil was expected given past research that has shown the translocation of RDX to plant tissues in many plant species (Just & Schnoor, 2004; Harvey, Fellows, Cataldo, & Bean, 1991; Thompson & Schnoor, 1997; Larson, Jones, Escalon, & Parker, 1999). The objective of the experiment was to verify the reduction in concentration of RDX in native EAFB soil. This objective has been completed with positive results. The result of zero detections in the blade or leaf of the plants contradicts literature which shows RDX translocation primarily to the leaves or blade of the plant (Brentner et al., 2009). Multiple transformation processes observed following the uptake and translocation to the leaves has also been demonstrated (Van Aken, Yoon, Just, & Schnoor, 2004). The analysis method used was only designed for RDX, no RDX metabolites would have been detected.

The lack of RDX degradation in the excised root microcosms suggest the plant exudates and/or root decomposition have little to no effect over a time period of 56 days. The result is supported by work previously completed (Anderson, 2010) where it was found that the naturally occurring microbial communities in EAFB Lakeland soil did not have the capacity to degrade RDX.

Table 2-1. Soil and texture analysis of Lakeland Soil.

Sand (%)	70
Silt (%)	4
Clay (%)	26
Classification	Loamy Sand

Table 2-2. Physical-chemical soil characteristics of Lakeland Soil.

Soil pH	5.1
Buffer pH	6.93
Bulk Density (g/cc)	1.45
Phosphorus (lb/acre)	16 (low)
Potassium (lb/acre)	36 (very low)
Calcium (lb/acre)	512 (med)
Magnesium (lb/acre)	38 (low)
Nitrate Nitrogen (lb/acre)	14
Ammonical Nitrogen (lb/acre)	0
CEC (meq/100g)	1.9
Organic Matter Content (%)	0.4

Figure 2-1. The experimental setup for the RDX microcosm study. Includes three replicates planted in soil from Eglin Air Force Base with Bahiagrass Pensacola, three with excised Bahiagrass Pensacola roots, and three unplanted. All replicates were covered with aluminum foil. Not shown: the poplar and excised poplar root microcosms.

Figure 2-2. Significant Bahiagrass Pensacola root density was achieved by completion of study.

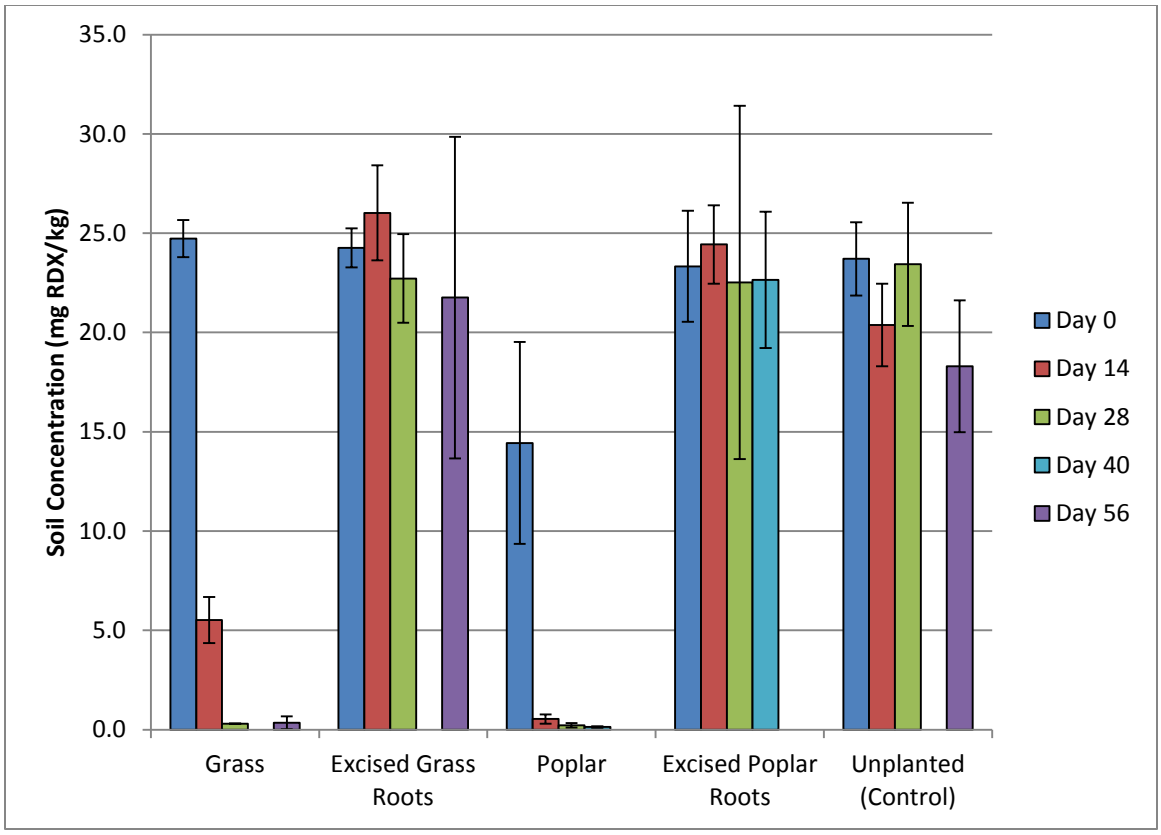


Figure 2-3. RDX concentration in soil of each microcosm over time.

CHAPTER 3
PHYTOREMEDIATION FIELD STUDY FOR THE TREATMENT OF EXPLOSIVE COMPOUNDS AT EGLIN AIR FORCE BASE, FL

3.1 Introduction

The task of maintaining military readiness has caused extensive contamination at military testing and training ranges throughout the United States (Hewitt et al., 2007; Hewitt, Jenkins, Walsh, Walsh, & Taylor, 2005; Jenkins et al., 2006; Walsh et al., 2003). This has been shown to be the case at Range C-62 at EAFB (Brentner, 2008a), the location chosen to demonstrate phytoremediation as a viable strategy for sustainable range management. Phytoremediation is an ideal alternative due to its comparatively low implementation cost and low impact on the environment; other treatment methods often require excavation of soils. Additionally, phytoremediation can be implemented over large areas and can continue to remain active over long periods of time – attributes necessary to meet the needs of a continuously operated testing or training range (Hannink et al., 2002).

It was decided the ideal location for the study would be adjacent to the open burn/open detonation (OB/OD) area on Range C-62 at EAFB (Anderson, 2010). The location of the OB/OD area in reference to the locations of the three field study plots is shown in Figure 3-1. The physical condition and appearance of the open detonation area is shown in Figure 3-2. The area adjacent to the OB/OD site was chosen for the study because the OB/OD area is used routinely for the demolition of unexploded ordinance (UXO). These demolitions, especially those resulting in the low-order detonation of

either the UXO or the Composition 4 (C4) used for detonation, result in widespread contamination of adjacent soils (Hewitt et al., 2007; Clausen et al., 2006).

An initial sampling of the soil adjacent to the OB/OD area found contamination of TNT, RDX, HMX, and TNB (Brentner, 2008a). Of the four compounds present in the soil, RDX is of particular concern due to its high solubility and mobility in the environment as compared to the others. It is also a main component of commonly used explosives including C4 (90% RDX) and Composition B (60% RDX) (Hewitt et al., 2007). The migration of RDX into the groundwater has been observed at the Iowa Army Ammunition Plant and the Massachusetts Military Reservation (Best et al., 1997; Clausen, Robb, Curry, & Korte, 2004). Anderson showed some RDX migration to nearby intermittent streams, possibly from groundwater transport (Anderson, 2010).

3.2 Project Overview

Bahiagrass Pensacola (*Paspalum notatum)* was the plant of choice for the implementation of phytoremediation near the OB/OD area at EAFB. A plant native to the region, it is a warm-season, drought tolerant grass (Jia, Dukes, & Jacobs, 2007) and is extensively used for erosion control at EAFB.

As seen in Figure 3-2, three 0.4 acre plots of Bahiagrass were installed for the study by Travis J. Anderson with collaborators via a contractor (Anderson, 2010). Plot #1, adjacent to the OB/OD site, was expected to have the most contamination. Plot #2, approximately 100 feet down-gradient from the OB/OD site, would have less contamination than Plot #1. Plot #3, approximately 300 feet to the North of the OB/OD site, served as a control, and was to have little to no contamination. The plots were planted with Bahiagrass sod on May 26-27, 2009.

3.3 Site Work and Preparation

The previous investigator on the project, Travis J. Anderson, completed all site preparation work. There was a meeting on Januray 14, 2009 with the Eglin Range Configuration Control Committee (RC3). The meeting briefed the committee on the scope of the phytoremediation project. The Eglin RC3 granted clearance for the planting of vegetation on the three plots near the OB/OD site. The meeting also resulted in ruling out the use of phreatophytic tree species such as poplar due to the risk of disturbing subsurface UXO through the required excavation. The decision was made to plant with Bahiagrass Pensacola sod as opposed to seed because of the faster establishment period, a greater chance of survival, and less chance of disturbing subsurface UXO. The installation of the sod resulted in an organic, nutrient rich soil layer of approximately 2 cm.

As shown in Figure A-1 and Figure A-2 of Appendix A, the Bahiagrass Pensacola was installed on May 26 and 27, 2009. The company hired for landscaping, Three Rivers RC&D, performed the site preparation and sod installation. A water truck was used for an initial watering in order to establish the sod. As shown in Figure B-1 of Appendix B, June and July had lower than average precipitation which delayed establishment and growth until August and September 2009 (Anderson, 2010). The proof of successful establishment is shown in Figure 3-3. The initial installation as well as the condition of Plot #1 over the duration of the study is shown in Figure A-1 through Figure A-9 in Appendix A. By the completion of the study, the root depth was approximately 6 inches and a blade height of approximately 10 inches.

3.4 Sampling Method

Sampling was conducted on each plot according to the systematic random sampling method. The strategy and design behind this method is to obtain soil sample increments positioned at collection points that are distributed relatively evenly throughout the sampling area (Hewitt et al., 2007). This was necessary due to the heterogeneity associated with explosive contamination on military ranges (Jenkins et al., 2006). This method, applied to the plots at EAFB, results in 100 discrete soil and plant samples from three separate passes over each plot in the planted region as shown in Figure 3-4. In addition, 40 discrete soil samples were obtained from a perimeter offset 12 feet from the edge of the planted region every 15 feet in order to serve as an unplanted control. During the May 26-27, 2009 sampling, the sampling method was applied to all three plots resulting in 140 soil samples and 100 plant samples per plot. In all subsequent samplings, only plot #1 was sampled to the full extent because of the small number of detections at the other two plots. Due to time constraints and lack of detections, samples were pared to only 34 discrete soil and plant samples for plots #2 and #3 along with an additional 14 discrete soil samples taken every 45 feet around the perimeter. The samples collected at plots #2 and #3 were still sampled according to the systematic random sampling method. As seen in Figure 3-4, only the first pass was completed.

The May 26-27, 2009 sampling was immediately prior to the planting of the Bahiagrass Pensacola. Therefore, the May 26-27, 2009 sampling should be considered the "time equals zero" sampling. Subsequent sampling occurred at 6 months (November 18-19, 2009), 12 months (May 24-25, 2010), and 18 months (November 13-14, 2010).

The four samplings resulted in 1,111 soil samples and 487 plant samples which were used to characterize each plot over the course of the study.

The soil samples were retrieved using a 2 cm diameter steel soil corer at a depth of 5 cm and were then placed in a re-sealable plastic bag. As shown in Figure 3-5, the soil cores were actually taken at a depth of 7 cm, but the top 2 cm was removed to avoid sampling the sod in which the Bahiagrass was initially established. The steel soil corer was wiped down with paper towels between samplings in order to minimize cross-contamination. Pruning shears were utilized to take cuttings of the Bahiagrass at each soil sampling location in the planted region of each plot.

3.5 Materials and Methods

3.5.1 Explosives Extraction from Soil

The extraction of explosives from soil was performed according to a modified version of EPA Method 8330B (USEPA, 2006). The soil was left to dry at room temperature until a constant weight was achieved. A mortar and pestle were used to crush the soil into fine grains. Two grams of soil was placed in a 15 mL vial with 10 mL of acetonitrile for the extraction of the energetic compounds. The vials were then placed in an ultrasonic bath for 18 hours. Samples were filtered with 0.20 µm Durapore© membrane filters. The sample filtrate was then used for analysis.

3.5.2 Explosives Extraction from Plant Material

The extraction of explosives from plant tissue was performed according to a modified version of EPA Method 8330B (USEPA, 2006). A sample of plant material was crushed and homogenized using a mortar, pestle, and liquid nitrogen. Two grams of

plant material was placed in a 15 mL vial with 10 mL of acetonitrile for the extraction of the energetic compounds. The vials were then placed in an ultrasonic bath for 18 hours. Samples were filtered with 0.20 µm Durapore© membrane filters. The sample filtrate was used for analysis.

3.5.3 Chemical Analyses

The explosives extracted from soil were analyzed using HPLC (HP Series 1100; Hewlett-Packard, Palo Alto, CA) using an Acclaim® Explosives E1 column (Dionex Corporation). The samples were analyzed with a mobile phase of methanol:deionized water 43:57 v/v at a flow rate of 1.0 mL/min. Detections were measured at a UV absorbance of 230 nm and 254 nm using a UV visible photodiode array detector (HP Series 1100).

Explosives extracted from plant samples were analyzed using liquid chromatography-mass spectrometry (LC/MS). An Acclaim Explosives E2 column (2.1 x 150 mm, 3µm; Dionex Corporation) was used on an Agilent 6140 Quadrupole LC/MS. The mass spectrometer was operated in negative-ion electrospray mode. A mobile phase of 2mM ammonium acetate in methanol:deionized water 48:52 v/v at a flow rate of 0.3 mL/min.

In addition to analysis using HPLC, soil samples from May 24-25, 2010 and November 13-14, 2010 were analyzed with LC/MS. This was a quality assurance measure to verify the detections and concentrations found using HPLC.

Calibration curves were constructed using standards before and after each batch of samples were run in order to ensure quality. Standards were also placed after every ten sample vials in order to verify retention times and elution order. The standards, EPA

8330-R Explosives Mix, was ordered from AccuStandard (New Haven, CT). The explosives mix included the following components: 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), nitrobenzene (NB), tetryl, TNT, RDX, and HMX. The standards were used to identify and quantify explosives in samples.

While HPLC was able to detect all of the explosives in the mixture, the method used for analysis with LC/MS was only able to detect TNT, RDX, HMX, 2-ADNT, 4-ADNT, 2,4-DNT, 2,6-DNT, TNB, and Tetryl.

3.6 Results

3.6.1 Plots #2 and #3

568 discrete soil samples and 336 discrete plant samples from plots #2 and #3 were analyzed over the course of the four samplings. One detection was found in both plots #2 and #3 during the May 26-27, 2009 sampling and four detections were found in the plots during the November 13-14, 2010 sampling.

3.6.2 Soil Analyzed by HPLC

The mean concentrations and standard deviations of each constituent detected by HLPC for all four samplings are given in Table 3-1 and Table 3-2. The means include non-detects as half of the limit of detection. Figure 3-17 and Figure 3-18 show the comparison explosive compound and metabolites mean concentrations for each sampling in the planted and unplanted regions of Plot #1.

3.6.2.1 May 26-27, 2009

The detections of explosive compounds in Plot #1 are shown in Figure 3-6 for the May 26-27, 2009 sampling. As seen in Figure 3-6, RDX and HMX were the two compounds most commonly detected and were also often found in the same discrete sample. Of the 100 discrete soil samples in the planted region of Plot #1, there were 18 detections of HMX ranging in concentration from 0.04 to 5.78 mg/kg, 30 detections of RDX ranging from 0.06 to 3.71 mg/kg, 3 detections of TNT ranging from 0.11 to 0.23 mg/kg, and 8 detections of TNT metabolites ranging from 0.10 to 29.91 mg/kg. Of the 40 discrete soil samples in the unplanted region, there were 12 detections of HMX ranging in concentration from 0.05 to 15.79 mg/kg, 13 detections of RDX ranging from 0.07 to 154.30 mg/kg, 2 detections of TNT from 0.12 to 0.21 mg/kg, and 7 detections of TNT metabolites ranging from 0.19 to 15.02 mg/kg.

3.6.2.2 November 18-19, 2009

The detections of explosive compounds in Plot #1 are shown in Figure 3-7 for the November 18-19, 2009 sampling. Again, the two most common compounds detected were RDX and HMX. Of the 100 discrete soil samples in the planted region of Plot #1, there were 22 detections of HMX ranging in concentration from 0.04 to 3.35 mg/kg, 33 detections of RDX ranging from 0.05 to 17.13 mg/kg, 3 detections of TNT ranging from 0.05 to 0.46 mg/kg, and 5 detections of TNT metabolites ranging from 0.04 to 0.50 mg/kg. Of the 40 discrete soil samples in the unplanted region, there were 14 detections of HMX ranging in concentration from 0.04 to 0.42 mg/kg, 19 detections of RDX ranging from 0.04 to 4.12 mg/kg, 1 detection of TNT at 0.04 mg/kg, and 3 detections of TNT metabolites ranging from 0.05 to 1.28 mg/kg.

3.6.2.3 May 24-25, 2010

The detections of explosive compounds in Plot #1 are shown in Figure 3-8 for the May 24-25, 2010 sampling. RDX and HMX were again the two most commonly detected compounds. From the 100 discrete soil samples taken, the planted region of Plot #1 had 18 detections of HMX ranging in concentration from 0.02 to 6.16 mg/kg, 13 detections of RDX ranging from 0.02 to 2.06 mg/kg, 7 detections of TNT ranging from 0.04 to 0.13 mg/kg, and 8 detections of TNT metabolites ranging from 0.07 to 1.71 mg/kg. Of the 40 discrete soil samples in the unplanted region, there were 15 detections of HMX ranging in concentration from 0.05 to 2.97 mg/kg. There were no detections of RDX, TNT, or TNT metabolites in the unplanted region during the May 24-25, 2010 sampling.

3.6.2.4 November 13-14, 2010

The detections of explosive compounds in Plot #1 are shown in Figure 3-9 for the May 24-25, 2010 sampling. HMX was the most commonly detected compound. Of the 100 discrete soil samples in the planted region of Plot #1, there were 4 detections of HMX ranging in concentration from 0.05 to 0.45 mg/kg, 3 detections of RDX ranging from 0.11 to 0.50 mg/kg, and 3 detections of TNT metabolites ranging from 0.80 to 5.13 mg/kg. Of the 40 discrete soil samples in the unplanted region, there were 8 detections of HMX ranging in concentration from 0.23 to 0.69 mg/kg, 1 detection of RDX at 0.84 mg/kg, and 4 detections of TNT metabolites ranging from 0.73 to 10.50 mg/kg. There were no detections of TNT in the planted or unplanted region during the November 13-14, 2010 sampling.

