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Phytoforensics on energetics: novel plant tissue measure approach and modeling

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NOVEL PLANT TISSUE MEASURE APPROACH AND MODELING

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ABSTRACT

Explosives and energetics are common soil and groundwater pollutions. This research was to develop novel phytoforensics approaches on energetics. Four different plants species, including woody perennial trees and monocot grasses, were planted both in soil and sand reactors with continuous exposure to a mixture of explosives in the greenhouse. Time dependent assessments were carried out to determine kinetic mechanisms of uptake, transport and accumulation. Plant concentrations were analyzed by both traditional solvent extraction and novel sap analysis methods. A dynamic soil-plant system model was developed to quantify the relationship between tissue concentration and soil pore water concentration for non-volatile organic chemicals with root pathway only. The model included processes of diffusion exchange between root and soil, mass flow in xylem, metabolism and chemical equilibrium in soil and plant interior.

The novel plant analysis method with sap extracted by freeze-centrifuge treatments was validated by solvent extract method on the range of plant species and tissues. The novel approach is rapid, cost effective and labor saving and does not require any soil or water sampling, thereby can access vast field samples not practical previously. The Stella® soil-plant system model was effective in estimating the concentrations in soil pore water, plant sap and tissue from dosing concentration input in the experimental settings of this work. The model can be applied for non-volatile compounds and different conditions with only minor adaptions and might be the base of improvement of soil-plant system models for phytoforensics. The phytoforensic approach was validated on RDX and HMX by both experimental results and simulated results as strong correlation were achieved between plant concentration and exposure concentration.

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NOMENCLATURE

Symbol	Description	
k_H	Henry's Law Constant	10
K_{ow}	The Octanol–water Partition Coefficient	10
K_{oc}	The Organic Carbon Partition coefficient	10
K_d	Distribution/ Partition Coefficient	15
C_e	Soil or Tissue Concentration	52
C_s	Chemical Absorbed in the Tissue	52
C_w	Sap or Pore Water Concentration	52
ρ_w	Sap Density	52
n	Water Content	52
m	Chemical Mass	59
M	Bulk Soil Mass or Fresh Tissue Biomass	59
k	Growth Rate	59
Q	Flow Rate	60
A	Area	60
T_c	Transpiration Conductance	60
E_c	Evaporation Conductance	60
A/V	Ratio of Area to Volume	60
ρ	Fresh Tissue or Bulk Soil Density	60
C_d	Dosing Concentration	61
P	Root Permeability	61
k_m	1^{st} Order Degradation (Transform) Rate	61

1. INTRODUCTION

1.1. BACKGROUNDING

Explosives and energetic compounds are problematic at many active or former military installations, munitions productions facilities, and industrial facilities due to decades of use and previous disposal and handling techniques. These contaminants pose a threat to ecosystem and human health[1-4]. However, the pollutants are often dispersed and usually difficult to access, making understanding the potential threat and designing efficient remediation approaches difficult. Traditional sampling methods of contaminant delineation are expensive, time-consuming and invasive. Great expense and effort has been put into developing methods for detecting and quantifying the energetic compounds that are fugitive in our environment. The US Air Force alone is estimated to spend over US$60 million annually on groundwater analysis for various compounds including solvents, energetic and munitions, yet contaminants go undetected and personnel are unnecessarily exposed to many undetected or poorly delineated contaminants. This contamination is often over broad areas at highly variable concentrations and many times include active ranges. The potential to contaminate drinking water supplies or surface water with the munitions contaminants are a specific public health and ecological concern. The scale in the problem in the US was outlined in a US GAO report in 2004, estimated that remediating and cleaning up unexploded ordnance, discarded military munitions and munitions constituents on its ranges would cost between US$16 billion and US$165 billion and covers an estimated 25 million acres (10 million hectares)[5].

Plants provide a vascular system connecting the subterranean world with the biosphere and atmosphere in active, solar-driven transport processes that link water,

carbon and nutrient cycles. Plants also collect some subsurface contaminants and transport them above ground via the same transport processes. Above ground tissues afford easy sampling access, which is the foundation of novel field "phytoforensics"[6, 7]. Phytoforensics utilizes plants present at sites as the biosensors, and then employs novel sampling methods and rapid, cost-effective chemical analysis of the plant tissues that offers: 1) understanding of contaminant distribution in plant tissues, 2) high spatial and concentration accuracy for site assessment, 3) low per-sample costs for analysis, 4) minimal invasion/disturbance to sites and 5) simplicity for application in a wide range of field settings. Phytoforensics can be an effective tool to screen broad areas and characterize source areas, and then sampling strategies can be efficiently generated for subsurface investigations and long-term groundwater monitoring. This approach has been deployed for the VOCs using headspace-SPME analysis [8-11], but considerable challenges are posed by energetic contaminants, as the low volatility prohibits the proven headspace methods.

Explosives are divided into three main groups, including nitroaromatics, nitramines and nitrate esters. Nitroaromatic explosives contain an aromatic ring with multiple nitro groups. The most widely used nitroaromatic explosive is 2,4,6-trinitrotoluene (TNT), containing three nitro groups. Additional nitroaromatic compounds found as contaminants in military training ranges include dinitrotoluenes (DNTs), used in propellants and being also byproducts associated with the manufacture and transformation of TNT, Aminodinitrotoluenes (ADNTs), diaminonitrotoluene and nitrobenzenes[12]. 2,4-Dinitroanisole (DNAN) is another nitroaromatic explosive, expected to replace traditionally used explosives such as TNT since it is an insensitive

munitions compound[13, 14]. Nitramines contain N-nitro groups. The most important military high explosive currently used is 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), which is often found, together with TNT, in ordnance, land mines and in the plastic explosive Composition. Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is used in many anti-tank weapons and military-grade RDX contains about 10% HMX impurity[12]. Nitrate ester explosives are esters of nitric acid, which commonly contain O-nitro groups. The main nitrate esters used as explosives are nitrocellulose, used in blasting gelatins, glyceroltrinitrate (nitroglycerine, GTN), added to nitrocellulose to improve stability and pentaerythritoltetranitrate (PETN), used in blasting caps and detonators[2, 12].