3.6.3 Soil Analyzed by LC/MS

The mean concentrations and standard deviations of each constituent detected by LC/MS for all four samplings are given in Table 3-3. Figure 3-25 and Figure 3-26 show the comparison of explosive compound and metabolite mean concentrations for the May 24-25, 2010 and November 13-14, 2010 samplings in the planted and unplanted regions of Plot #1.

3.6.3.1 May 24-25, 2010

The detections of explosive compounds in Plot #1 are shown in Figure 3-10 for the May 24-25, 2010 sampling. HMX and RDX were the most commonly detected compounds. Of the 100 discrete soils samples in the planted region of Plot #1, there were 72 detections of HMX ranging in concentration from 0.002 to 8.161 mg/kg, 26 detections of RDX ranging from 0.03 to 1.87 mg/kg, and 34 detections of TNT metabolites (predominantly 2-ADNT; 4-ADNT) ranging from 0.003 to 1.821 mg/kg. Of the 40 discrete soil samples in the unplanted region, there were 35 detections of HMX ranging in concentration from 0.002 to 0.846 mg/kg, 12 detections of RDX ranging from 0.03 to 0.33 mg/kg, and 18 detections of TNT metabolites (exclusively 2-ADNT; 4-ADNT) ranging from 0.003 to 0.016 mg/kg. There were no detections of TNT in the planted or unplanted region during the May 24-25, 2010 sampling.

3.6.3.2 November 13-14, 2010

The detections of explosive compounds in Plot #1 are shown in Figure 3-11 for the November 13-14, 2010 sampling. HMX was the most commonly detected compound. Of the 100 discrete soil samples in the planted region of Plot #1, there were 54 detections of HMX ranging in concentration from 0.003 to 0.381 mg/kg, 3 detections

of RDX ranging from 0.10 to 0.75 mg/kg, 2 detections of TNT ranging from 0.05 to 0.10 mg/kg, and 17 detections of TNT metabolites ranging from 0.006 to 2.405 mg/kg. Of the 40 discrete soil samples in the unplanted region, there were 27 detections of HMX ranging in concentration from 0.004 to 0.790 mg/kg and 13 detections of TNT metabolites ranging from 0.002 to 4.654 mg/kg. There were no detections of TNT or RDX in the unplanted region during the November 13-14, 2010 sampling.

3.6.4 Plants Analyzed by LC/MS

3.6.4.1 November 18-19, 2009

The May 26-27, 2009 sampling was completed before the Bahiagrass Pensacola sod was installed, therefore the first sampling of plants was completed during the November 18-19, 2009 sampling. The detections of explosive compounds found in the discrete plant samples taken from Plot #1 are shown in Figure 3-12 for the November 18-19, 2009 sampling. HMX and RDX were the only two compounds found in the plants. Of the 100 discrete plant samples, there were 3 detections of HMX ranging in concentration form 0.001 to 0.002 mg/kg and 22 detections of RDX ranging from 0.003 to 0.049 mg/kg.

3.6.4.2 May 24-25, 2010

The detections of explosive compounds found in the discrete plant samples taken from Plot #1 are shown in Figure 3-13 for the May 24-25, 2010 sampling. HMX and RDX were the only two compound found in the plants. Of the 100 discrete plant samples, there were 6 detections of HMX ranging in concentration from 0.033 to 0.670 mg/kg and 5 detections of RDX ranging from 0.014 to 0.122 mg/kg.

3.6.4.3 November 13-14, 2010

The detection of an explosive compound found in the discrete plant samples taken from Plot #1 is shown in Figure 3-14 for the November 13-14, 2010 sampling. Of the 100 discrete plant samples, HMX was the only compound detected at a concentration of 0.141 mg/ kg.

3.7 Discussion

3.7.1 Explosives in Soil Samples Analyzed by HPLC

Figure 3-6 through Figure 3-9 show the detections of TNT, metabolites of TNT, RDX, or HMX in soil from analysis with HPLC. From these figures, it can be seen that the overall trend is toward fewer detections in both the planted and unplanted regions of Plot #1 for all compounds.

3.7.1.1 TNT plus Metabolites

Figure 3-15 and Figure 3-16 show the frequency histogram of TNT and TNT metabolite concentrations by HPLC analysis in the planted and unplanted regions, respectively, for the four samplings between May 26-27, 2009 and November 13-14, 2010. The most commonly detected TNT metabolites were 2-ADNT, 4-ADNT, 2,4-DNT, and 2,6-DNT. 2-ADNT and 4-ADNT have been shown to be formed from the aerobic reduction of TNT by plants (Hannink et al., 2002). 2,4-DNT and 2,6-DNT have also been observed as TNT metabolites (Schneider et al., 1996; Thompson et al., 1998).

Until the November 13-14, 2010 sampling, the TNT plus metabolite concentrations appear to be trending towards more detections at lower concentrations in the planted region of Plot #1 (see Figure 3-15). However, the November 13-14, 2010

results show only three detections, all of which are relatively greater in concentration compared to the majority of previous detections. In the unplanted region, the trend again appears to be greater detections at low concentrations over time (see Figure 3-16). There were no detections during the May 24-25, 2010 and November 13-14, 2010 samplings.

In Figure 3-17, which provides a better breakdown of TNT and each metabolite, it can be seen that the mean TNT concentration remains relatively constant in the planted region. The figures depicting mean concentrations of the contaminants were constructed by including all non-detections as one-half the limit of detection. Due to the high number of non-detects and the relative greater concentration found in the samples where explosives were detected, the standard deviation of the means are very high as seen in Table 3-1 through Table 3-3. Even with the high standard deviations, the mean concentrations are still useful in the comparison of the mean concentration to half of the limit of detection (i.e. if the mean concentration of the contaminant is equal to half the limit of detection, little or no detections were found of significant concentration in the given sampling). Figure 3-17 depicts a high mean concentration of 2,4-DNT in the initial May 26-27, 2009 sampling, the November 13-14, 2010 sampling showed little to no presence of TNT metabolites, and the May 24-25, 2010 and November 13-14, 2010 samplings showed modest increases in the mean concentrations of 2,4-DNT and 2-ADNT. Figure 3-18 shows similar trends in the unplanted region: the mean TNT concentration remains relatively constant, a large mean concentration of 2,4-DNT is found in the initial sampling, and the mean metabolite concentrations increase in the final sampling.

These results may suggest the microbial communities in the soil are successfully degrading the compound since the TNT concentrations are remaining relatively constant throughout the study in both the planted and unplanted regions and the TNT metabolite concentrations are increasing. This result would confirm previous work accomplished in the laboratory, which showed 88% to 89% reduction in TNT concentrations over 28 days and 93% to 97% reduction over 56 days in unplanted Lakeland soil (Anderson, 2010). It is unclear if the implementation of phytoremediation is enhancing this process because rates could not be determined for the planted versus unplanted regions of Plot #1 due to the high number of samples below the limit of detection. This would greatly sway any mean, median, or parametric statistical analysis performed.

Although phytoremediation may not be enhancing the degradation or transformation of TNT, it appears that the organic carbon associated with plant roots and sod may be slowing the migration of TNT and metabolites. The mobility of TNT should decrease in the presence of organic carbon due to its affinity to partition to it as shown by the partition coefficients presented in Table 1-1 of Chapter 1. The decrease in mobility is shown through the comparison of detections of the planted and unplanted regions of the May 24-25, 2010 and November 13-14, 2010 samplings, exhibited in Figure 3-15 and Figure 3-16. Though the number of detections cannot be compared directly because of the differing frequency at which the samples were taken from each region (100 samples from the planted and 40 samples from the unplanted), it is clear that the fraction of detections to overall samples differ greatly in the planted and unplanted regions during the May 24-25, 2010 and November 13-14, 2010 samplings. The figures show that in the planted region sampled in May 24-25, 2010, 15% of the samples were above the limit of

detection, whereas in the unplanted region, none of the samples were above the limit of detection. This was again seen in the November 13-14, 2010 sampling. In the planted region, 3% of the samples were above the limit of detection whereas in the unplanted region, none of the samples were above the limit of detection.

3.7.1.2 RDX

Figure 3-19 and Figure 3-20 show the frequency histogram of RDX concentrations by HPLC analysis in the planted and unplanted regions, respectively, for the four samplings between May 26-27, 2009 and November 13-14, 2010. In the planted region, the trend between the May 26-27, 2009 and November 18-19, 2009 was a greater number of detections at lower concentrations. Between November 18-19, 2009 and May 24-25, 2010 the number of detections in the planted region decreased from 33 detections to 13 detections and finally to 3 detections in November 13-14, 2010 (see Figure 3-19). In the unplanted region of Plot #1, the same trend is followed. There were a greater number of detections at lower concentrations between May 26-27, 2009 and November 18-19, 2009. Between November 18-19, 2009 and May 24-25, 2010 the number of detections decreased from 19 to zero detections and the final sampling in November 13-14, 2010 resulted in only 1 detect. This trend is also seen in the mean RDX soil concentrations in the planted and unplanted regions shown in Figure 3-17 and Figure 3-18, respectively.

A general linear model was applied to the data in order to determine if plant type (planted or unplanted), time (sampling date), or a combination of the two had a statistically significant effect on RDX concentration in the soil of Plot #1. Non-detections were not included because no parametric test can be performed due to the lack

of normality in the distribution of the data as seen in Figure C-1 and Figure C-2. The results of the model are shown in Figure C-3 of Appendix C. The results show that time was statistically significant (P-value < 0.001) in the reduction of RDX concentrations in the soil while the plant type (unplanted or planted) had no significance.

Since the number of detections as well as concentrations is decreasing in both the planted and unplanted regions, it is believed that the RDX is migrating downward and into the groundwater faster than the Bahiagrass Pensacola plants can translocate the compound. This is conceivable given the solubility of RDX in water (Clausen et al., 2006) and its mobility in the environment (Dontsova, Yost, Simunek, Pennington, & Williford, 2006). Also, between the November 18-19, 2009 and May 24-25, 2010 sampling, EAFB received its 5th wettest December on record and its 6th wettest January on record as shown by Figure B-1 and Figure B-2 of Appendix B. These record rainfalls would have occurred while the Bahiagrass Pensacola was dormant, allowing for very little active uptake and phytoremediation to occur. In addition, Plot #1 was ignited on March 15, 2010 by the active use of the adjacent OB/OD site as shown in Figure A-7 of Appendix A. It is not believed that the grass fire had an effect on the reduction in RDX concentration in Plot #1. The soil sample is taken 2 cm below the ground surface. It is unlikely that a grass fire could become hot enough to combust underlying explosive compounds.

Additionally, the results of the field study are counter to those found in the laboratory study as described in Chapter 2. In the laboratory study, the RDX soil concentrations were reduced by 98.6% after 56 days in the planted Bahiagrass microcosm while the unplanted control saw no reductions. The difference in results is likely due to

the laboratory microcosms being kept moist, yet unsaturated in order to keep the system aerobic. This led to little to no flow-through of water, which did not allow for the migration of RDX. This allowed for a much longer contact time between the roots and the soil and water and therefore led to greater treatment. In order to better represent the natural system, it is recommended that future laboratory studies incorporate flow-through. The mass of RDX in the flow-through water, the soil, and the plant material would better represent the processes occurring at EAFB.

The reduction in mobility due to the presence of organic carbon via the sod and root layer was again seen for RDX. The mobility of RDX should decrease in the presence of organic carbon due to its affinity to partition to it, although not to the degree of TNT, as shown by the partition coefficients presented in Table 1-1 of Chapter 1. The decrease in mobility is shown through the comparison of detections of the planted and unplanted regions of the May 24-25, 2010 and November 13-14, 2010 samplings, exhibited in Figure 3-19 and Figure 3-20. Though the number of detections cannot be compared directly because of the differing frequency at which the samples were taken from each region (100 samples from the planted and 40 samples from the unplanted), it is clear that the fraction of detections to overall samples differ in the planted and unplanted regions during the May 24-25, 2010 and November 13-14, 2010 samplings. The figures show that in the planted region sampled in May 24-25, 2010 13% of the samples were above the limit of detection, whereas in the unplanted region, none of the samples were above the limit of detection. This was again seen in the November 13-14, 2010 sampling. However, there were fewer detections in both the planted and unplanted region, so the comparison is not as drastic. In the planted region, 3% of the samples were above the

limit of detection whereas in the unplanted region, approximately 2% of the samples were above the limit of detection.

3.7.1.3 HMX

Figure 3-17 and Figure 3-18 show the mean HMX soil concentrations by HPLC analysis in the planted and unplanted regions, respectively, for the four samplings between May 26-27, 2009 and November 13-14, 2010. From Figure 3-17 and Figure 3-18, the figures show no discernible trend in both the planted and unplanted regions of Plot #1 from the mean concentrations of HMX in soil. The mean concentrations oscillate over the course of the four samplings.

Figure 3-21 and Figure 3-22 are histograms showing the distribution of HMX concentrations in soil in the planted and unplanted regions of Plot #1. In the planted region there appears to be a trend of greater detections at lower concentrations between the May 26-27, 2009 and November 18-19, 2009 samplings (see Figure 3-21). Between the May 24-25, 2010 and November 13-14, 2010 samplings, there are fewer detections. The detections are at lower concentrations when compared to the initial sampling. In the unplanted region, the trend of greater detections at lower concentrations extends from May 26-27, 2009 to May 24-25, 2010. The November 13-14, 2010 results are distributed differently than the other three samplings. Unlike the other three samplings, the November 13-14, 2010 had no detections less than 0.2 mg/kg (see Figure 3-22).

A general linear model was applied to the data in order to determine if plant type (planted or unplanted), time (sampling date), or a combination of the two had a statistically significant effect on HMX concentration in the soil of Plot #1. Non-detections were not included because no parametric test can be performed due to the lack

of normality in the distribution of the data as seen in Figure C-4 and Figure C-5. The results of the model are shown in Figure C-6 of Appendix C. The results show that time was statistically significant (P-value = 0.011) in the reduction of HMX concentrations in the soil while the plant type had no significance.

While HMX is much more recalcitrant due to its poor solubility compared to TNT and RDX, it is somewhat mobile once solubilized and has been shown to accumulate in leaves (Hannink et al., 2002; Groom et al., 2002; Yoon et al., 2002; Yoon et al., 2006). However, the same trend was observed as with RDX. More detections of HMX were observed at lower concentration over the study in both the planted and unplanted regions of Plot #1. Therefore, phytoremediation using Bahiagrass Pensacola must not be having a substantial effect on the reduction of HMX concentrations in the soil. The evidence points to HMX migrating downward in profile and diluting the soil concentration.

The organic carbon found in the sod and root zone did not appear to have an effect on the mobility of HMX. This is surprising given that the partition coefficients, found in Table 1-1 of Chapter 1, are of the same order of magnitude as those for RDX, which did appear to be hindered by the presence of organic carbon. The differing behavior may be caused by the decreased mobility of HMX due to its decreased solubility as compared to TNT and RDX.

3.7.2 Explosives in Soil Samples Analyzed by LC/MS

Figure 3-10 and Figure 3-11 show the detections of TNT, metabolites of TNT, RDX, or HMX in soil from analysis with LC/MS. From these figures, it can be seen that the general trend is toward fewer detections in both the planted and unplanted regions of Plot #1. The main purpose of the analysis with LC/MS was to reaffirm detections and

concentrations of explosive compounds in the soil. Certainty in trends cannot be determined with only two samplings analyzed.

3.7.2.1 TNT plus Metabolites

Figure 3-23 and Figure 3-24 show the frequency histogram of TNT and TNT metabolite concentrations by LC/MS analysis in the planted and unplanted regions, respectively, for the samplings in May 24-25, 2010 and November 13-14, 2010. The two figures show a trend of an increase towards greater TNT plus metabolite concentrations. Figure 3-25 and Figure 3-26 also show that the mean TNT concentration remained approximately the same as the mean concentration of TNT metabolites increased in both the planted and unplanted regions of Plot #1. This was also the case with the HPLC results. Therefore, the same conclusion is reached: microbial communities in the soil are successfully transforming the TNT. It is again unclear if the implementation of Bahiagrass Pensacola for phytoremediation is having an effect on the rate of transformation because the process is occurring in both the planted and unplanted regions.

3.7.2.2 RDX

Figure 3-27 and Figure 3-28 show the frequency histogram of RDX concentrations by LC/MS analysis in the planted and unplanted regions, respectively, for the May 24-25, 2010 and November 13-14, 2010 samplings. As seen in both figures, there is no clear change in distribution between the two samplings. Fewer detections were observed in both the planted and unplanted regions of Plot #1 during the November 13-14, 2010 sampling compared to the May 24-25, 2010 sampling. This follows the

same trend observed in the HPLC data indicating that the RDX is migrating downward in both the planted and unplanted regions.

A general linear model was applied to the data in order to determine if plant type (planted or unplanted), time (sampling date), or a combination of the two had a statistically significant effect on RDX concentrations in the soil of Plot #1. Non-detections were not included because no parametric test can be performed due to the lack of normality in the distribution of the data as seen in Figure C-7 and Figure C-8. The results of the model are shown in Figure C-9 of Appendix C. The results show that neither time nor plant type was statistically significant in the reduction of RDX concentrations in soil according to the LC/MS data.

From the histograms shown in Figure 3-27 and Figure 3-28, it is believed that the RDX is migrating downward into the groundwater faster than the Bahiagrass Pensacola plants are able to uptake the compound. This same conclusion was reached from the analysis of the HPLC data.

3.7.2.3 HMX

Figure 3-29 and Figure 3-30 show the frequency histograms of HMX concentrations by LC/MS analysis in the planted and unplanted regions, respectively, for the May 24-25, 2010 and November 13-14, 2010 samplings. In the planted region, there appears to be a trend towards greater detections at lower concentrations when comparing the May 24-25, 2010 and November 13-14, 2010 samplings. In the unplanted region, the trend of greater detections at lower concentrations is not apparent. In fact, there appears to be more detections of higher concentrations. The reduction of HMX concentrations in the planted region and the increase of HMX concentrations in the unplanted region are

also shown by the mean HMX soil concentrations in Figure 3-25 and Figure 3-26, respectively.

A general linear model was applied to the data in order to determine if plant type (planted or unplanted), time (sampling date), or a combination of the two had a statistically significant effect on HMX concentrations in the soil of Plot #1. Non-detections were not included because no parametric test can be performed due to the lack of normality in the distribution of the data as seen in Figure C-10 and Figure C-11. The results, shown in Figure C-12 of Appendix C, show that the plant type crossed with time is statistically significant (P-value = 0.003). This means that the planted and unplanted regions of Plot #1 had a statistically significant impact on the change in concentration of HMX in soil over time.

The results shown in Figure 3-29 and Figure 3-30, Figure 3-25 and Figure 3-26, and Figure C-12 all indicate that implementation of Bahiagrass Pensacola for phytoremediation is indeed reducing HMX concentrations in soil in the planted region of Plot #1. This conclusion differs from the one reached following the analysis of the data from samples analyzed using HPLC. The conclusions may be different because of the much higher sensitivity that LC/MS has to HMX as compared to HPLC.

As shown in Table 3-1 and Table 3-3, the limit of detection of HMX using LC/MS and HPLC are 0.002 and 0.04 mg/kg, respectively. The higher sensitivity provides a greater ability to detect trends in the data given the widespread contamination of HMX at low concentrations in Plot #1. However, only two samplings were analyzed using LC/MS. In order to prove that the trend holds true, at least one more sampling should be analyzed. If the Bahiagrass Pensacola is reducing HMX concentrations in the

soil of Plot #1, it is likely that the HMX is translocating to the blades without any transformation. Leaching of HMX from dying or dead plant material has also been observed (Yoon et al., 2002). This is a cause for concern if phytoremediation is to be implemented for the treatment of HMX.

3.7.3 Explosives in Plant Samples

Figure 3-12 through Figure 3-14 show the detections of HMX and RDX in the discrete plant samples taken during the November 18-19, 2009 through November 13-14, 2010 samplings. The general trend is toward fewer detections in the plants of Plot #1. In November 18-19, 2009 there were 22 detections of RDX and 3 detections of HMX. Between the November 18-19, 2009 and May 24-25, 2010, Plot #1 burned down due to detonations of UXO at the adjacent OB/OD site on March 15, 2010 as shown in Figure A-7. The vegetation was substantially re-established by the May 24-25, 2010 sampling as shown in Figure A-8. However, any explosives accumulated in the grass from the November 18-19, 2009 sampling to March 15, 2010 would not have been observed in the May 24-25, 2010 sampling. The May 24-25, 2010 sampling resulted in 5 detections of RDX and 6 detections of HMX. In November 13-14, 2010, there was only 1 detection of HMX. The results suggest RDX is migrating downward faster than the plants can uptake the contaminant which would support the conclusions drawn from the HPLC data for RDX in sections 3.7.1.2 on page 35. There were too few detections of HMX to determine a trend.

Though the detection of explosive compounds in Bahiagrass was significant because it is the first time uptake and translocation of RDX and HMX has been documented during a phytoremediation field study on military ranges, the fraction of total

mass found in Bahiagrass as compared to that found in soil is almost insignificant as shown in Table 3-4. Several assumptions were made in order to determine the mass fractions. One assumption was that the depth of contamination was 5 cm deep – a property of soil contaminated with explosives as described in published material (Hewitt et al., 2007). The amount of plant material present at each sampling was estimated to be 0.1 tons per acre. The mean soil concentration of each contaminant used to calculate the total mass in the soil and grass included non-detects as half the limit of detection.

Table 3-1. Mean and standard deviation from HPLC analysis of explosive compound and metabolite detections in Plot #1 during the May and November 18-19, 2009 sampling. The analysis included non-detect samples as half the value of the limit of detection.

	LOD/2	May 26-27, 2009				November 18-19, 2009			
		Planted		Unplanted		Planted		Unplanted	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
HMX	0.02	0.13	0.60	0.46	2.49	0.07	0.34	0.06	0.09
RDX	0.08	0.23	0.54	4.08	24.37	0.30	1.71	0.27	0.70
TNT	0.10	0.11	0.01	0.11	0.02	0.11	0.04	0.10	0.01
TNB	0.05	0.06	0.03	0.08	0.15	0.06	0.05	0.05	0.00
2-ADNT	0.16	0.16	0.01	0.16	0.00	0.16	0.00	0.16	0.00
4-ADNT	0.21	0.21	0.00	0.21	0.01	0.21	0.00	0.21	0.00
2,4-DNT	0.09	0.44	3.02	0.49	2.36	0.10	0.01	0.12	0.19
2,6-DNT	0.15	0.17	0.17	0.17	0.13	0.15	0.00	0.15	0.00

Table 3-2. Mean and standard deviation from HPLC analysis of explosive compound and metabolite detections in Plot #1 during the May and November 13-14, 2010 sampling. The analysis included non-detect samples as half the value of the limit of detection.

	LOD/2	May 24-25, 2010				November 13-14, 2010			
		Planted		Unplanted		Planted		Unplanted	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
HMX	0.02	0.13	0.63	0.12	0.46	0.03	0.06	0.09	0.16
RDX	0.08	0.13	0.24	0.08	0.00	0.09	0.05	0.10	0.12
TNT	0.10	0.10	0.01	0.10	0.00	0.10	0.00	0.10	0.00
TNB	0.05	0.05	0.00	0.05	0.00	0.05	0.00	0.05	0.00
2-ADNT	0.16	0.16	0.00	0.16	0.00	0.22	0.50	0.46	1.64
4-ADNT	0.21	0.21	0.00	0.21	0.00	0.21	0.00	0.21	0.00
2,4-DNT	0.09	0.11	0.16	0.09	0.00	0.12	0.24	0.23	0.73
2,6-DNT	0.15	0.15	0.01	0.15	0.00	0.15	0.00	0.15	0.00

Table 3-3. Mean and standard deviation from LC/MS analysis of explosive compound and metabolite detections in Plot #1 during the May and November 13-14, 2010 sampling. The analysis included non-detect samples as half the value of the limit of detection.

	LOD/2	May 24-25, 2010				November 13-14, 2010			
		Planted		Unplanted		Planted		Unplanted	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
HMX	0.001	0.153	0.832	0.041	0.133	0.021	0.055	0.069	0.161
RDX	0.079	0.123	0.227	0.085	0.052	0.087	0.069	0.079	0.000
TNT	0.005	0.005	0.000	0.005	0.000	0.006	0.010	0.005	0.000
TNB	0.026	0.027	0.010	0.026	0.000	0.027	0.012	0.026	0.000
2-ADNT	0.005	0.007	0.007	0.005	0.002	0.007	0.011	0.007	0.008
4-ADNT	0.003	0.006	0.010	0.004	0.004	0.003	0.002	0.005	0.006
2,4-DNT	0.009	0.009	0.000	0.009	0.000	0.015	0.047	0.031	0.143
2,6-DNT	0.100	0.117	0.172	0.100	0.000	0.147	0.300	0.214	0.720

Table 3-4. Percent total mass of RDX and HMX in the soil and plant material. Assumptions include a contaminated depth of 5 cm and 0.1 ton per acre of growth. Non-detects were included in means as half the limit of detection.

	$M_{T,RDX}$		$M_{T,HMX}$	
	Soil	Grass	Soil	Grass
November 2009	99.993%	0.007%	99.9996%	0.0004%
May 2010	99.981%	0.019%	99.996%	0.004%
November 2010	99.974%	0.026%	99.998%	0.002%

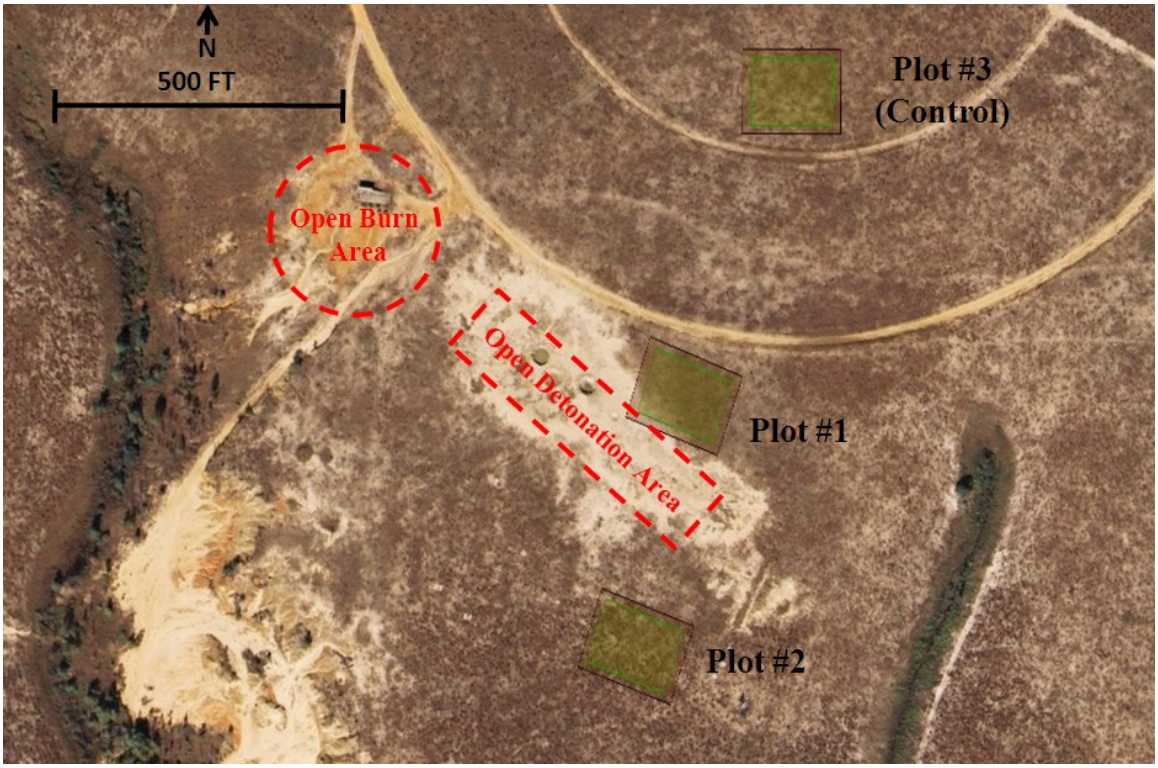


Figure 3-1. Location of Plots #1-3 and the OB/OD areas.

Source: Anderson, T. J. (2010). Phytoremediation of energectic compound at Eglin Air Force Base. Master's Thesis, University of Iowa;

W. Pizzolato, personal correspondence, 2008.

Figure 3-2. Craters at the OB/OD site caused by UXO detonations on November 13, 2010. Photo taken by Matthew B. Flannigan.

Figure 3-3. Bahiagrass Pensacola root depth (6 in) and blade height (10 in) on November 13, 2010. Photo taken by Matthew B. Flannigan. In this photo: Kat Williams.

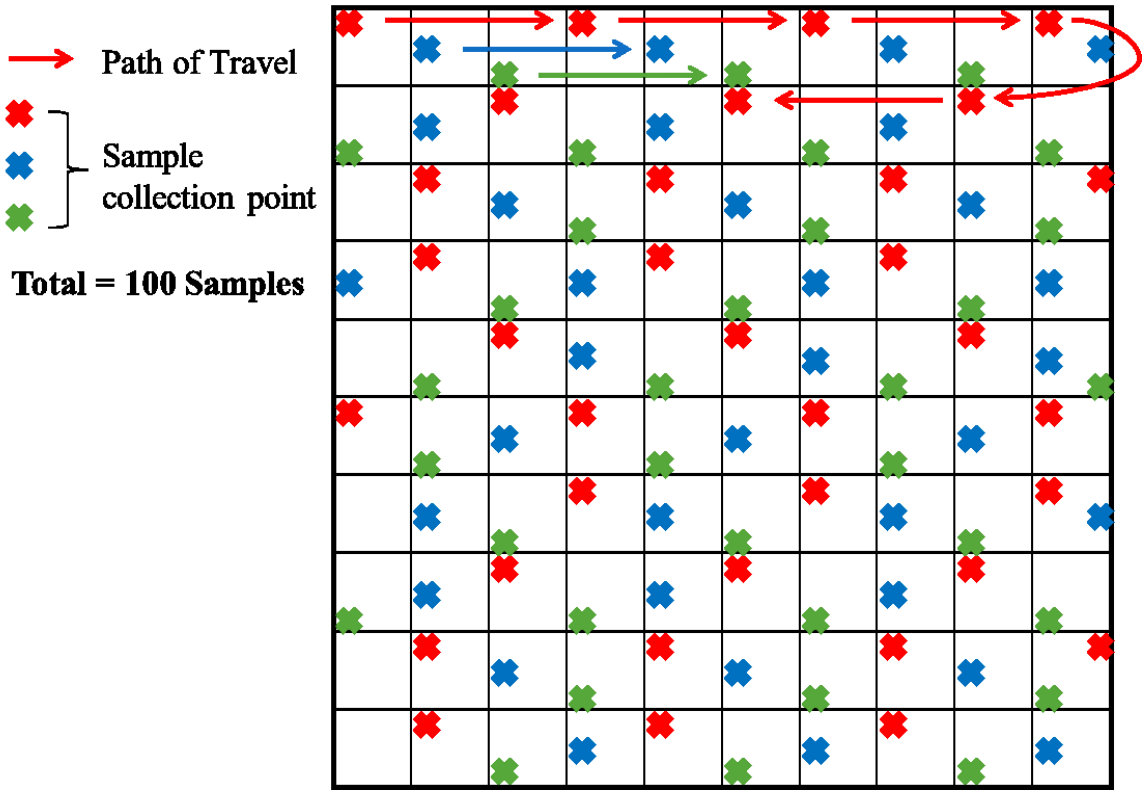


Figure 3-4. Systematic random sampling method used for plots #1-3. The entire 100 samples were taken at Plots #2 and #3 for only the May 26-27, 2009 sampling. Each subsequent sampling only the first 34 were retrieved.

Source: Anderson, T. J. (2010). Phytoremediation of energetic compound at Eglin Air Force Base. Master's Thesis, University of Iowa;

Jenkins, T. F., Hewitt, A. D., Grant, C. L., Thiboutot, S., Ampleman, G., Walsh, M. E., et al. (2006). Identity and distribution of residues of energetic compounds at army live-fire training ranges. [Article]. Chemosphere, 63(8), 1280-1290.

Figure 3-5. Soil corer used to collect Lakeland soil samples at the Range C-62 site on November 13, 2010. Photo taken by Matthew B. Flannigan.

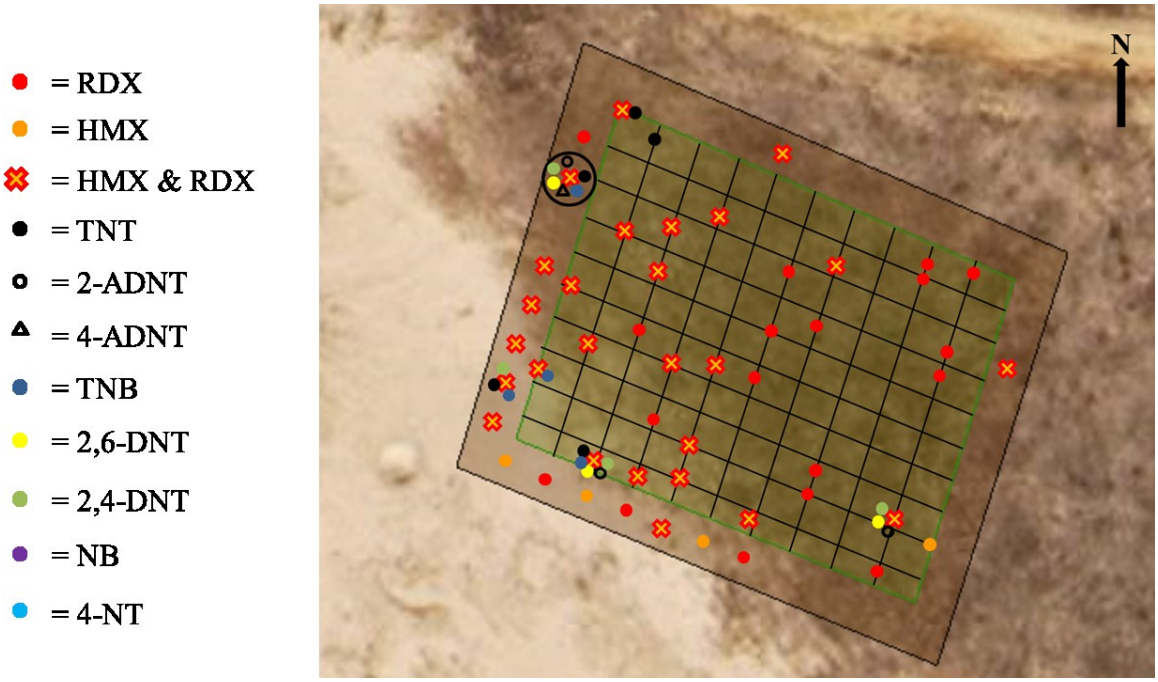


Figure 3-6. Plot #1 detections in soil for the May 26-27, 2009 sampling analyzed with HPLC. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

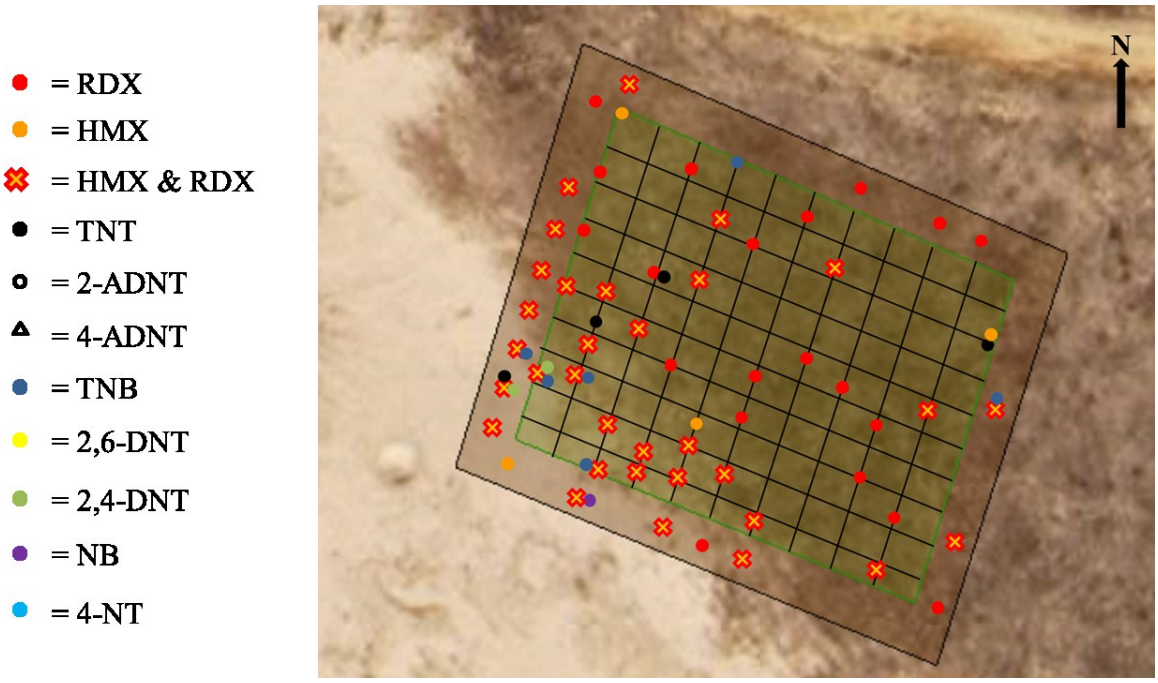


Figure 3-7. Plot #1 detections in soil for the November 18-19, 2009 sampling analyzed with HPLC. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