This research was to develop novel phytoforensics approaches on energetics. Studies ranged from whole plant studies conducted in the greenhouse (explosives) to full scale field assessment (perchlorate). Four explosives covering main groups including RDX, HMX, TNT and PETN were investigated in this research with four plant species, including both woody perennial trees and annual grasses. Trees are expected to offer the greatest potential for gathering contaminant information, as they utilize more groundwater than grasses and have a greater capacity to serve as a reservoir of contaminants. The perennial trees hybrid poplar (*Poplusdeltoides x nigra,* clone 34) and laurel leaf willow (*Salix pentandra*) were selected in this application as they are common species used in phytoremediation and can be propagated from clone. Artillery ranges to eventually be tested are expected to be void of large trees and many contaminated areas have been stripped of perennial vegetation; so the range grasses big bluestem (*Andropogon gerardii*) and perennial ryegrass (*Lolium perenne L*) also were selected to

offer the widest range of plants expected at contaminated sites. These plant species targeted a scientifically important range of plants that offered a spectrum of information on munitions-plant interactions that can be compared to existing literature.

An advanced analysis method by mass-spectrometry (MS) detector has been developed in collaborative work at Missouri University of Science and Technology(S&T). This LC-MS/MS analysis method can analyze 7 explosives simultaneously within 6 minutes and with lowest method detection limits (MDLs) published for these compounds[15]. This unique analytic technique is fast and highly sensitive, which allows determination of explosives even at sub-ppb levels.

Current plant tissue analysis methods involve solvent extraction[16, 17]. Such methods are not environmentally friendly, requiring a large quantity of solvent, and are time and labor intensive. Thus novel sampling approaches are needed and are herein developed and validated coupled with the LC-MS/MS analysis method mentioned above. Novel plant tissue analysis methods are to extract and analyze sap directly. As most of plant tissues have more than 40% (W:W) moisture content, ample aqueous volume is available in plant sample. The solid-liquid separation methods include filtration, squeezing, capillary action, and centrifugal separation and compaction. The centrifugation method is simple without any requirement of specialized apparatus. Plants retain water in tissues efficiently and direct centrifugation may not work on plant tissue so that a pretreatment method is needed to enhance liquid draining off. The freezing process disrupts the cellular structure by expanding the water trapped in the tissues, allowing water to drain readily after thawing. Then high speed centrifugation separates liquids from tissues. Thus, freezing-thawing-centrifugation treatments are assumed to be

able to effectively remove sap from plant tissue. Centrifugal filters are applied in the centrifuge tube to separate liquids and tissues if necessary.

Although a variety of research has been made to understand the fate of explosives in plants, the information is still not enough to develop phytoforensic modeling on explosives. Some plant models have been developed for pesticide and other organic compounds, however, the limitations of modeling application are still evident. The main reasons are that the complex physiological processes in different plant species are not completely understood and some parameters are difficult to obtain. In part, the arduous and labor intensive sampling and analysis methods have limited the data that can be efficiently quantified. The data from laboratory and field study to calibrate and validate current modeling are limited. Research proposed and carried out herein can measure the sap concentration directly in a variety of plant species and specific tissues, making it feasible to better elucidate and understand the fate and distribution of the contaminants in plants and develop an applied phytoforensic model for explosives.

1.2. TECHNICAL GOAL AND OBJECTIVES

Developing novel phytoforensics approaches for energetics is the overarching goal of this work. To reach this goal, specific hypothesis-based objectives have been generated, including:

Objective 1: Develop and Validate Novel Plant Analysis Methods. Chemicals are present in the plant tissue fluid at equilibrium with concentrations sorbed to plant tissue. Contaminant concentrations in the fluid are proportional to the levels in the plant tissue,

and are thought to eventually be proportional to the explosive contamination level in the soil/groundwater. Direct plant-tissue fluid sampling followed by accurate analysis can quantify energetic contaminants concentration and uncover the soil/sediment contamination.

Objective 2: Assess the Impact of Bacteria Degradation and Soil Sorption. The impact of soil must be understood completely to relate plant tissue concentration to groundwater concentration. Different soils impact contaminant fate and must be investigated individually. Sorption and biodegradation impacts vary with soil type and impact contaminant uptake by plants. The diverse biological activity of the high organic soil and sorption processes impact contamination transport into roots and must be understood in relation to plant concentrations.

Objective 3: Determine the Time Dependent *In-planta* Concentration. In phytoforensic sampling any time variation must be known and either understood or avoided if possible. Steady state in plants is preferred to delineate the contaminants concentration in the subsurface by evaluating *in-planta* concentration. Although in the strict sense, steady- state in plants is maybe unrealistic due to complex physiological processes and the constantly changing environment conditions in the field, a relative stable *in-planta* concentration, such as in the range of ≈ 30% fluctuation, still can be attained. Different plant species may need longer or shorter term to reach apparent steady state due to different plant physiology and environmental conditions.

Objective 4: Determine the Spatial Dependent *In-planta* Concentration. The energetic compounds distribution in the whole tree must be understood to select phytoforensic sampling target tissue and position. The leaf or grass frond as terminal

tissue of transpiration can have significantly different concentration compared to the transporting tissue of transpiration (i.e. stem or branch). The concentration differences among the plant compartments also result from the difference of partitioning between tissue and sap as well as different metabolism rates and mechanisms. Finally, the interior difference of transport tissue must be better clarified for non-volatile energetic contaminants.

Objective 5: Determine Relationships between *In-planta* Concentration and Exposure Concentration. A clear understanding of the relationship between *in-planta* concentration and surrounding concentration in soil and groundwater constitutes the basis for feasibility of phytoforensics on energetic contaminants. Differences in plant types and soil properties will result in varying relationships that have not been observed or studied.

Objective 6: Develop Mathematical Phytoforensic Models. The correlation between *in-planta* concentration and surrounding concentration is impacted by many parameters including soil properties, plant types and environmental conditions. The relation derived from the specific experimental measurements and data is not applied widely. A mathematical model composed by a series of mathematical equations describing transport and transformation processes is needed to enhance the understanding of this complex soil-plant interaction system and is widely applied into a variety of field assessment for phytoforensics and design for phytoremediation projects.

Objective 7: Apply to a Full-scale Field Assessment. The scientific reduction approach applied in the controlled greenhouse and laboratory studies represents the actual processes in an uncontrolled field site, and the results can be translated to relate tissue concentrations and subsurface contaminant concentrations across a vast area.

1.3. SIGNIFICANCE

Intellectual Merit: This research represents the first scientific endeavor for phytoforensics of energetics and battlefield contaminants. The novel sap sampling and analysis methods will benefit to analyze plant tissues rapidly and with greater sensitivity than current methods. Plant-explosive interactions had been investigated by various researchers, yet the knowledge gaps on spatial distribution and temporal change of *in-planta* concentration had not been determined prior to this research.

Broader Impacts: The novel sampling methods are rapid, solvent free, cost effective and labor saving, thereby accessing vast field sampling not available previously. Phytoforensic techniques allow for faster and more accurate site assessment, being less intrusive and much lower costs. A special benefit for explosives is to alleviate UXO concerns in site assessment efforts, which make preliminary screening of suspected contamination sites totally infeasible. The findings of this work also benefit for the development of phytoremediation and protection of environment and human health.

1.4. COLLABORATOR WORKS

The funded project was large including many collaborators over the one year of funded research. As a large collaborative project, not all data were solely produced due to my efforts. Herein, the works from collaborators are noted: 1) centrifugation method optimization works were done by Dr. Adcharee Karnjanapiboonwong and Ruipu Mu; 2) the sap sampling of two planting experiments, hydroponic planting experiment and small scale tree and grass experiment, were done in collaboration with Xiaojing Wang and Dr.

Karnjanapiboonwong ; 3) all LC-MS/MS analysis of collected samples was carried out by Ruipu Mu, and IC analysis for perchlorate samples from the field site was carried out by Danielle Marie West.

2. REVIEW OF LITERATURE

2.1. PROPERTIES AND ENVIRONMENTAL FATE

Chemical structures decide physical and chemical properties, which further decide behaviors in the environment. The physical and chemical properties as well as constants of the main explosives are summarized in Table 2.1 from wide sources[4, 13, 18-24].

The Henry's Law constant (k_H) facilitates the estimation of the mass transfer of a compound between water and air. All main explosives possess low vapor pressures and moderately low Henry's Law constants. Thus, the volatilization from soil or groundwater to the atmosphere is negligible.

TNT, DNAN and GTN show the moderate water solubility, while RDX, HMX and PETN show low water solubility. However, fortunately, nitroaromatics and nitrate esters explosives have stronger sorption to the soil matrix to limit a high degree of mobility compared with nitramines explosives according to their K_{ow} and K_{oc} values. The octanol–water partition coefficient (K_{ow}) is the ratio of the concentration of a chemical solute in octanol and in water for equilibrium at a specified temperature. K_{ow} serves as an indicator for the tendency of the chemical to bioaccumulate. The organic carbon partition coefficient (K_{oc}) is the ratio of the concentration of a chemical solute in organic carbon and in pore water at equilibrium in the soil, serving as an indicator for the tendency of chemical sorption to soils or sediments. The low log K_{ow} values of RDX and HMX indicate nitramines can be readily transported down to groundwater and spread to wide areas. But the low solubility, especially for HMX, limits its concentration in groundwater.

Reduction is the key chemical transformation pathway of highly oxidized energetic compounds under anoxic and suboxic conditions. One-electron standard

reduction potentials is a thermodynamic parameter for predicting the rate and extent of reductive transformation for energetic residues. Faster reduction was observed for explosives having more positive value of one-electron reduction potentials[13]. Biodegradation is discussed in the later sections in detail. As for abiotic transformation, photolysis is the primary process at the soil surface for TNT[18]. An alkaline condition can rapidly initiate base-catalyzed hydrolysis and can rapidly transform TNT and other energetic material[25]. RDX also was reported to undergo direct or indirect photolysis in aqueous solution[26]. The direct photolysis of HMX in soil has not been observed [18].

2.2. INTERACTION BETWEEN SOIL AND EXPLOSIVES

2.2.1. Transformation in Soil. All laboratory findings [18, 27-30] showed the high consistence on the conclusion that "TNT degrades rapidly in the order of hours to days in spiked sediment, soils, and natural waters" mentioned in the publication by Conder.et al[31]. The transformation decreased at higher TNT soil concentrations. TNT transformation is more rapid under highly reducing conditions (Eh =-150 mV) than under oxidizing conditions (Eh =+500 mV). It is also widely agreed that TNT readily biotransforms under both aerobic and anaerobic conditions to products such as hydroxylamine and amine derivatives. However, there are contrary views on the mobility of the transformation products. One point of view is that the transformation products can irreversibly bind to organic soil fractions, resulting in a reduction of bioavailability and

Table 2.1 Physical and Chemical Properties of Main Explosives[4, 13, 18-24]