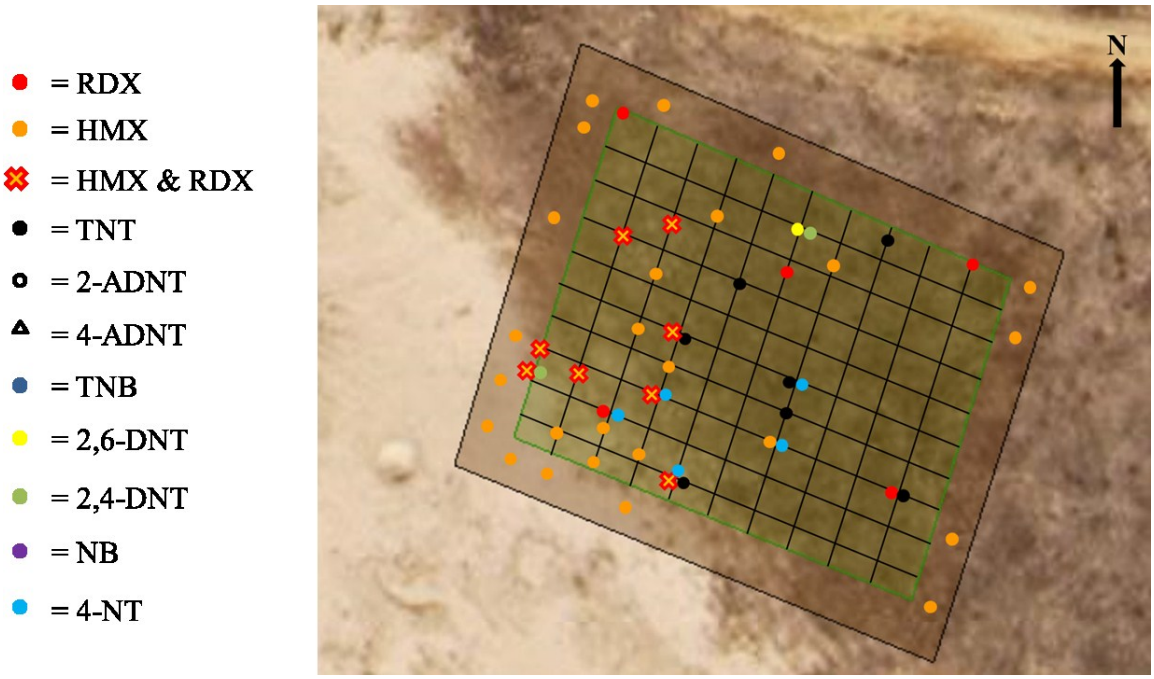


Figure 3-8. Plot #1 detections in soil for the May 24-25, 2010 sampling analyzed with HPLC. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

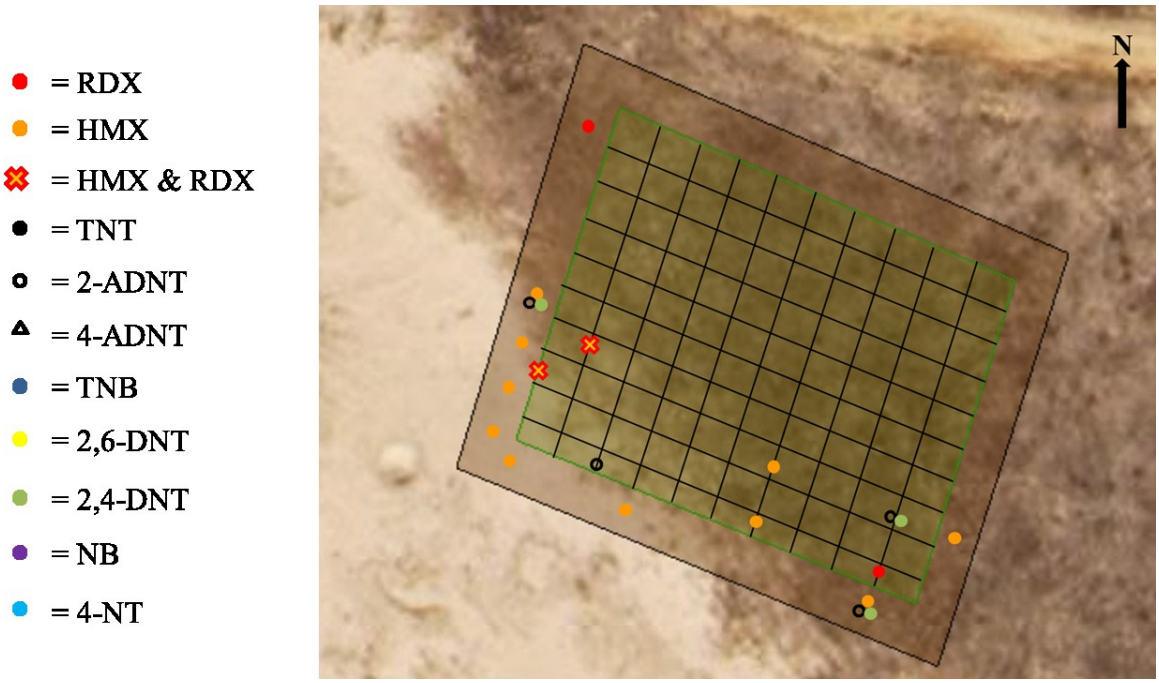


Figure 3-9. Plot #1 detections in soil for the November 13-14, 2010 sampling analyzed with HPLC. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

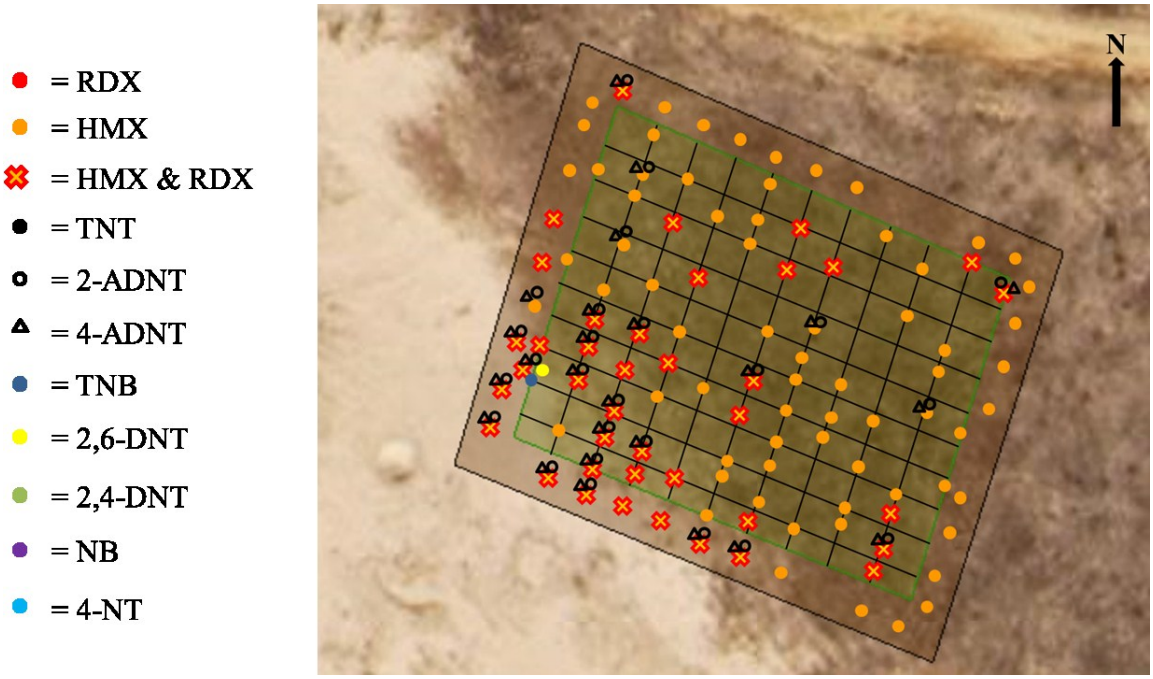


Figure 3-10. Plot #1 detections in soil for the May 24-25, 2010 sampling analyzed with LC/MS. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

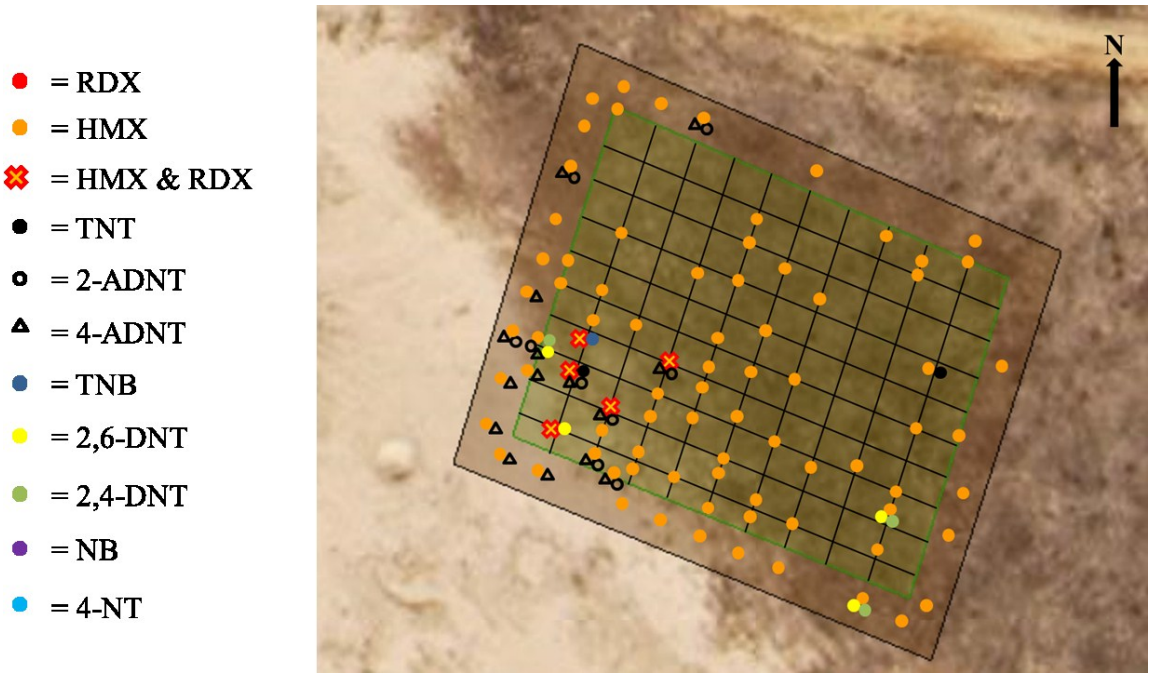


Figure 3-11. Plot #1 detections in soil for the November 13-14, 2010 sampling analyzed with LC/MS. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

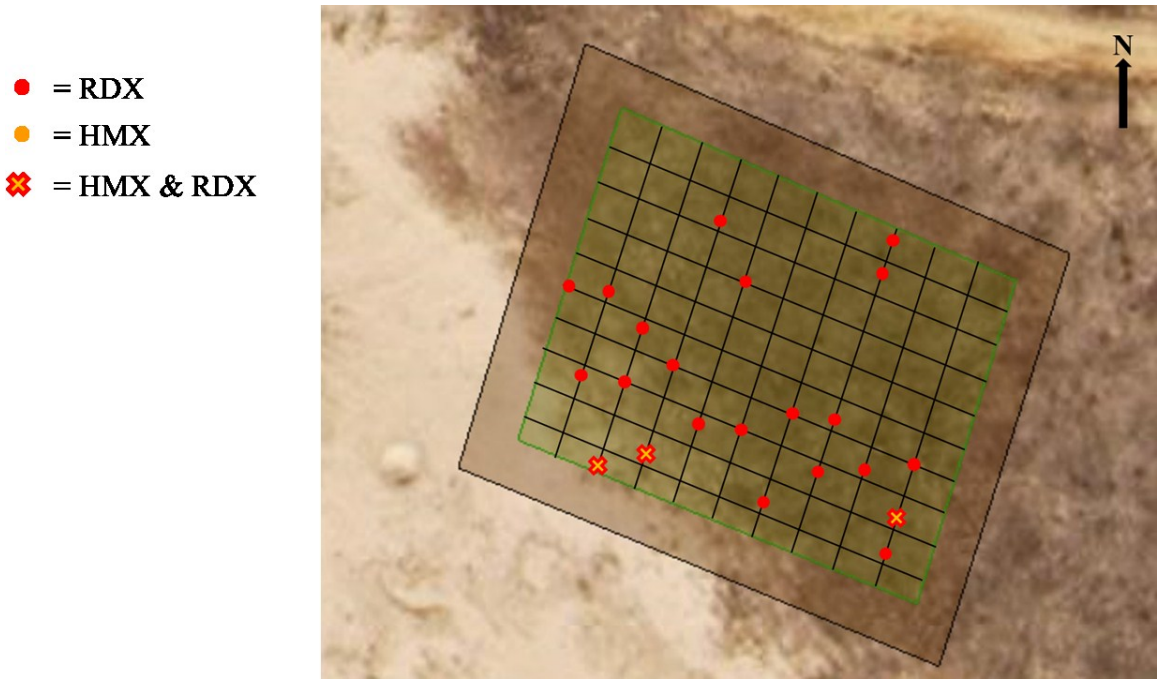


Figure 3-12. Plot #1 detections in plant tissue for the November 18-19, 2009 sampling analyzed with LC/MS. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

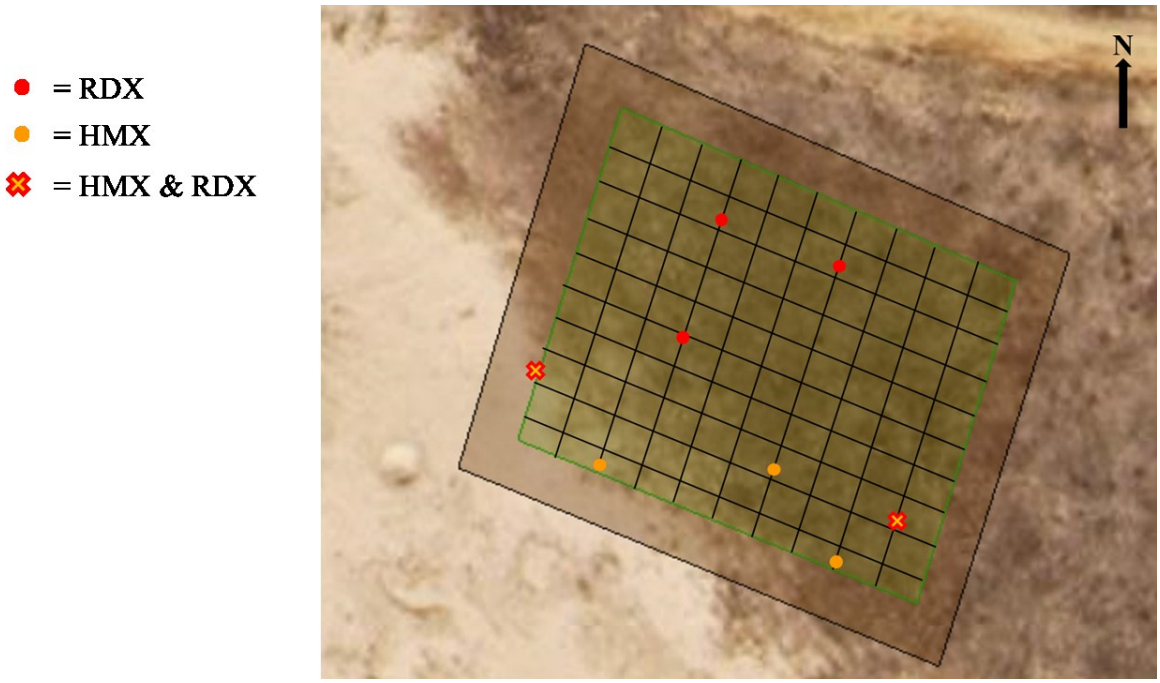


Figure 3-13. Plot #1 detections in plant tissue for the May 24-25, 2010 sampling analyzed with LC/MS. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

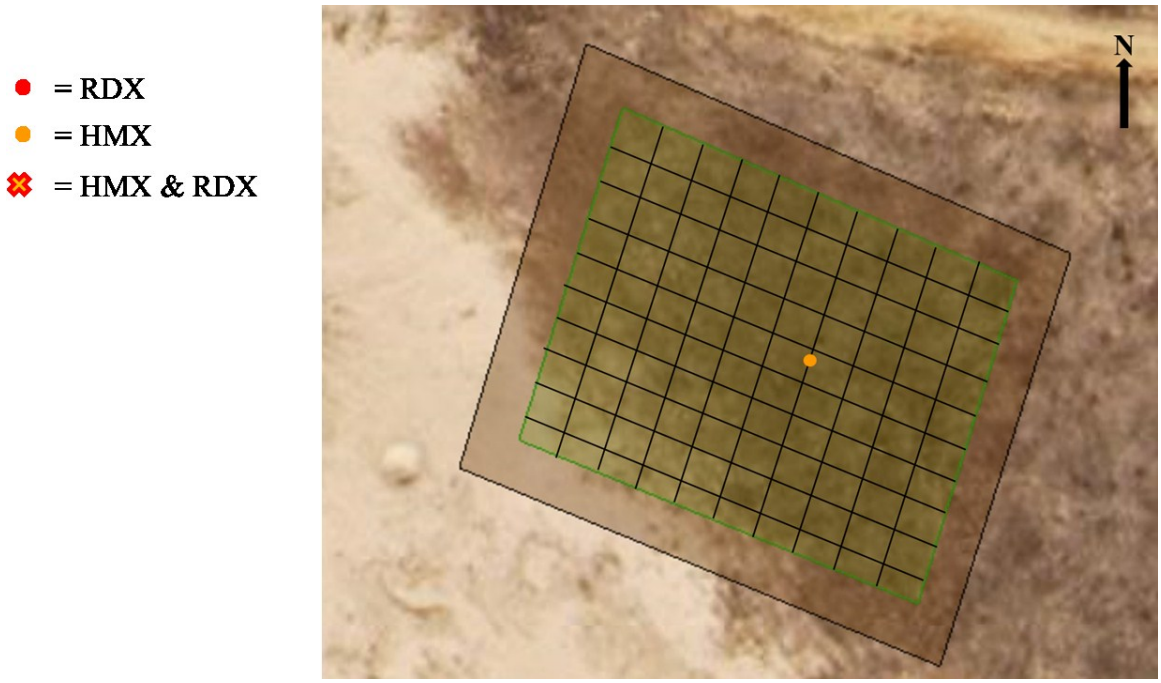


Figure 3-14. Plot #1 detections in plant tissue for the November 13-14, 2010 sampling analyzed with LC/MS. The region shaded is green is the planted portion. The fringe around the edge is unplanted (12 ft wide).