Compound	TNT	DNAN	RDX	HMX	GTN	PETN
Molecular weight	227.13	198.14	222.12	296.15	227.09	316.14
Molecular formula	$C_7H_5N_3O_6$	$C_7H_6N_2O_5$	$C_3H_6N_6O_6$	$C_4H_8N_8O_8$	$C_3H_5N_3O_9$	$C_5H_8N_4O_{12}$
Vapor pressure (mmHg)	1.99×10^{-4}; 8.02×10^{-6}	N.A.	1.0×10^{-9}; 4.0×10^{-9}	3.3×10^{-14}; 2.41×10^{-8}	2.6×10^{-6}; 2.0×10^{-4}	3.8×10^{-10}; 1.36×10^{-7}
Henry's Law constants (atm-m3/mol)	4.57×10^{-7}; 2.1×10^{-8}	1.35×10^{-5}	1.2×10^{-5}; 2.0×10^{-11}	2.6×10^{-15}; 8.67×10^{-10}	N.A.	1.32×10^{-9}
Solubility in H2O (mg/L, 20°C)	100-130	234-267 (25°C)	38-42; 59.7 (25°C)	6.6	1380 1800 (25°C)	43 (25°C)
One-e^- reduction potentials (volts)	-0.30	-0.40	-0.55	-0.66	N.A.	N.A.
Partition coefficients						
Log K_{ow}	1.6-1.84	1.61	0.81-0.87	0.06-0.19	1.62	2.38 Estimation
Log K_{oc}	2.1-3.2	N.A.	0.8-4.2	2.8	2.77	N.A.

toxicity[32]. The irreversible binding inhibited the advanced mineralization of TNT. Results of nuclear magnetic resonance techniques using stable isotopes of nitrogen and carbon confirmed covalent bonding of TNT transformation products to functional groups on humic acid. But some research indicated that TNT transformation products may either be more mobile than TNT or showed similar mobility proven by the increased phyto-toxicity of the soil after weathered and aged treatment[3, 18, 29].

Little is known about the fate of DNAN and its transformation products in the natural environment. The one-electron reduction potentials values indicate 2,4-DNAN has similar reduction tendency as 2,4-DNT, but lower than that of TNT (Table 2.1). DNAN was observed to experience different degradation pathways under aerobic and anaerobic conditions. DNAN was transformed to diaminoanisole ($C_7H_{10}N_2O$) in an anaerobic bioreactor[33]. The product formed azobond polymers after exposure to air. Perreault et al reported aerobic biotransformation of DNAN in spiked soil microcosms. The initial 4µmol DNAN was completely transformed in 8 days in soil slurries supplemented with carbon and nitrogen sources and in 34 days in slurries supplemented with carbons alone while DNAN persisted in unamended microcosms. A strain of *Bacillus* (named 13G) that transformed DNAN by co-metabolism was isolated from the soil. HPLC and LC–MS analyses of cell-free and resting cell assays of *Bacillus* 13G with DNAN showed the formation of 2-amino-4-nitroanisole as the major end-product. The isolated *Bacillus* strain 13G did not grow or mineralize significant quantities of DNAN, suggesting cometabolism rather than a growth-linked process[14].

RDX is known to be transformed by both bacterial and fungal strains, and significant mineralization into CO_2 was reported. A *Rhodococcus* sp. isolated from a

RDX-contaminated soil was shown to achieve a 30% mineralization of ^{14}C-RDX in pure culture, and the well-studied white-rot fungus *Phanerochaete chrysosporium* was shown to mineralize 76% of ^{14}C-RDX in soil slurries. Involvement of cytochrome P450 in microbial transformation of RDX was demonstrated[34].

Due to different conformations, HMX with a crown structure is chemically more stable and therefore less amenable to biodegradation than RDX with a chair structure[35]. The persistence of HMX in the contaminated firing range soil or hydroponic solution was indicated along with the significant disappearances of TNT and RDX[18, 36]. Transformation products of HMX were rarely detected in environmental samples. In laboratory studies HMX is stable under a broad range of redox and pH conditions. First order transformation rate constants for HMX ranging from 0 to 0.09 h^{-1} were measured in soil column and shake-test experiments[3]. Alavi et al tested the degradation of HMX, RDX, TNT, and DNT in two different fine sandy loam soils with the initial concentration of 1mg/L. The rate constant was 0.0066-0.017 day^{-1} for HMX and 0.008-0.14 day^{-1} for RDX compared with 1.86-2.38 day^{-1} for TNT[37].

GTN appears to be easily biodegraded by bacteria through sequential denitration steps[20, 22]. The concentrations of GTN at the end of the one-month weathering and aging process ranged from 0% to 40% of initial concentrations (4-673 mg/kg) in freshly amended soil treatments[20].

Although lacking proof from previous publications, PETN might be able to be biodegraded according to the similar chemical structure as GTN. The existing concentrations at battlefields were relative low so that few researches concerned this compound.

2.2.2. Sorption in Soil. The sorption ability of the soil is one factor which can not be ignored for the fate of compounds. Sorption decreases the transport and the bioavailability to plants. Different soils show the huge differences in sorption and biodegradation potential, which result in difficulty in doing direct comparison of different field and laboratory results. However, compared with millions of plant species, the interaction of soil and explosive is much easier to elaborate and understand, especially for soil sorption. Soil distribution coefficient (K_d) is the product of partition coefficients (K_{oc}) (Table 2.1) and the organic matter content of soil. Table 2.2 summarized K_d of TNT, RDX and HMX from the recent literatures. It is deserved to mention that the measured log K_{ow} for HMX is less than other explosives, TNT, and RDX (Table 2.1). However, the K_d of HMX is intermediate between the values for TNT and RDX (Table 2.2).

In addition to the organic matter content, other properties of soil are also considered to strongly determine adsorption. High soil clay content significantly reduced plant uptake by holding explosives in the soils[3, 38]. RDX did not exhibit specific adsorption to clay surfaces as shown for nitroaromatic compounds; however, it can participate in hydrogen bonding with clays[39]. Clay played a significant role in the sorption of HMX[40]. Monovalent and multivalent cations are other factors to affect the sorption of TNT and RDX to soils. Soils with monovalent cation clays (K^+, NH_4^+, and Na^+) were reported to have adsorption constants ranging up to 21-500 L/kg, whereas clays with multivalent cations (Ca^{2+}, Mg^{2+}, and Al^{3+}) had much lower adsorption constants ranging up to 1.7 L/kg[25]. However, although ionic strength is considered to usually affect sorption, Alavi et al concluded that no trend was found in the ionic strength

and pH on the adsorption of RDX, HMX and TNT in two different fine sandy loam soils[37]. Competitive adsorption between TNT, TNT degradation products, and other explosives has been postulated to affect sorption and transport[3, 25].