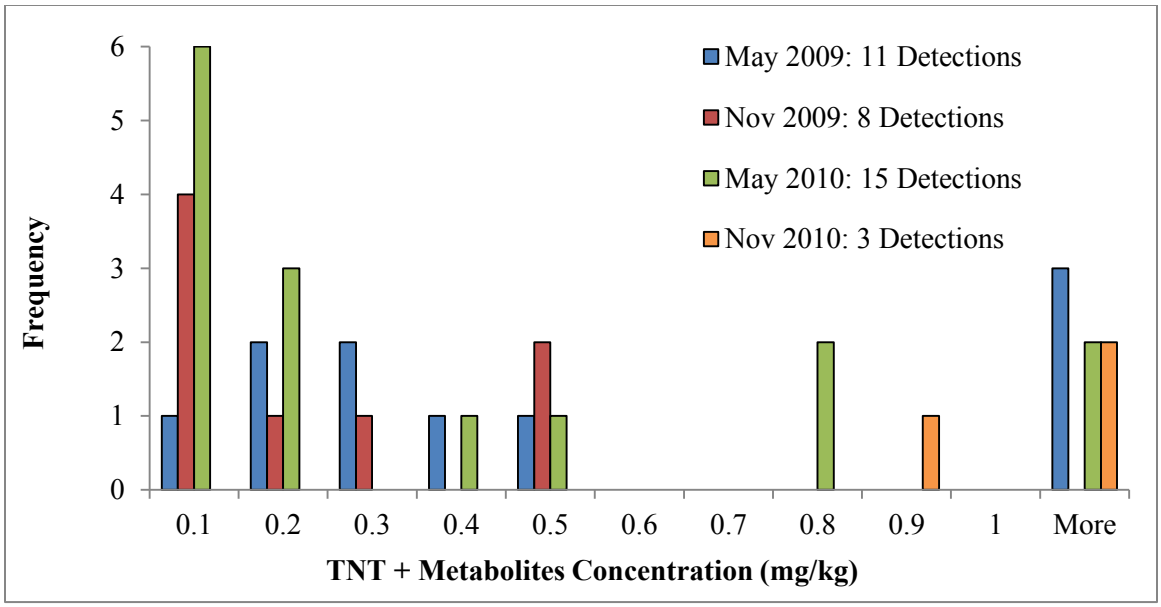


Figure 3-15. Frequency histogram of TNT plus metabolite soil concentrations found in the planted region of Plot #1 with HPLC through four successive samplings over one and a half years after planting.

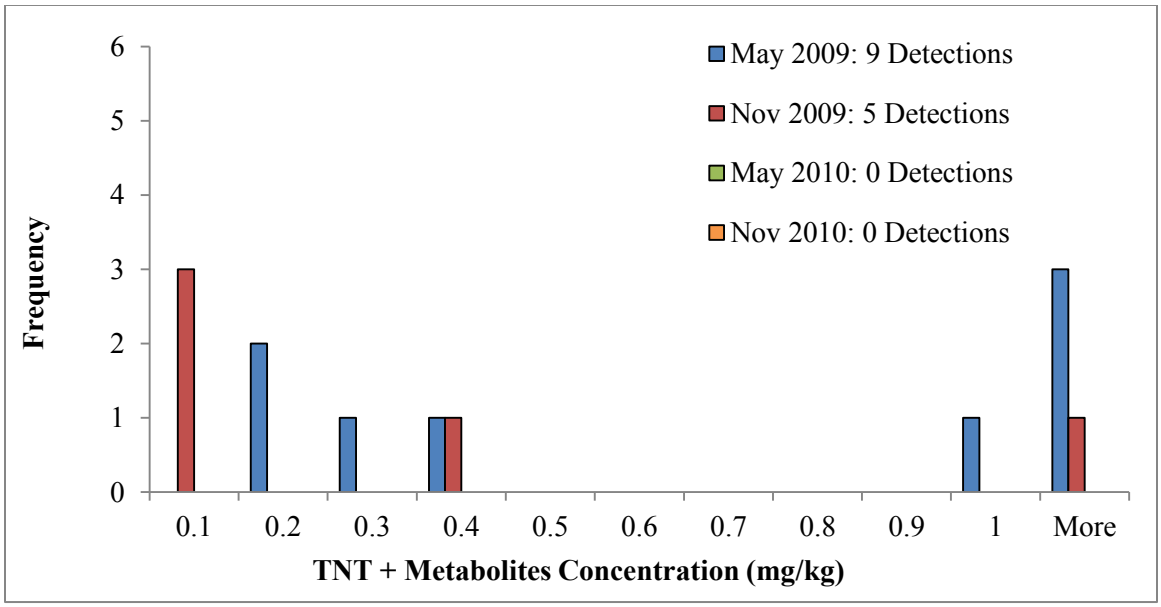


Figure 3-16. Frequency histogram of TNT plus metabolite soil concentrations found in the unplanted region of Plot #1 with HPLC through four successive samplings over one and a half years after planting.

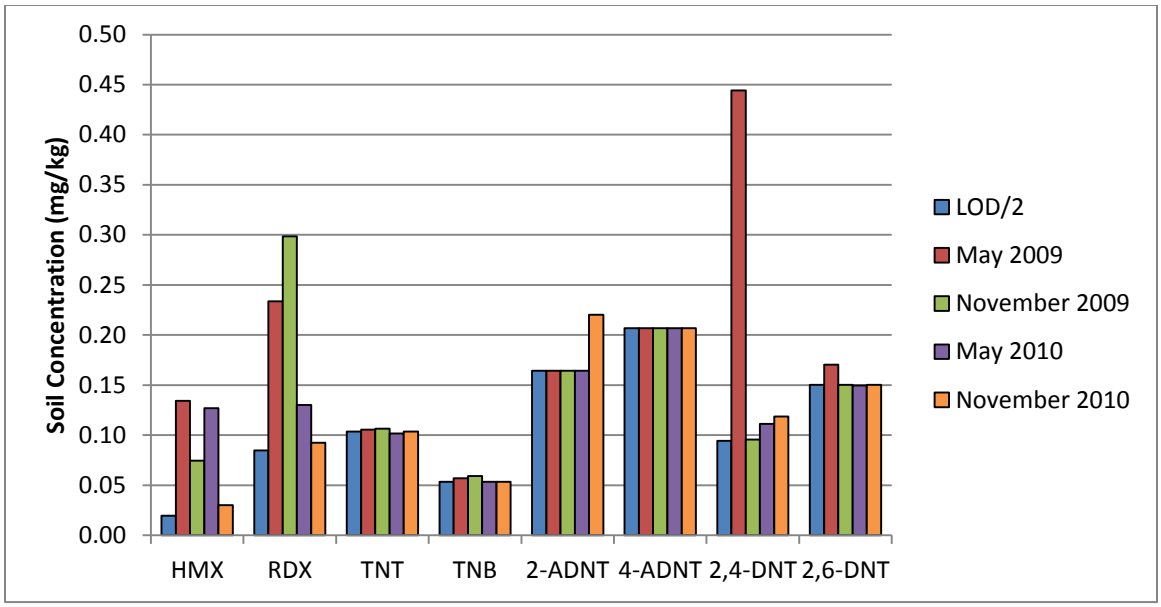


Figure 3-17. Mean concentration from HPLC analysis in the planted region of each constituent during the four samplings. Half the limit of detection serves as a reference for non-detect concentrations.

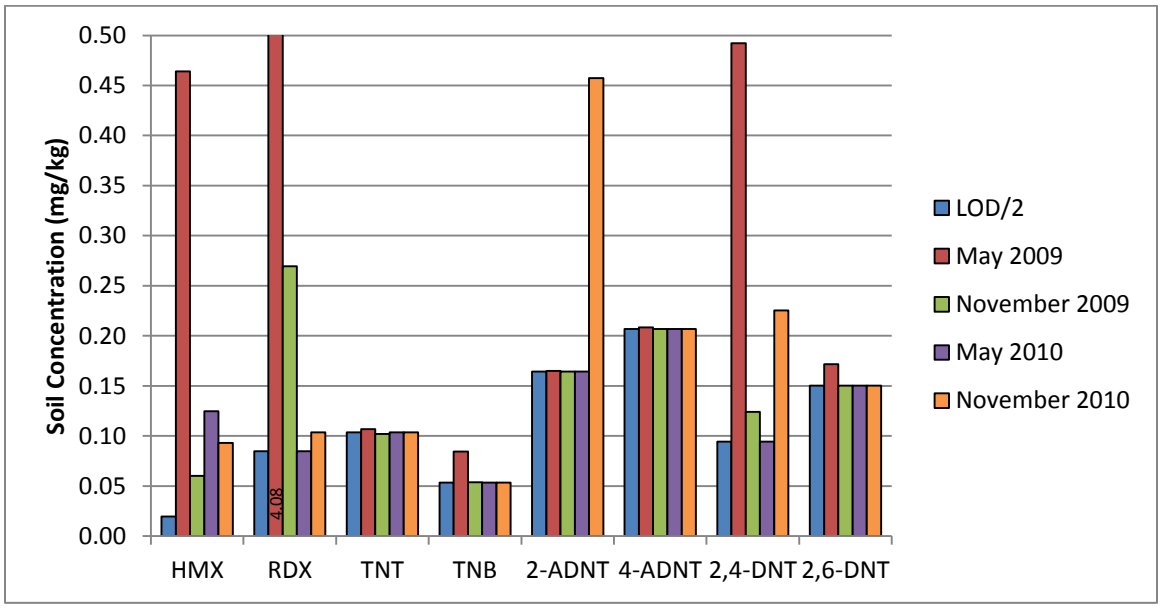


Figure 3-18. Mean concentration from HPLC analysis in the unplanted region of each constituent during the four samplings. Half the limit of detection serves as a reference for non-detect concentrations.

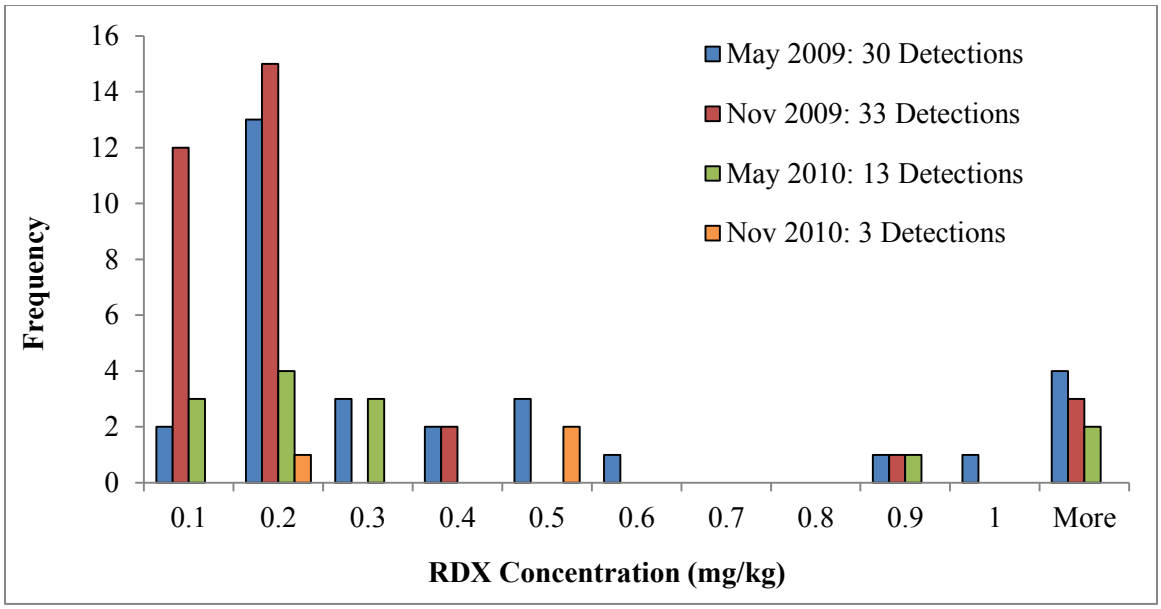


Figure 3-19. Frequency histogram of RDX soil concentrations found in the planted region of Plot #1 with HPLC through four successive samplings over one and a half years after planting.

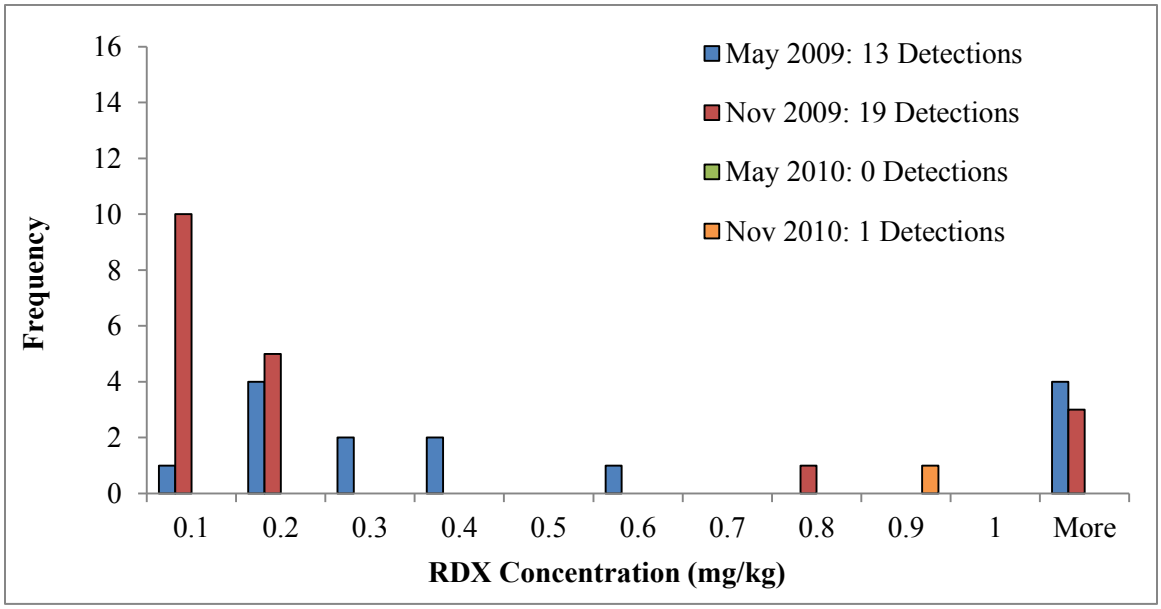


Figure 3-20. Frequency histogram of RDX soil concentrations found in the unplanted region of Plot #1 with HPLC through four successive samplings over one and a half years after planting.

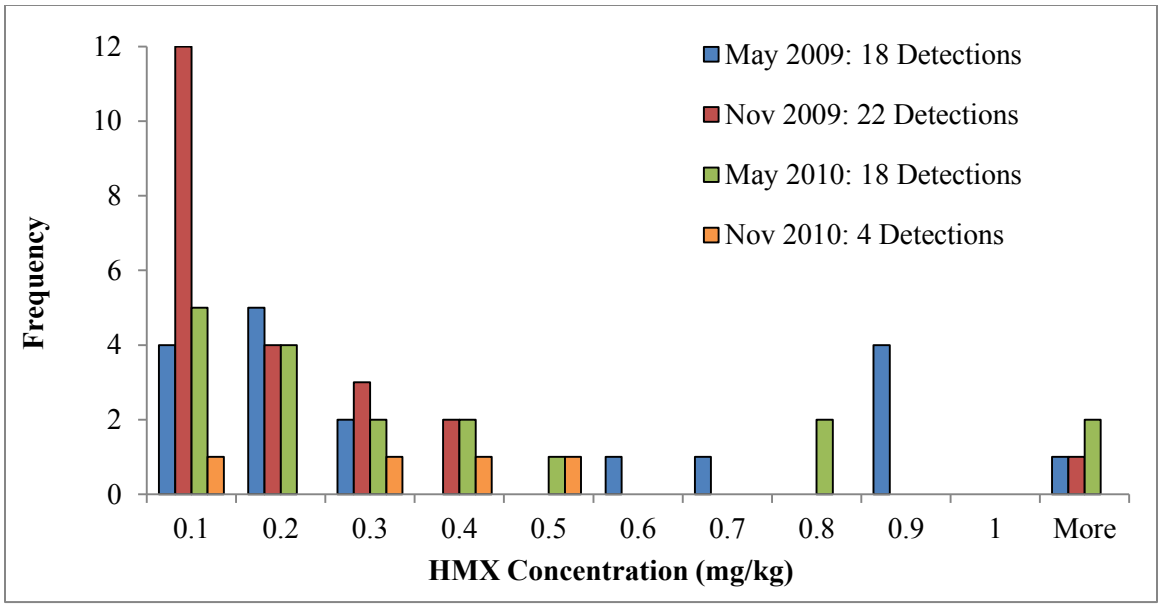


Figure 3-21. Frequency histogram of HMX soil concentrations found in the planted region of Plot #1 with HPLC through four successive samplings over one and a half years after planting.

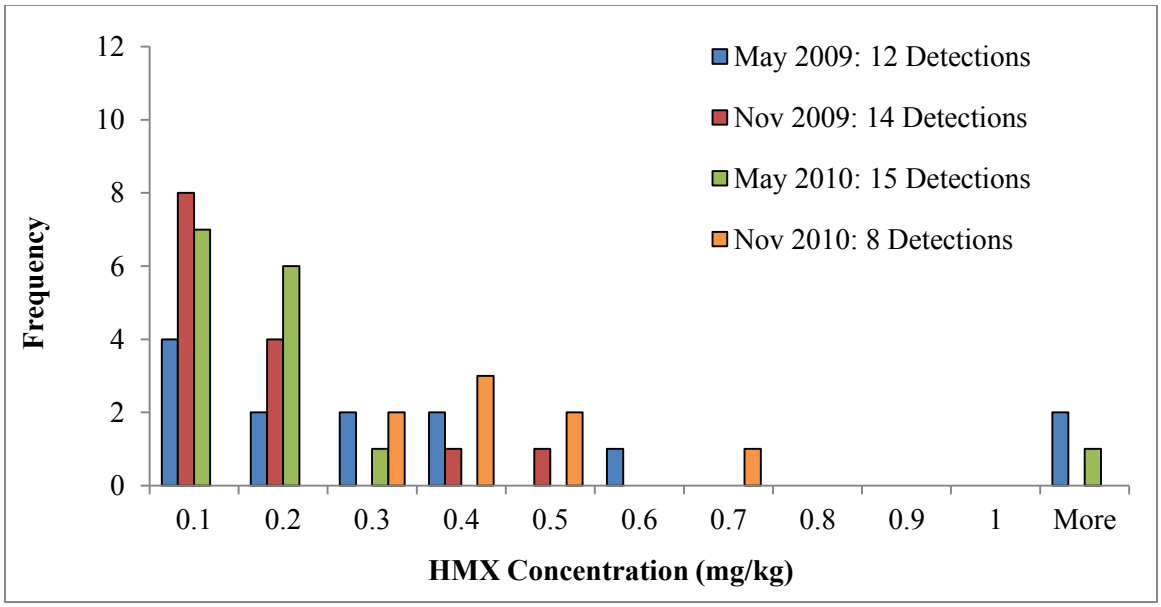


Figure 3-22. Frequency histogram of HMX soil concentrations found in the unplanted region of Plot #1 with HPLC through four successive samplings over one and a half years after planting.