Batch sorption studies are widely used to determine the isotherm type and sorption constants. Soil characteristics have influence on the isotherm type, for example at the same range the TNT sorption isotherms were linear for soil with low organic carbon content and nonlinear for soil with high organic carbon content. Most reports showed TNT sorption characteristics were nonlinear and best represented by the Freudlich or Langmuir isotherm [25, 39, 41].

Dontsova et al reported linear RDX sorption isotherms in the range of 1-10mg/l[41]. However, Larson et al found that RDX sorption was best characterized by the Freudlich isotherm in the range of 1-25mg/l for six different soils studied[25]. Also Alavi et al used the Freundlich isotherm to describe batch sorption experiments where three different soils were tested with RDX, DNT, and TNT at a range of 0.5-5mg/L and HMX at a range of 0.2-2mg/L[37].

Sorption of high explosives was rate limited. The contribution of chemical nonequilibrium was confirmed by interrupted flow experiments. Adsorption coefficients of both RDX and TNT determined by HYDRUS-1D model in column tests were smaller than those from batch tests for the same soils, probably because they included irreversible attenuation and more complete mixing occurring than in batch experiments[41].

Table 2.2 Distribution Coefficient (K_d) from Sorption Isotherm

K_d (L/kg)			Organic carbon (% dry weight)	Texture	Reference
TNT	RDX	HMX			
0.27-12	0.16-2.2	0.086-5.02			[25, 41]
285.23	36.19		35.4	Clay 55%	[39]
17.90	2.03		4.23	Clay 18%	[39]
1.59	0.57		1.84	Sand 89%; silt 6%; clay 5%	[16]
1.77	0.78		0.89	Clay 20%	[39]
1.6	0.65		0.78	Sand 87.5%; silt 7.5%, clay 5%	[41]
2.4	0.48		0.2	Sand 11.4%, silt 84.1%, clay 4.5%	[41]

2.3. INTERACTION BETWEEN PLANTS AND EXPLOSIVES

2.3.1. Toxicological Profiles. Extensive research has been devoted to defining the toxic effect of TNT and RDX on numerous plants. Little information is available on the toxic effect of nitrate esters on plants. The toxic effects depend on plant species that will be discussed in this section and soil characteristics that have been discussed in the last section. Standardized toxicity assays, such as ISO 1993, 1995; OECD 1984; US EPA 1989a, 1989b, are used to assess the effects of pure and mixed contaminants on terrestrial plants using seed germination, growth, or root elongation as endpoints. Compared to germination, seedling (shoot or root) growth is more sensitive, probably because the

seeds use the energy reserves in the cotyledons for germination[28]. The effective concentrations causing a 20% decrease in biomass, EC20s, are often used as a measure for the lowest observable effect concentration (LOEC)[42]. Transpiration changes are also suggested as phytotoxicity test indicators[43]. Ali et al proposed the reduction state of photosystem II and non-photochemical energy dissipation as a useful tool in bioassay toxicity testing of TNT- polluted soil. The change of chlorophyll-a fluorescence kinetics and corresponding fluorescence parameters were investigated on the lettuce (*Lactuca sativa*) exposed to TNT at concentrations of 32–1000 mg/kg. The fluorescence parameters, related to the reduction state of photosystem II and to non-photochemical dissipation of light energy, showed a strong relation between the inhibitory effect of photosystem II activity and concentration of TNT as well as the biomass growth[44]. Table 2.3 summarizes the published data on the toxic effect of TNT, RDX and GTN from recent literature.

Price et al suggested that TNT was toxic to plants at levels typically ranging from 100 to 500 mg TNT/kg soil depending on plant species and soil characteristics[38]. However, according to the collection of published information, the toxic threshold concentrations are in the range of 1.4 to 311mg/kg, mostly concentrating in the range of 30-50mg/kg (Table 2.3).

Plants reveal much higher tolerance to nitramine explosives. The published screening benchmark for RDX in soil for terrestrial plants is 100 mg/kg, based on the LOEC of 100 mg RDX/kg for cucumber (*Cucumis sativa*) in aged soil. However, some plants tolerated concentrations up to 1540 mg RDX/kg soil in a 55-day exposure test[42]. Rocheleau et al also found that exposures to RDX and HMX had no significant inhibitory

effects on ryegrass (*Lolium perenne*) growth in standard 21-day exposures at soil RDX concentrations even up to 10000 mg/kg[40]. Hydroponic studies demonstrated bioaccumulation of RDX to as much as 15 times the concentrations found in the hydroponic solution with little or no indication of plant toxicity[38]. For HMX, a 28-day hydroponic test indicated that HMX was not toxic to actively growing hybrid poplar cuttings, even under saturated conditions[19]. A Lettuce (*Lactuca sativa*) and barley (*Hordeum vugare*) were not affected by an HMX exposure up to 3,320±1,019 mg/kg using silica or 1,866±438 mg/kg using a forest soil[28].

For GTN, the nitrate ester explosive, the toxic threshold concentrations are in the range of 12 to 200mg/kg (Table 2.3). Rocheleau et al used the same toxicity test approach to study the effects of TNT and GTN on plants, which made the results comparable. The threshold concentration of GTN are close to that of TNT in barnyard grass (*Echinochloa crus-galli*, monot), whereas higher than that of TNT in alfalfa (*Medicago sativa*, dicot)[20, 29]. These results indicated plants were more tolerated with GTN than with TNT. However, the studies on nitrate ester were still very limited. More study on various plants species are needed to make the more solid conclusions. A study with mustard seedlings (*Sinapis alba*) in hydraulic planting showed that GTN transformation products, dinitroglycerin (DNG) and glycerolmononitrate (MNG), had lower toxicity compared with the effects of the parent material[20].

The toxic effects strongly depend on plant species. The classification index of dicots or monocots cannot be used as absolute indicators for toxic effects, although generally the dicotyledonous species are more sensitive than monocotyledonous species, such as for TNT, dicots cress (*Lepidium*) and turnip (*Brassica rapa*) compared to oat

(*Avena sativa*) and wheat (*Triticum aestivum*)[28]; alfalfa (*Medicago sativa*) compared to ryegrass (*Lolium perenne*)[27]; for RDX dicot soybean (*Glycine max*) compared to monocot Maize (*Zea mays*)[45, 46]. Other studies found no indication of a general difference in TNT sensitivity or TNT uptake and transformation between dicots and monocots, such as common bean (*Phaseolus vulgaris*) compared to wheat (*Triticum aestivum*)[27-29]. It was even reported that the dicot alfalfa (*Medicago sativa*) grown showed a higher tolerance to TNT than did the monocot chives (*Allium schoenoprasum*)[28]. In addition, a study indicated that the plants tolerance to elevated concentrations of TNT and RDX were in the order (most to least tolerant) of maize (*Zea mays*, monocot)> tomato (*Solanum lycopersicum*, dicot)> nutsedge (*Cyperus esculentus* , monocot)> lettuce (*Lactuca sativa*, dicot)[3].

In the screening experiments, upright brome (*Bromus erectus*), perennial ryegrass (*Lolium perenne*) and sweet vernal grass (*Anthoxanthum odoratum*) were identified as TNT tolerant plants among sixteen tested grasses[47]. For RDX, white clover (*Trifolium repens*, dicot) and lettuce (*Lactuca sativa*, dicot) were the RDX tolerant plants, while sunflower (*Helianthus annuus*, dicot) and sainfoin (*Onobrychis viciifolia*, dicot)were RDX sensitive plants among fifteen tested plants. Overall, dicots were more sensitive to RDX than monocots based on growth responses and developmental effects[48].

2.3.2. Uptake and Distribution. The organic compounds with log K_{ow} at the range of 1 to 3.5 are widely accepted to be more easily taken up by plants. The organic compounds with small log K_{ow} are difficult to diffuse into the organic membrane. On the contrary, compounds with very low water solubility will strongly be bounded to cell membrane, which inhibits the advance transport in plants. According to the value of log

K_{ow} (Table 2.1), explosives belonging to nitroaromatics and nitrate esters groups are prone to be extracted by plants. For nitramines group, RDX has a log K_{ow} value close to 1, whereas HMX has a lower logK_{ow} value, suggesting HMX is more recalcitrant to be taken up.

Hydroponic planting is a general approach to study phytoextraction. Extensive research has demonstrated many explosives can be taken up by numerous plants, including terrestrial herbaceous plants, woody plants, aquatic plants and other submersed and immersed wetland plants[18, 43, 49-52]. The degree to which this occurred differed for different explosives. For example, Moon et al. observed that a rapid decrease in the concentration of TNT, less than 5.4±4.7% of the initial concentration of 5.3 mg/L, along with RDX, 60% of the initial concentration of 3.6 mg/L, and HMX, 95% of the initial concentration of 2.4mg/L, after 3 days when poplar cuttings were exposed to the combinations of three explosives[36]. These study conclusions agreed with theory based on log K_{ow} value. Also Moon et al suggested that the presence of other explosives in the solution did not affect the uptake of individual compounds[36].

Methods of using radio labeled compounds are often used to study the translocation of compounds after being extracted. Mass balance is applied to calculate the distribution in the whole plants. The previous studies showed the fates of explosives in plant vary greatly.

In the laboratory, researchers collected a wide variety of abundant plant species and found that most TNT labels were located in the roots and TNT was highly metabolized to bound residues and more polar products[30, 32, 36, 45, 52, 53]. The parent compound of TNT was rarely recovered from plants. Both reduction and oxidation

pathway of TNT are assumed in plants. The taken up label translocated into aerial parts was less than 25% for 4 agronomic plants, maize (*Zea mays*), wheat (*Triticum aestivum*), rice (*Oriza sativa*), and soybean (*Glycine max*) in a 42-day- long exposure[45]. Trees translocated even less efficiently, only 3.3% to 14.4% of ^{14}C were located in aboveground tree portions for hybid poplar tree (*Populus deltoides x nigra*, DN34)[32, 36], hybrid willow (*Salix spec.*, clone EW-20) and Norway spruce (*Picea abies*)[53]. A field survey of native plants at a TNT contaminated site indicated no explosives in aboveground plant tissues, but accumulation of TNT, 2-ADNT and 4-ADNT in some roots, which confirmed the laboratory studies[3].

For nitramine explosives, researchers collected a wide variety of plant species and found the accumulation of RDX and HMX in the leaf tissues[19, 36, 38, 45, 50]. RDX was mainly found in its parent form [45], however an increasing trend of polar metabolite production with time was observed[50]. No HMX transformants in leaf tissue were found and HMX was concluded to be more recalcitrant than RDX[18, 19, 36, 40]. The taken up label translocated into aerial parts was more than 80% for 4 agronomic plants, Maize (*Zea mays*), wheat (*Triticum aestivum*), rice (*Oriza sativa*), and soybean (*Glycine max*) in a 42-days RDX exposure[45]. Similarly, Common bean (*Phaseolus vulgaris*) showed 69%, 18% and 13% of the taken up label accumulated in leaf tissues, stem and root tissues, respectively, after 7 days RDX exposure[50]. Up to 60% of the RDX taken up by hybrid poplar trees (*Populus deltoides x nigra*, DN34) accumulated in leaf tissues after 7 days [^{14}C]RDX solution exposure[50]. Phosphor imager autoradiography showed higher concentration of ^{14}C labels was distributed to the edge of leaves and older leaves. Stronger ^{14}C activity specifically around chloroplasts and lignified tissues detected by

microscope-level autoradiographs of the leaf sections, indicating that translocation of RDX or its metabolites into chloroplasts or conjugation of these molecules into plant structure, such as cell wall[54].