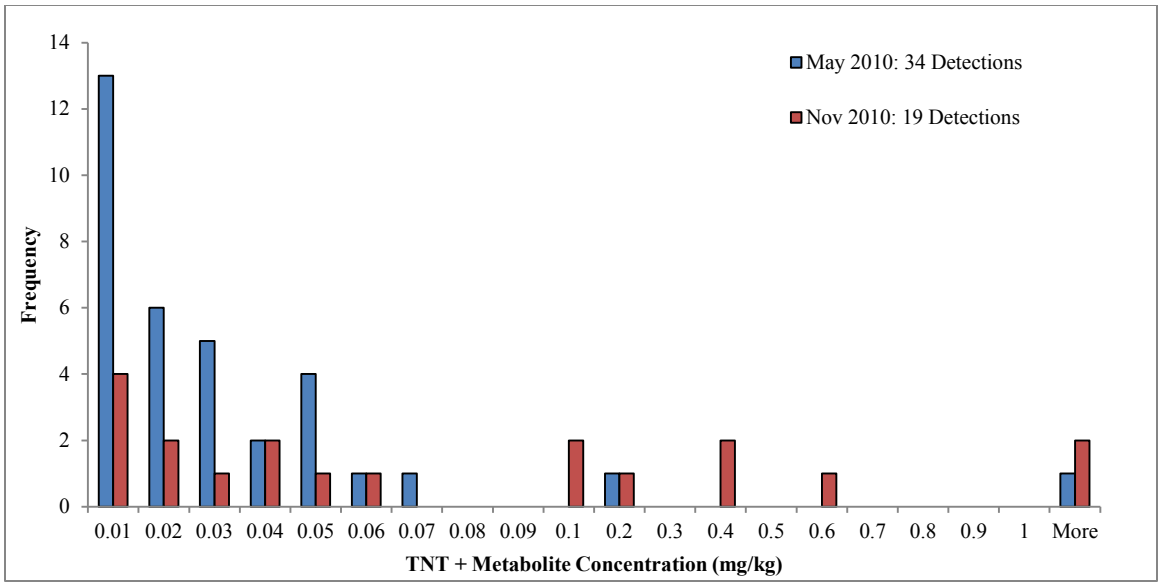


Figure 3-23. Frequency histogram of TNT plus metabolite soil concentrations found in the planted region of Plot #1 with LC/MS only through the May and November 13-14, 2010 samplings.

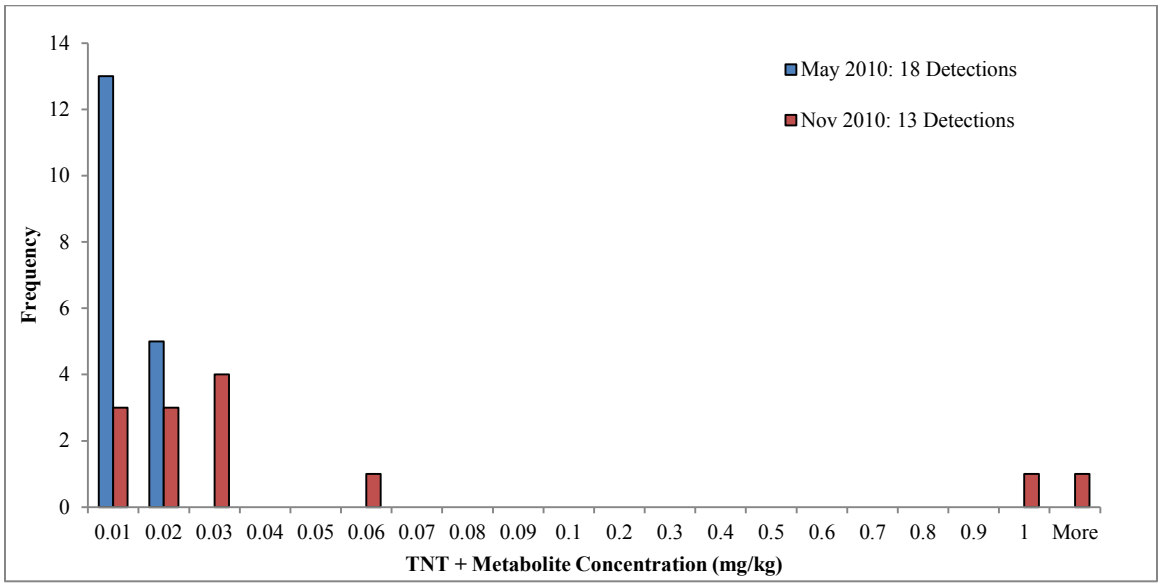


Figure 3-24. Frequency histogram of TNT plus metabolite soil concentrations found in the unplanted region of Plot #1 with LC/MS only through the May and November 13-14, 2010 samplings.

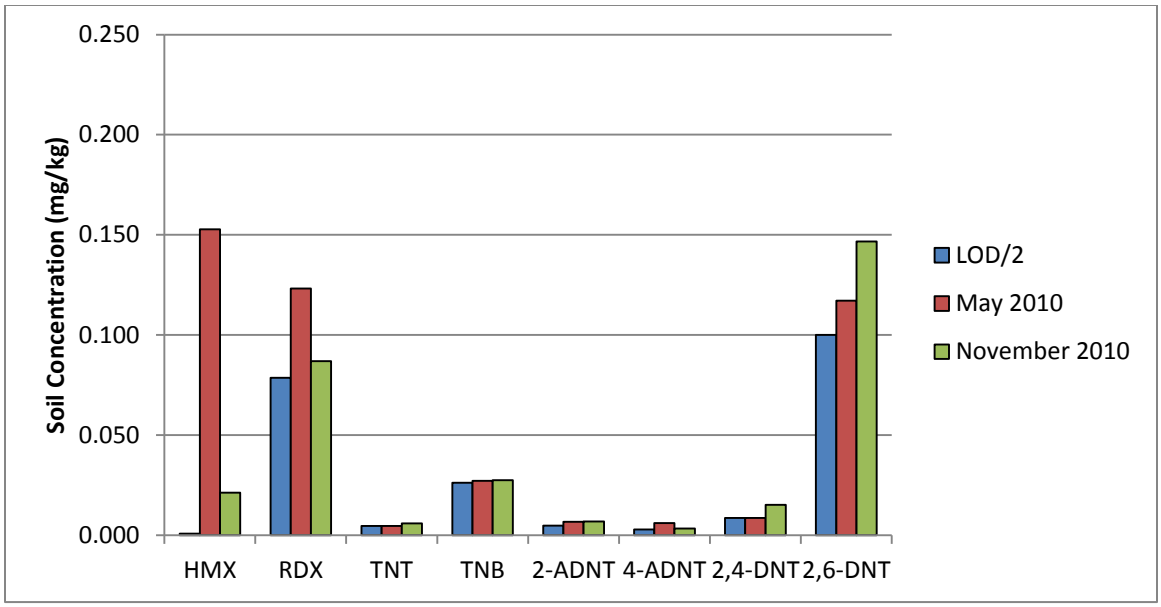


Figure 3-25. Mean concentration from LC/MS analysis in the planted region of each constituent during the May 24-25, 2010 and November 13-14, 2010 samplings. Half the limit of detection serves as a reference for non-detect concentrations.

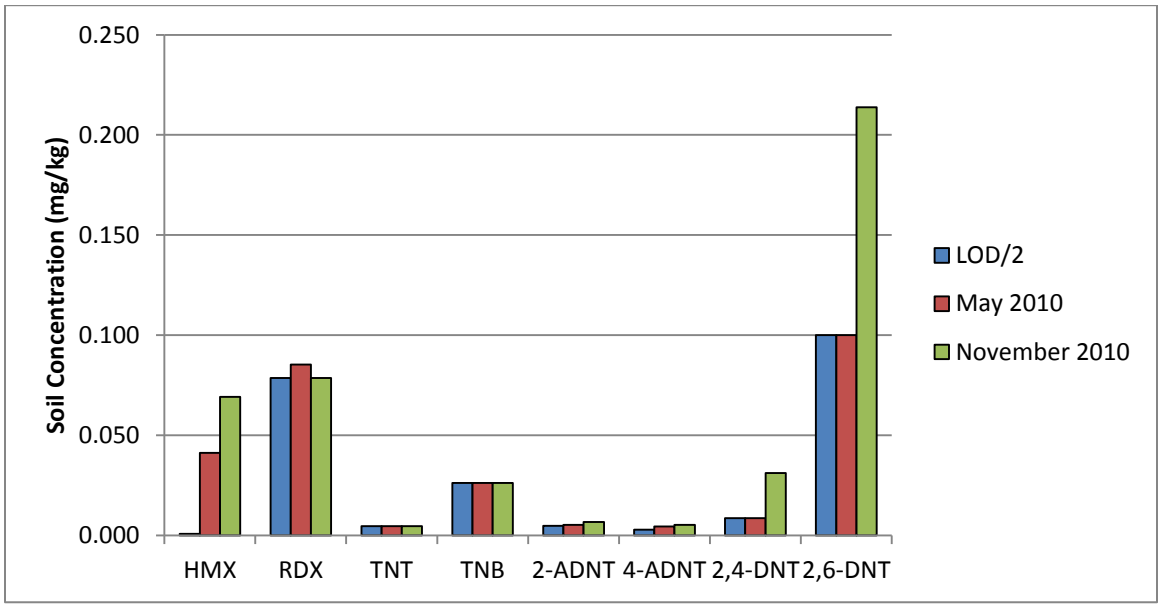


Figure 3-26. Mean concentration from LC/MS analysis in the unplanted region of each constituent during the May 24-25, 2010 and November 13-14, 2010 samplings. Half the limit of detection serves as a reference for non-detect concentrations.

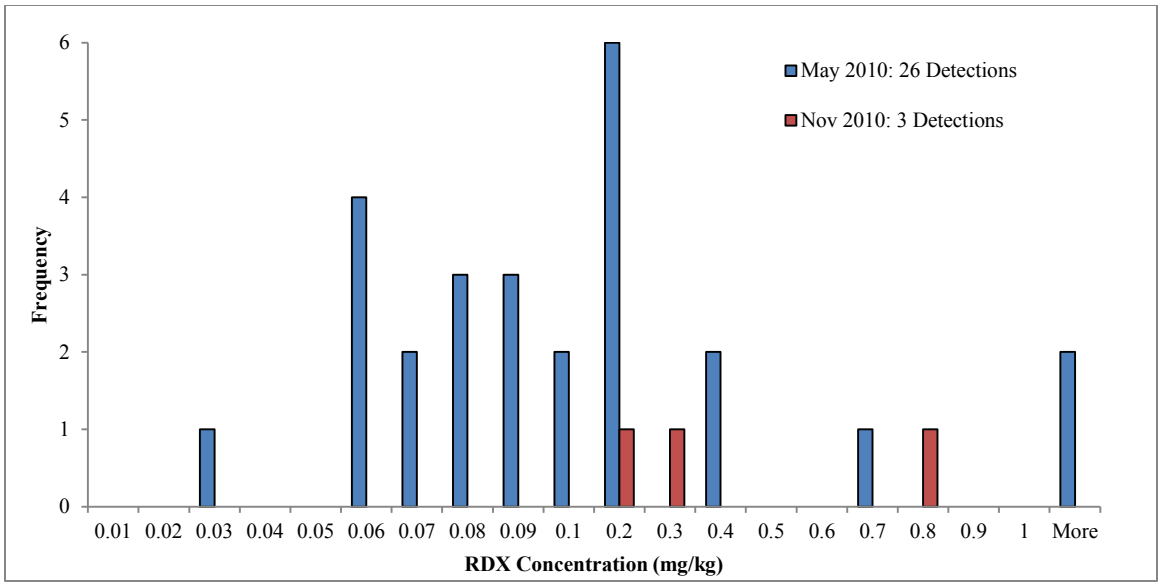


Figure 3-27. Frequency histogram of RDX soil concentrations found in the planted region of Plot #1 with LC/MS only through the May and November 13-14, 2010 samplings.

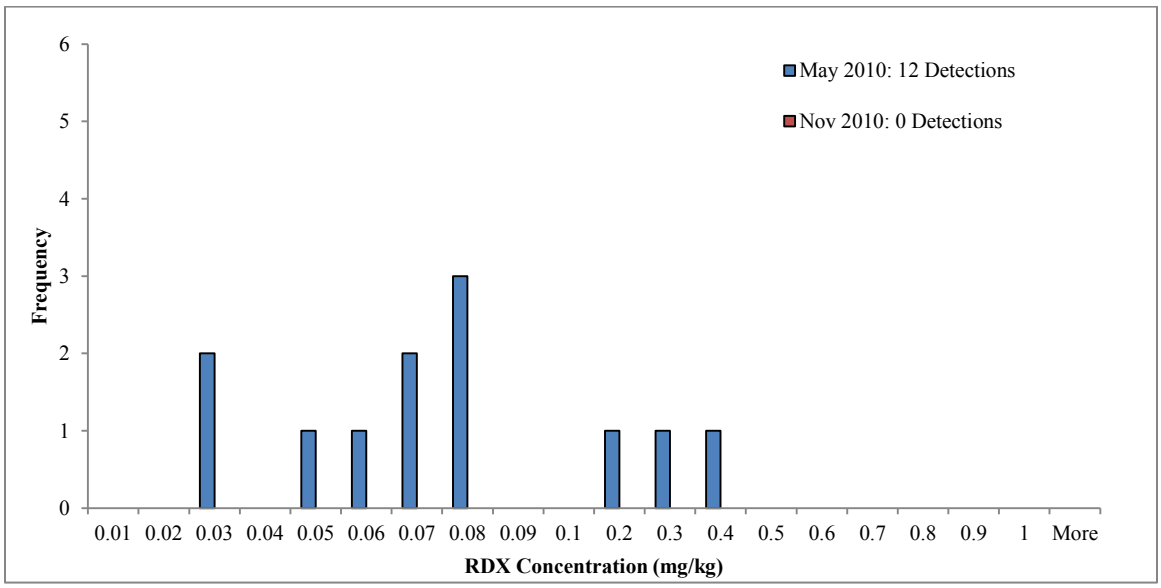


Figure 3-28. Frequency histogram of RDX soil concentrations found in the unplanted region of Plot #1 with LC/MS.

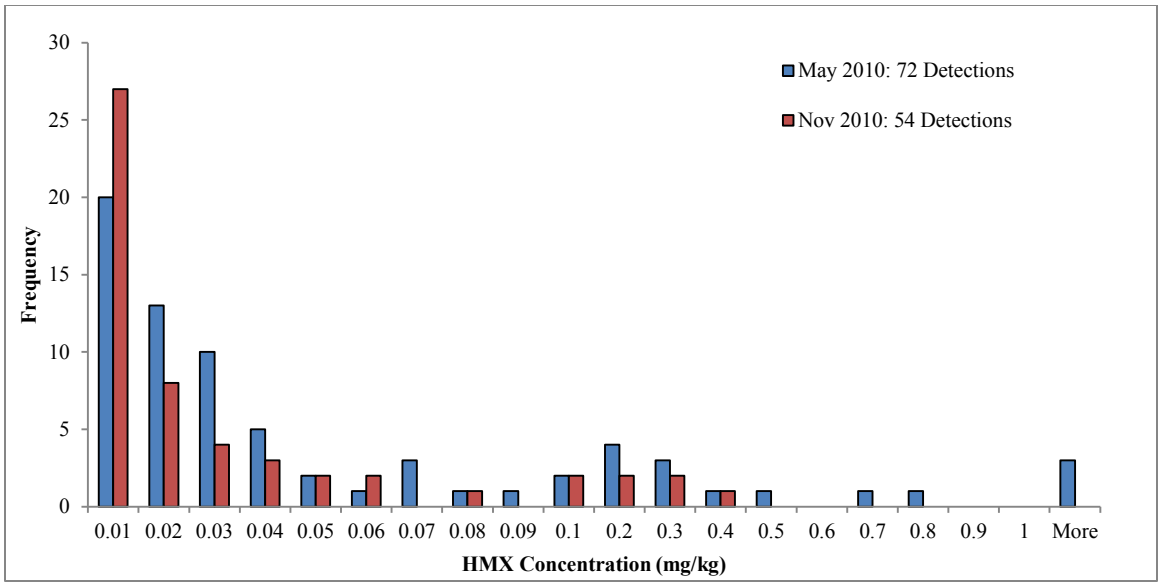


Figure 3-29. Frequency histogram of HMX soil concentrations found in the planted region of Plot #1 with LC/MS only through the May and November 13-14, 2010 samplings.

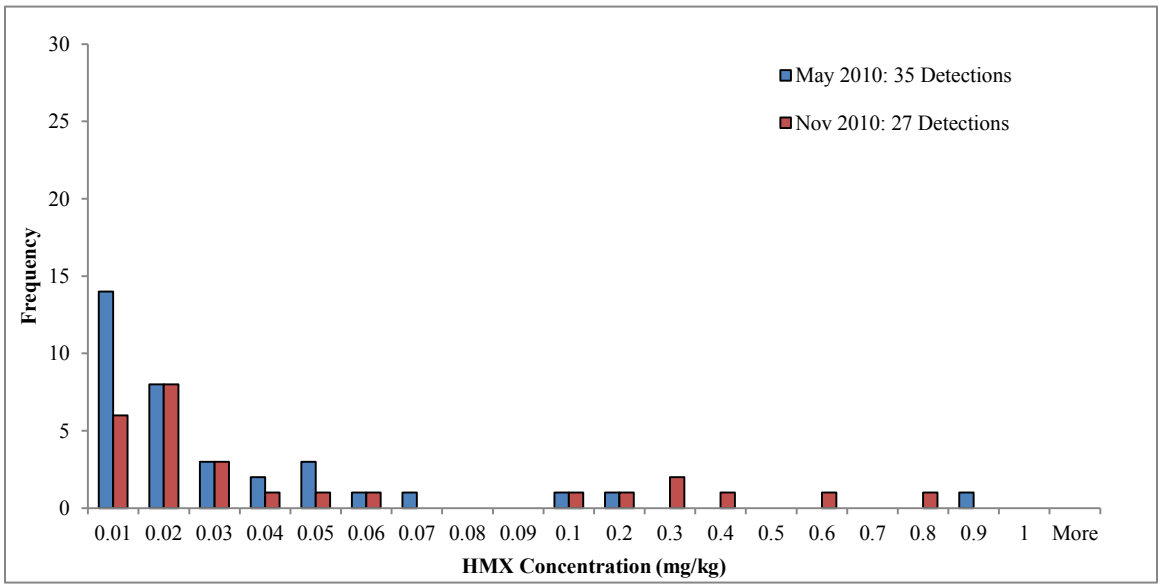


Figure 3-30. Frequency histogram of HMX soil concentrations found in the unplanted region of Plot #1 with LC/MS only through the May and November 13-14, 2010 samplings.

CHAPTER 4
CONCLUSIONS

4.1 Research Objectives

The specific objectives and the results of each of objective are listed below. Further conclusions from each chapter are presented in the following sections.

- Determine if Bahiagrass Pensacola significantly improves the biodegradation of explosives in soil at EAFB through comparisons of the planted and unplanted regions of three plots located within Range C-62.

The field study conducted at EAFB successfully determined the fate of TNT and RDX in the presence of Bahiagrass Pensacola. The biodegradation of TNT was observed in both the planted and unplanted regions of the field study plot. It was unclear whether phytoremediation was enhancing the bioremediation of TNT. The suspected dissolution of RDX was observed in both the planted and unplanted regions. It appeared that the migration of RDX downward occurred faster than the Bahiagrass could translocate the contaminant. Further investigation is warranted into the fate of HMX. From the statistical analysis of the detections, the samples analyzed by HPLC indicated HMX was migrating downward faster than phytoremediation could take effect. However, the statistical analysis of the detections as analyzed by LC/MS indicated the planted region was indeed having an effect on HMX soil concentrations.

- Determine whether plants can significantly uptake and degrade explosives in the field.

The field study conducted at EAFB successfully showed uptake and translocation of HMX and RDX into Bahiagrass Pensacola. The decrease in RDX detections in the

plants over the course of the study bolster the conclusion that the RDX is migrating downward faster than the Bahiagrass could translocate the contaminant. There were too few HMX detections to determine a trend. Both RDX and HMX were detected in plant material, but not in significant quantities. Estimates indicate the mass in the plant material was less than 0.1% of the total mass of RDX and HMX in the soil and plants.

- Compare fate and transport processes in laboratory studies using actual soils from the site of the field study with the field demonstration results.

Previous investigations under the SERDP grant ER-1499 showed the bioremediation of TNT and the recalcitrance of RDX in unplanted soil from the field study site (Anderson, 2010). The bioremediation of TNT was also observed in the field study. The study discussed in Chapter 2 showed the rapid reduction in RDX soil concentrations in soil from the field study site planted with Bahiagrass Pensacola and Hybrid Poplar. However, the field study showed that the RDX migrated downward before the Bahiagrass could uptake or degrade the compound. The difference in results was likely due to a experimental design in the laboratory setting that did not accurately reflect the conditions in the field.

4.2 Phytoremediation of RDX in Soil From Eglin Air Force Base using Bahiagrass and Poplar Plants

The laboratory microcosm study showed significant reductions in the concentration of RDX in native EAFB soil in the presence of Bahiagrass Pensacola and hybrid poplar. The concentration of RDX in the presence of Bahiagrass decreased an average of 98.6% after 56 days. The concentration of RDX in the presence of hybrid poplar decreased an average of 99.1% after 40 days. There was no reduction in RDX soil

concentrations in the excised root microcosms which suggest plant exudates and decomposing root material did not enhance biodegradation in the soil. There were no RDX detections in the root and blade or leaf tissue samples. This is contradictory to published material on the uptake of RDX that has shown that due to its high solubility and mobility in the environment, RDX is readily translocated to leaves. In the future, it is suggested that laboratory studies incorporate flow-through in order to better represent conditions at EAFB.

4.3 Phytoremediation Field Study for the Treatment of Explosive Compounds at Eglin Air Force Base, FL

Important findings were made involving the three most prevalent explosive compounds found at military testing and training ranges: TNT, RDX, and HMX. The number of TNT detections was low throughout the entire study. The mean concentrations of TNT and TNT metabolites showed that mean TNT concentrations remained low (at or near the limit of detection) while mean TNT metabolite concentrations increased over time in both the planted and unplanted regions of Plot #1. This was shown in data analyzed by both HPLC and LC/MS. From these results, it was concluded that the microbial communities in the soil are successfully degrading the compound. This result is supported by work completed in the lab, which showed the degradation of TNT and the formation of metabolites in unplanted EAFB soil (Anderson, 2010). It is unclear if the implementation of phytoremediation is enhancing this process because rates could not be determined between the planted and unplanted regions of Plot #1.

The frequency histograms of each sampling, the mean concentrations of contaminants, and the general linear model applied to the data showed the RDX concentrations in soil were decreasing in both the planted and unplanted regions of Plot #1. This suggests the RDX is migrating downward and likely into the groundwater faster than the Bahiagrass is able to uptake and translocate the compound. This is conceivable given the solubility of RDX in water and its mobility in the environment. The results of the field study are contradictory to the results from the laboratory microcosm study. This is likely due to the watering regime of the laboratory study. Special care was given so that the soil was moist while ensuring only a minimal amount of water drained from the pots. RDX was not given the ability to be transported out of the root zone. This is not the case in the environment where the water will drain through the soil and provide recharge to groundwater.

There were mixed results for the fate of HMX in the field study. The data from the analysis of samples by HPLC indicated that the HMX was migrating downward with little or no treatment from the Bahiagrass as exhibited by the histograms and general linear model. However, the data from the analysis by LC/MS, which is much more sensitive to HMX, showed that the HMX was indeed being treated by the Bahiagrass because the concentrations were increasing in the unplanted region while the concentrations in the planted region were decreasing. This was shown by the histograms of each sampling, mean HMX concentrations of each sampling, and a general linear model. However, only two samplings were analyzed using LC/MS. Additional samplings would need to be analyzed to verify this trend.

HMX and RDX were detected in the discrete plant samples retrieved during each sampling. This is significant because it is the first time uptake and translocation of RDX and HMX has been documented during a phytoremediation field study on military ranges. But, the RDX detections in Bahiagrass decreased as the detections in the soil decreased.

Assuming that the airborne deposition rates of explosive compounds were relatively similar in both the planted and unplanted regions of Plot #1, the data suggests that the organic carbon associated with the Bahiagrass roots and sod were effective in preventing or retarding the downward migration and percolation of contaminants. This alone, even without unequivocal proof of phytoremediation, is very positive.

Overall, the objective of implementing phytoremediation as a strategy for the containment or treatment of explosive contaminants on testing and training ranges at EAFB produced negative results. It does not appear TNT is capable of migrating offsite in significant quantities due to its low mobility and biodegradation. However, both HMX and especially RDX pose a great risk of migrating offsite in significant quantities due to their high mobility. Additionally, it does not appear that phytoremediation using Bahiagrass Pensacola in Lakeland soil at EAFB can significantly treat or contain HMX or RDX.

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APPENDIX A
INSTALLATION AND CONDITION OF BAHIAGRASS PENSACOLA SOD AT EGLIN AIR FORCE BASE

Figure A-1. Installation of Bahiagrass Pensacola sod on May 26, 2009.

Source: Anderson, T. J. (2010). Phytoremediation of energetic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

Figure A-2. Installation of Bahiagrass Pensacola sod on May 26, 2009.

Source: Anderson, T. J. (2010). Phytoremediation of energectic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

Figure A-3. Plot #1 on May 26, 2009.

Source: Anderson, T. J. (2010). Phytoremediation of energectic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

Figure A-4. Plot #1 on June 24, 2009.

Source: Anderson, T. J. (2010). Phytoremediation of energectic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

Figure A-5. Plot #1 on September 1, 2009.

Source: Anderson, T. J. (2010). Phytoremediation of energectic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

Figure A-6. Plot #1 on November 17, 2009.

Source: Anderson, T. J. (2010). Phytoremediation of energectic compound at Eglin Air Force Base. Master's Thesis, University of Iowa.

Figure A-7. Plot #1 on March 15, 2010. Photo taken by William "Sandy" Pizzolato.

Figure A-8. Plot #1 on May 22, 2010. Photo taken by Matthew B. Flannigan.

Figure A-9. Plot #1 on November 13, 2010. Photo taken by Matthew B. Flannigan.

APPENDIX B

SUMMARY OF CLIMATE DATA

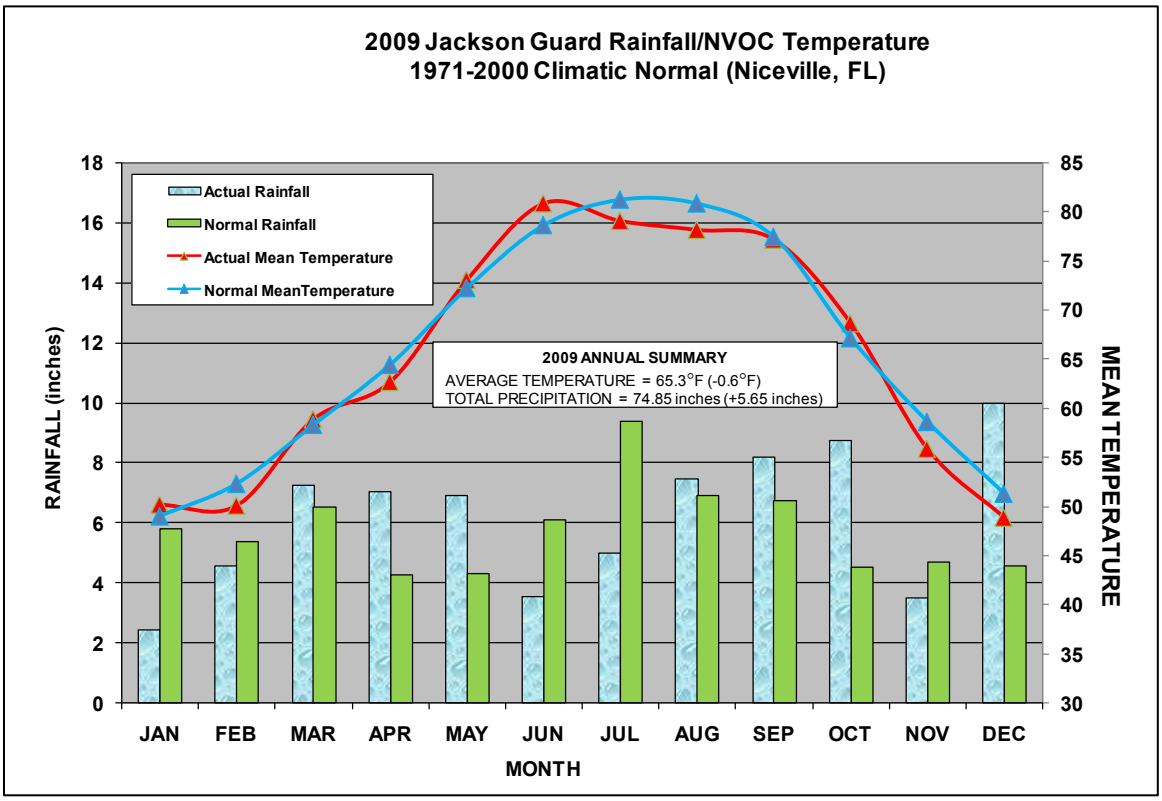

Figure B-1. Summary of 2009 climate in Niceville, FL.

Source: W. Pizzolato, personal correspondence, January 4, 2010.

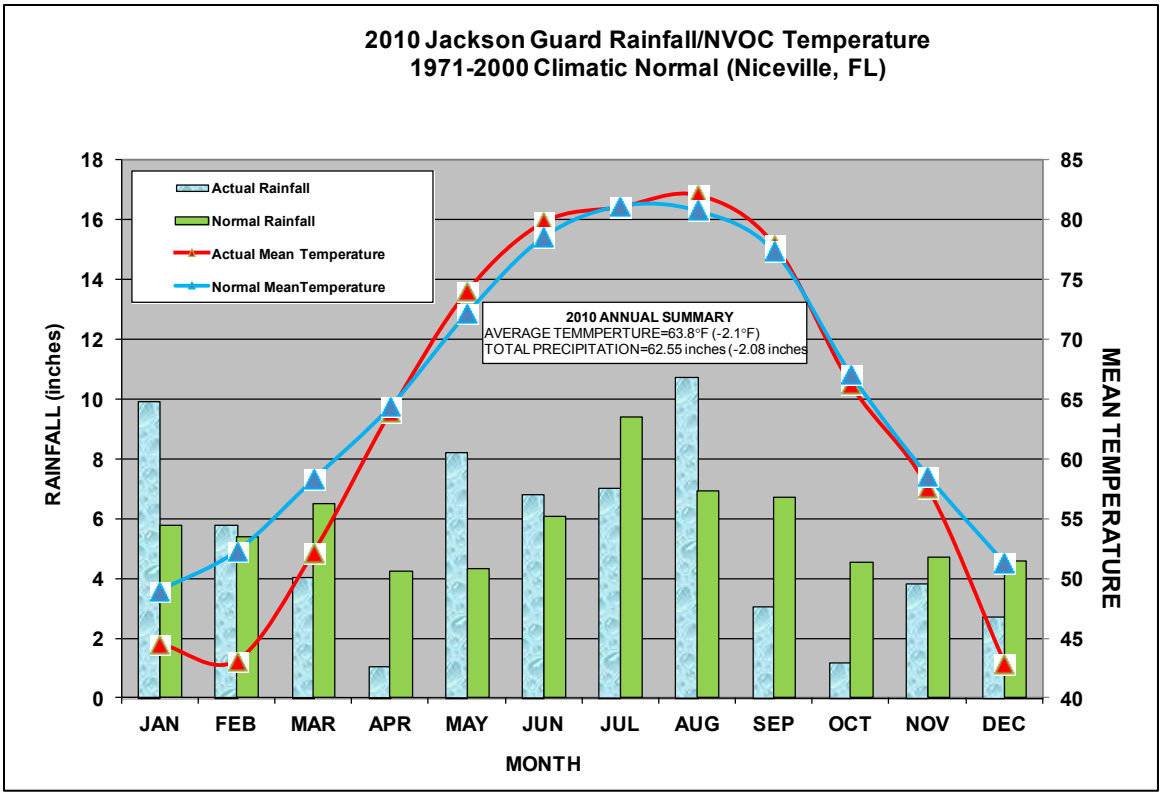

Figure B-2. Summary of 2010 climate in Niceville, FL.

Source: W. Pizzolato, personal correspondence, January 8, 2011.

APPENDIX C
STATISTICAL ANALYSIS OF RESULTS

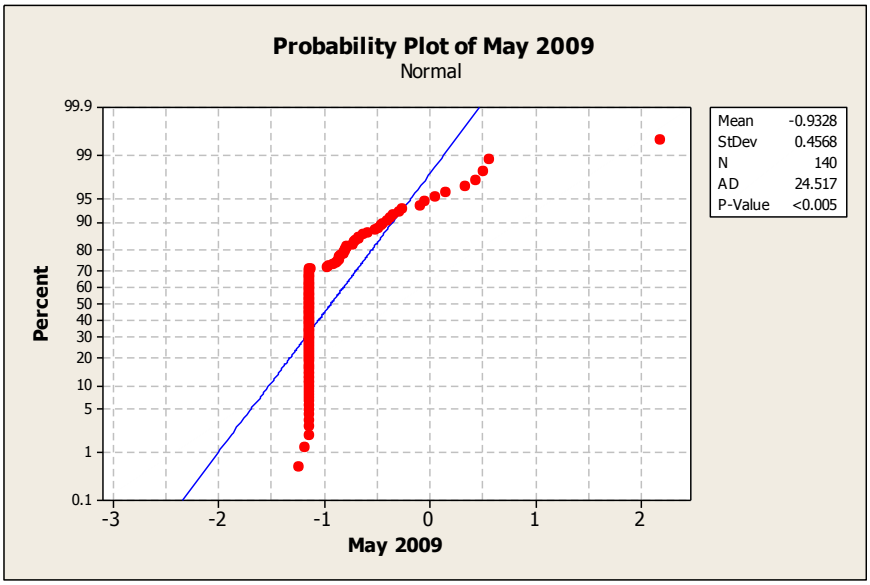

Figure C-1. Normal probability plot of log transformed RDX soil concentrations from the May 26-27, 2009 sampling analyzed using HPLC.

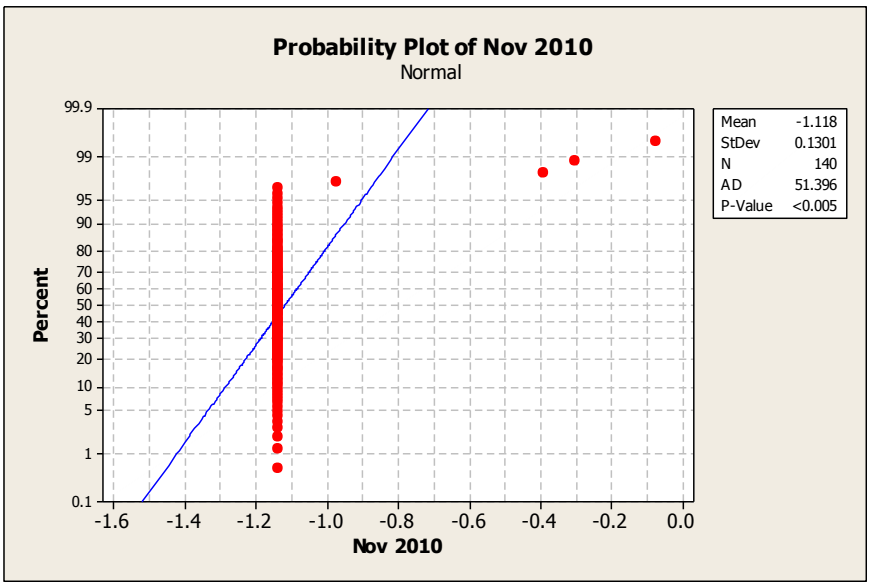

Figure C-2. Normal probability plot of log transformed RDX soil concentrations from the November 13-14, 2010 sampling analyzed using HPLC.