HMX translocation was investigated in hybrid poplar trees. 70%, 6.8%, 2.5%, 13.9%, and 7.2% of the taken up [^{14}C]HMX was translocated and accumulated in leaves, roots, new stems, bottom old stems, and upper old stems, respectively. The distribution of label among plant tissues kept constant for three sampling time (30days, 50days, and 60days)[19]. When poplar cuttings were exposed to the combinations of three explosives: TNT, RDX and HMX, 64.1% of the taken up ^{14}C-RDX and 57.9% of the taken up ^{14}C-HMX were recovered in leaves after 30 days[36].

Much less research was done on nitrate ester explosives. Riefler et al reported that GTN was readily taken up by yellow nutsedge (*Cyperus escalantus*), common rush (*Juncus effuses*), and yellow foxtail (*Setaria glacula*) from hydroponic solution containing initial GTN concentration of 10 mg/L. 12% and 5% of the initial GTN accumulated in yellow nutsedge and common rush respectively after 5 days of exposure with slightly higher concentration in the leaves. No GTN was found in yellow foxtail tissues[22]. Similarly, Rocheleau et al reported that no GTN was detected in ryegrass(*Lolium perenne*) after 35 days soil exposure[20].

Due to the accumulation of nitramine explosives in the plant leaf tissue, determination of their tissue accumulation concentration becomes meaningful in regards two objectives. One is to assess the ecological risks due to the possibility for these compounds to enter food chains. The other is for phytoforensic application mentioned in the beginning of this review. Table 2.4 summarized the exposure concentration, tissue

concentration and bioconcentration factors (BCF) of RDX and HMX from the recent literatures. Briefly, tissue concentration ranged from 62-5217 mg/kg for RDX and 26-380 mg/kg for HMX in monocotyledonous species, where the perennial ryegrass (*Lolium perenne*) revealed highest accumulation capabilities on both RDX and HMX [16, 18, 27, 38, 40, 42, 46, 55]. In dicotyledonous species except for woody trees the reported tissue concentration ranged from 15-4355 mg/kg for RDX and 50-220 mg/kg for HMX, where the alfalfa (*Medicago sativa*) revealed highest accumulation capabilities on both RDX and HMX [18, 38, 42, 46]. In the hybrid poplar planted in solution with 7.9-26 mg/l RDX for 7 days, the leaves concentrations of RDX were 354-723 mg/kg, while the woody stems concentrations of RDX were 46-121 mg/kg[50].

For phytoforensic application, the correlation between tissue concentration and exposure concentration is the key point. Some observations from uptake and transport studies indeed provided promising information. Chen et al. studied the uptake of RDX by four different plant species from hydroponic solutions and soil: maize (*Zea mays*), soybean (*Glycine max*), wheat (*Triticum aestivum*), and Sudan grass (*Sorghum bicolor*). Results showed that the accumulation of RDX in the plant tissue was concentration-dependent linearly from 6 to 21 mgRDX/L solution or from 12.5 to 100 mgRDX/kg soil[46]. In another study by Best et al, although the relationships built were not linear, RDX, RDX-metabolite hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), and accompanying HMX concentrations in plants were significantly related to concentrations in soil after 55 days of exposure. In response to exposure to RDX-contaminated soil, the RDX concentrations in plants increased initially and decreased subsequently[42].

For phytoforensic application, another key point is the temporal profile and the spatial distribution of tissue concentration. Time dependent plant response in terms of accumulation of the explosives parent and degradation compounds was demonstrated in a 99-day exposure. The shoot RDX and MNX and root RDX of Indian grass (*Sorghastrum nutans*) and dark green bulrush (*Scirpus atrovirens*) were significantly affected by exposure period, indicating that accumulation of RDX increased with time and that MNX levels increased due to degradation of RDX in the shoots[16, 55]. The RDX concentration in the top third of the shoot was 2 to 8 times greater than the concentrations detected in middle third and 6-30 times greater than the concentrations detected in bottom thirds of the shoot in actively growing as well as in mature dark green bulrush after 112 days of exposure. RDX concentrations in plant decreased drastically in all the three treatments after exposure was terminated, indicating effective metabolism[55].

2.3.3. Metabolism Pathways. Plants have amazing capabilities to degrade extracted toxic compounds although the mineralization does not occur in plants as in microbes. The formation of conjugates of xenobiotic compounds is important in plants, leading to their lower toxicity to the plant. The model often proposed is that of the 'green liver'. Toxic compounds taken up by plants are metabolized in three stages: transformation, conjugation, and sequestration.

Numerous in vitro tissue culture tests proved that plants had the capability to metabolize TNT. The initial concentration of TNT at 50 mg/l was fully degraded during 6 hours in tissue cultures of rhubarb (*Rheum palmatum*)[56]. In most studies, TNT reductive transformation by plants has been accompanied by the appearance of 2 and 4-ADNT that are TNT monoamino derivatives. However, their levels were very low, less

than 10%, even 1%, of the transformed TNT concentration [32, 56, 57]. Diaminonitrotoluenes (4,6 and 2,4 DANT) and hydroxyaminodinitrotoluene (HADNT) were also reported as TNT metabolites in some studies. A number of unidentified metabolites were widely reported in plant extracts[32, 53]. These unknown extractable metabolites were very variable, not identical among plant species. However, all of them were of a very polar nature. A common viewpoint is that they are the conjugates formed in the plant metabolism[4, 57].