General Linear Model: Log(RDX Conc) versus Plant Type, Time

```
Factor       Type   Levels  Values
Plant Type   fixed       2  0, 1
Time         fixed       4  1, 2, 3, 4
```

Analysis of Variance for Log(RDX Conc), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Type	1	0.0136	0.0331	0.0331	0.10	0.755
Time	3	5.4930	6.5429	2.1810	6.48	0.000
Plant Type*Time	3	1.3041	1.3041	0.4347	1.29	0.281
Error	109	36.7133	36.7133	0.3368		
Total	116	43.5240				

S = 0.580361 R-Sq = 15.65% R-Sq(adj) = 10.23%

Unusual Observations for Log(RDX Conc)

Obs	Log(RDX Conc)	Fit	SE Fit	Residual	St Resid	
34	2.18838	-0.33822	0.16096	2.52659	4.53	R
75	0.93317	-0.93296	0.09953	1.86613	3.26	R
85	0.25903	-0.91039	0.12373	1.16942	2.06	R
95	0.61520	-0.91039	0.12373	1.52559	2.69	R
113	-1.69897	-1.69897	0.58036	0.00000	*	X
114	-0.39124	-0.55511	0.33507	0.16386	0.35	X
115	-0.30228	-0.55511	0.33507	0.25283	0.53	X
116	-0.97180	-0.55511	0.33507	-0.41669	-0.88	X
117	-0.07786	-0.07786	0.58036	0.00000	*	X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

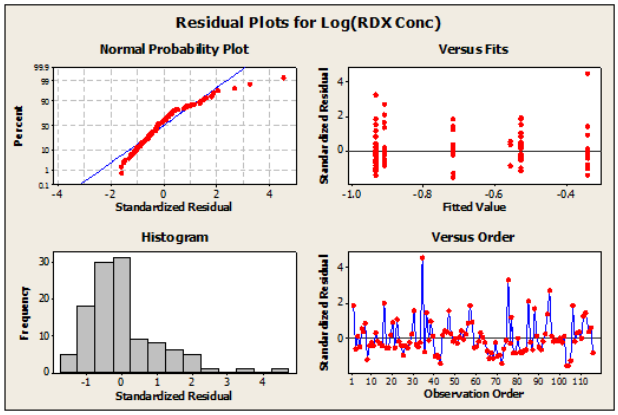

Figure C-3. Minitab© output of the application of a General Linear Model on log-transformed RDX soil concentrations analyzed using HPLC.

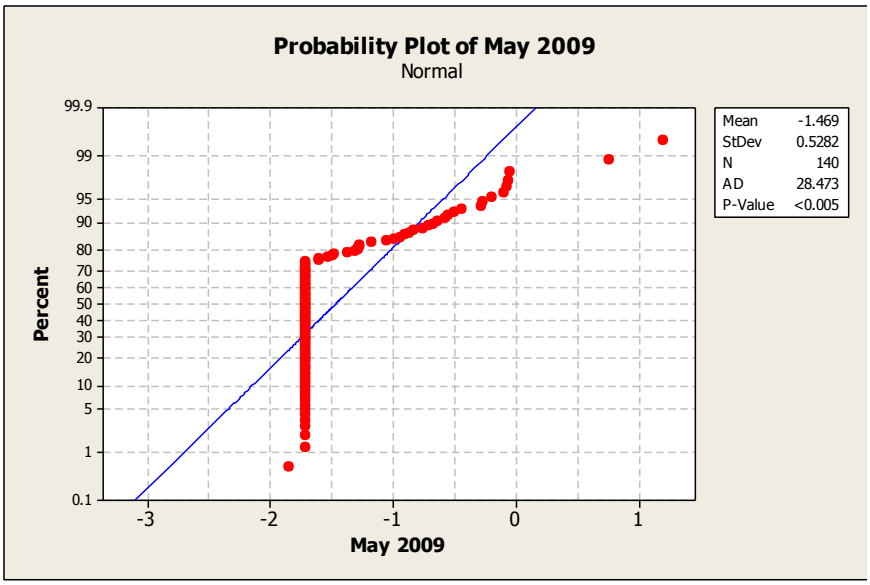

Figure C-4. Normal probability plot of log transformed HMX soil concentrations from the May 26-27, 2009 sampling analyzed using HPLC.

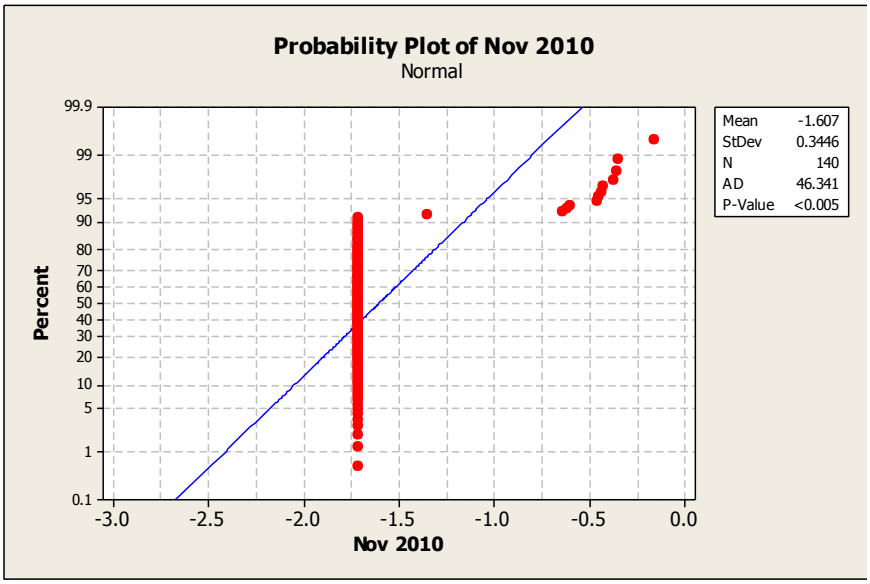

Figure C-5. Normal probability plot of log transformed HMX soil concentrations from the November 13-14, 2010 sampling analyzed using HPLC.

General Linear Model: Log(HMX Conc) versus Plant Type, Time

Factor	Type	Levels	Values
Plant Type	fixed	2	0, 1
Time	fixed	4	1, 2, 3, 4

Analysis of Variance for Log(HMX Conc), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Type	1	0.0025	0.0012	0.0012	0.00	0.950
Time	3	4.2433	3.5280	1.1760	3.88	0.011
Plant Type*Time	3	0.4804	0.4804	0.1601	0.53	0.663
Error	117	35.4273	35.4273	0.3028		
Total	124	40.1534				

S = 0.550270 R-Sq = 11.77% R-Sq(adj) = 6.49%

Unusual Observations for Log(HMX Conc)

Obs	Log(HMX Conc)	Fit	SE Fit	Residual	St Resid	
8	0.76176	-0.76567	0.11732	1.52743	2.84	R
11	-1.84128	-0.76567	0.11732	-1.07561	-2.00	R
27	1.19835	-0.78916	0.14707	1.98751	3.75	R
61	0.26240	-1.09749	0.10590	1.35989	2.52	R
81	0.78958	-0.68725	0.12970	1.47684	2.76	R
113	0.47205	-0.89512	0.14208	1.36717	2.57	R
114	-0.60071	-0.68040	0.27514	0.07969	0.17	X
115	-1.34677	-0.68040	0.27514	-0.66636	-1.40	X
116	-0.42660	-0.68040	0.27514	0.25380	0.53	X
117	-0.34753	-0.68040	0.27514	0.33287	0.70	X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

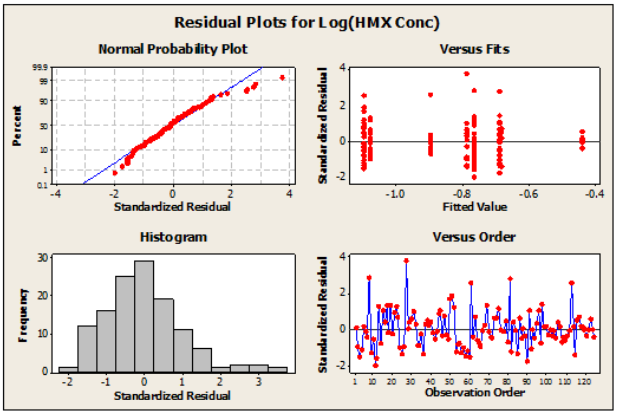

Figure C-6. Minitab© output of the application of a General Linear Model on log-transformed HMX soil concentrations analyzed using HPLC.

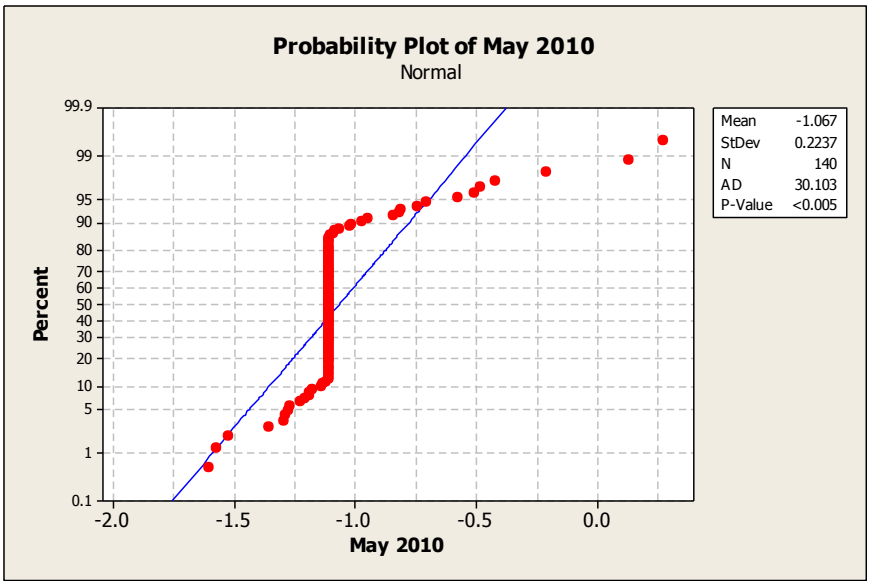

Figure C-7. Normal probability plot of log transformed RDX soil concentrations from the May 24-25, 2010 sampling analyzed using LC/MS.

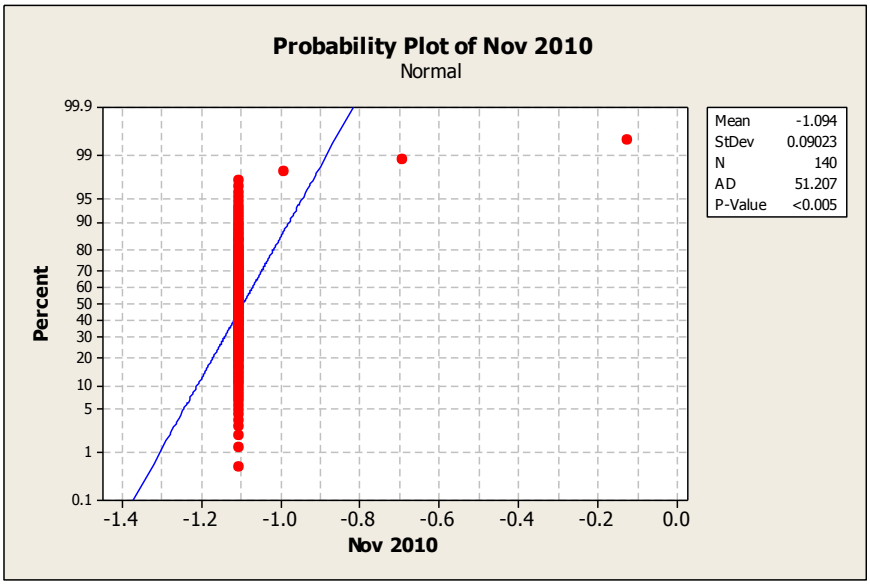

Figure C-8. Normal probability plot of log transformed RDX soil concentrations from the November 13-14, 2010 sampling analyzed using LC/MS.

General Linear Model: Log(RDX Conc) versus Plant Type, Time

Factor	Type	Levels	Values
Plant Type	fixed	2	0, 1
Time	fixed	2	1, 2

Analysis of Variance for Log(RDX Conc), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Type	1	0.5202	0.1318	0.1318	0.79	0.381
Time	1	0.3262	0.2618	0.2618	1.56	0.219
Plant Type*Time	1	0.0008	0.0008	0.0008	0.00	0.947
Error	38	6.3708	6.3708	0.1677		
Total	41	7.2180				

S = 0.409453 R-Sq = 11.74% R-Sq(adj) = 4.77%

Unusual Observations for Log(RDX Conc)

Obs	Log(RDX Conc)	Fit	SE Fit	Residual	St Resid	
13	0.27255	-0.89295	0.08030	1.16550	2.90	R
23	0.12957	-0.89295	0.08030	1.02253	2.55	R
39	-0.98898	-0.60096	0.23640	-0.38802	-1.16	X
40	-0.69135	-0.60096	0.23640	-0.09039	-0.27	X
41	-0.12255	-0.60096	0.23640	0.47841	1.43	X
42	-0.80327	-0.80327	0.40945	-0.00000	*	X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

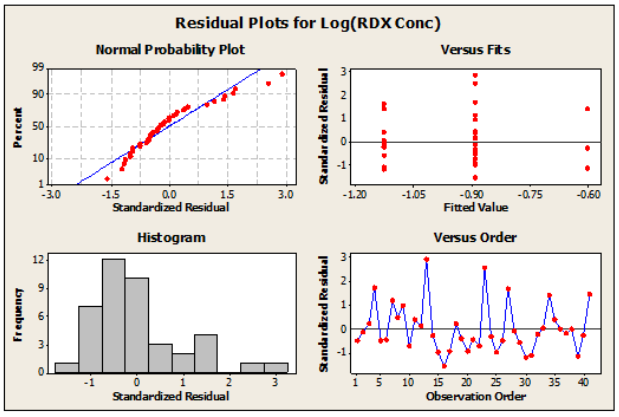

Figure C-9. Minitab© output of the application of a General Linear Model on log-transformed RDX soil concentrations analyzed using LC/MS.

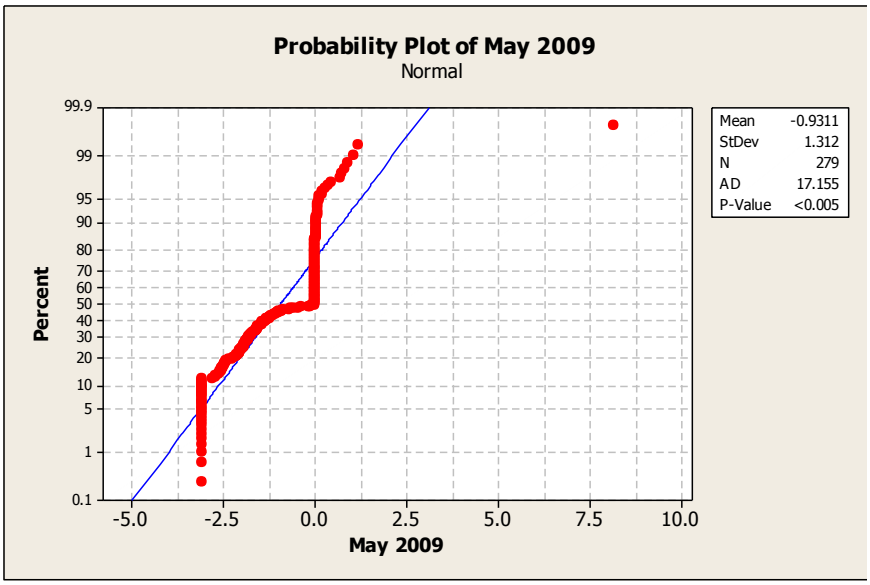

Figure C-10. Normal probability plot of log transformed HMX soil concentrations from the May 24-25, 2010 sampling analyzed using LC/MS.

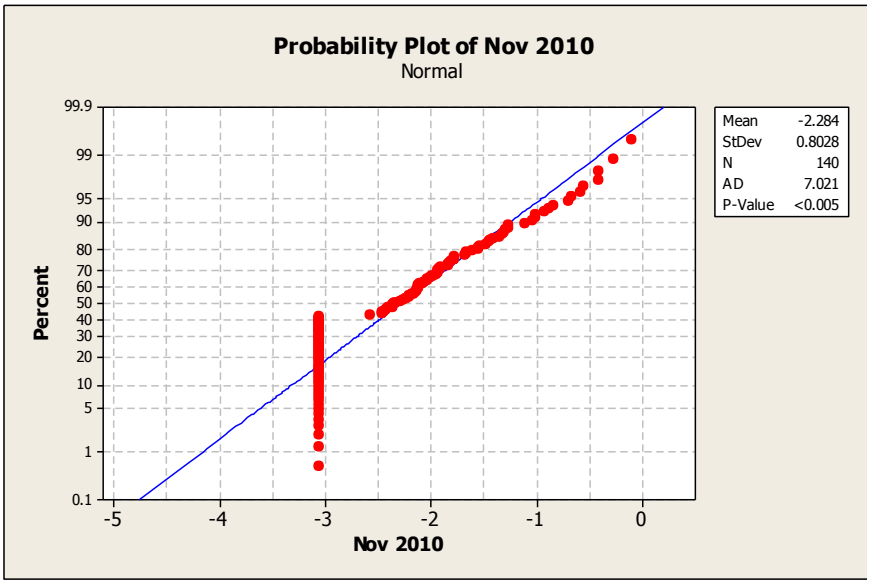

Figure C-11. Normal probability plot of log transformed HMX soil concentrations from the November 13-14, 2010 sampling analyzed using LC/MS.

General Linear Model: Log(HMX Conc) versus Plant Type, Time

Factor	Type	Levels	Values
Plant Type	fixed	2	0, 1
Time	fixed	2	1, 2

Analysis of Variance for Log(HMX Conc), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Type	1	0.0704	0.0000	0.0000	0.00	0.996
Time	1	0.2770	0.0292	0.0292	0.07	0.791
Plant Type*Time	1	3.8667	3.8667	3.8667	9.36	0.003
Error	184	75.9903	75.9903	0.4130		
Total	187	80.2044				

S = 0.642644 R-Sq = 5.25% R-Sq(adj) = 3.71%

Unusual Observations for Log(HMX Conc)

Obs	Log(HMX Conc)	Fit	SE Fit	Residual	St Resid	
6	0.91177	-1.54199	0.07574	2.45376	3.85	R
16	-0.16020	-1.54199	0.07574	1.38179	2.17	R
45	0.07445	-1.54199	0.07574	1.61644	2.53	R
67	-0.12980	-1.54199	0.07574	1.41219	2.21	R
72	0.03033	-1.54199	0.07574	1.57232	2.46	R
98	-0.07259	-1.84930	0.10863	1.77671	2.81	R
136	-0.41844	-1.82306	0.08745	1.40462	2.21	R
181	-0.10255	-1.51474	0.12368	1.41219	2.24	R

R denotes an observation with a large standardized residual.

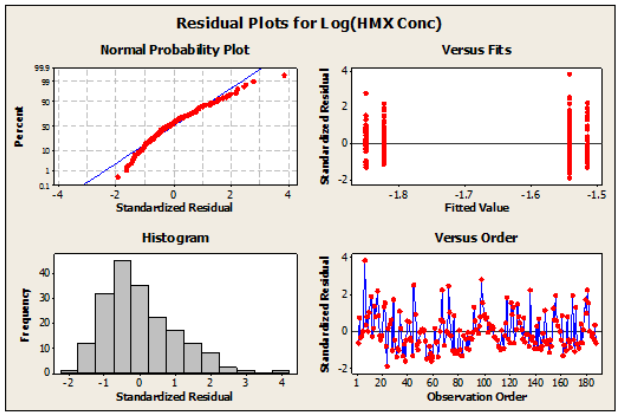

Figure C-12. Minitab© output of the application of a General Linear Model on log-transformed HMX soil concentrations analyzed using LC/MS.