In addition to the usual reductive pathway, the oxidation pathway also was reported for TNT. Trinitrobenzene (TNB), the product from the oxidation of toluene methyl group, was identified coupling with the degradation of TNT[56]. Bhadra et al isolated and characterized six oxidation products of TNT transformation in the aquatic systems of parrot feather (*Myriophyllum aquaticum*). These monoaryl compounds showed clear evidence of methyl oxidation and/or aromatic hydroxylation [58].

Besides using chemical approaches by looking for the transformation and conjugation products to understand the pathway of plant metabolism, a lot of studies were performed also from the biological approaches, such as enzyme reaction and gene study. As mentioned above, gene studies are beyond the limits of this review. Here, only the enzyme reaction studies are discussed. The nitroreductase enzyme participating in reduction of the TNT nitro group has been widely identified in algae, ferns, monocots, dicots, and trees. Nitroreductase activity was revealed in root cell cytosol and expression was strongly induced by plant cultivated in TNT-containing media. In contrast to the strongly induced nitroreductase, levels of oxidationenzymes, peroxidase and

phenoloxidase, changed very little with TNT addition. This suggested that the main pathway of TNT transformation in plant cells was nitro group reduction[52].

The efforts to elucidate the metabolism processes of the nitramine explosives in plants were evidently less compared with TNT. The three-step pathway of RDX reported by Van Aken et al. was widely accepted. The process involved (i) a light-independent reduction of RDX to MNX and hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX) by intact plant cells. (ii) a plant/light mediated breakdown of the heterocyclic ring of RDX, MNX, or DNX into C1-labeled metabolites, such as formaldehyde CH_2O and methanol CH_3OH; and (iii) a further light-independent mineralization of C1- labeled metabolites by intact plant cells [34]. Many reports identified MNX as the metabolite of RDX in plant tissue[16, 36, 40, 42, 55]. No pathway for HMX was reported.

Just et al presented the concept of phytophotolysis for the first time from a study on RDX degradation process in reed canary grass leaves, as an alternative approach along with more established enzyme-catalyzed processes. Direct photolysis of RDX via ultraviolet irradiation passing into the leaves was hypothesized to be responsible for the observed RDX transformations. In addition, membrane-bound “trap chlorophyll” in the chloroplasts might shuttle electrons to RDX as an indirect photolysis transformation mechanism[26].

The information on nitrate esters metabolism in plants is more limited. DNG and MNG were identified as the metabolites of GTN; and pentaerythritoltrinitrate (PETriN), pentaerythritoldinitrate (PEDN), pentaerythritolmononitrate (PEMN) and pentaerythritol were the transformation products of PETN in tissue cultures of rhubarb (*Rheum palmatum*). PETN disappeared more quickly than GTN did[56, 59].

The pictorial possible plant degradation pathways can be found in the following references: [4, 12, 32, 53] for TNT; [4, 12, 26, 34] for RDX; [56] for PETN.

In addition to the degradation by plant metabolism, it is also reported that the xenobiotic compound can be degraded by the symbiotic bacterium in plants. A pink-pigmented symbiotic bacterium was isolated from hybrid poplar tissues (*Populus deltoides x nigra* DN34). The bacterium in pure culture was shown to degrade TNT, RDX and HMX at a purely cometabolic since the bacterium was unable to use heterocyclic nitramines as carbon and/or nitrogen sources[35].

2.4. PLANTS MODELING

Models are developed targeting to understand complex processes; to predict unknown parameters; and to make the strategic decision based on projections. Plant uptake models are originated to understand herbicide or pesticide fate in plants for herbicide or pesticide design and are developed to make risk assessment of a wide range of environmental contaminants and to design phytoremediation projects. Plant uptake models predict the uptake, translocation, and elimination of chemicals by plants and describe the correlation between plant tissue concentration including roots, stems, leaves and fruits and surrounding concentration in soil, solution and atmosphere. Currently, a variety of models are available, which can be roughly classified into 3 groups based on the theoretical basics.

Table 2.3 Toxic Effect of Main Explosives on Plants

Name	Type[a]	TNT		RDX	GTN	
		Threshold conc.[b]	Lab description	Lab description	Threshold conc.	Lab description
Yellow Nutsedge (*Cyperus esculentus*)	M	5 mg/L	42 days hydroponic study[43]			
Upright Brome (*Bromus erectus*)	M		Tolerance to 41 g/kg soil in 16 grasses screen test[47]			
Barnyard Grass (*Echinochloa crus-galli*)	M	56mg/kg in freshly amended soil; 11mg/kg in aged soil	16 days exposure, EC20- shoot dry mass[29]		13mg/kg for freshly amended soil; 12mg/kg for aged soil	16 days exposure, EC20- shoot dry mass[20]
Barley (*Hordeum vulgare*)	M	55.9mg/kg in silica soil; 291.9mg/kg in forest soil	14 days exposure, emergence and growth[28]			
Sweet Vernal Grass (*Anthoxanthum odoratum*)	M		Tolerance to 41 g/kg soil in 16 grasses screen test[47]			
Oat (*Avena sativa*)	M	311mg/kg	LOEC[28]			
Perennial Ryegrass (*Lolium*	M	3.75mg/kg in aged soil	55 days exposure, EC20[27]	Tolerating concentrations up to 1540 mg RDX/kg	20mg/kg for freshly amended	19 days exposure,

Table 2.3 Toxic Effect of Main Explosives on Plants (cont.)

Name	Type[a]	TNT		RDX	GTN	
		Threshold conc.[b]	Lab description	Lab description	Threshold conc.	Lab description
perenne)				soil in the 55 days exposure[42]	soil; 26mg/kg for aging soil	EC20- shoot dry mass[20]
		61mg/kg in freshly amended soil, 13mg/kg in aged soil	19 days exposure, EC20- shoot dry mass[29]	Tolerating concentrations up to 10000 mg RDX/kg soil in the 21 days exposure[40]		
			Tolerance to 41 g/kg soil in 16 grasses screen test[47]			
Asian Rice (*Oryza sativa*)	M			Bleaching and necrosis in 42 days exposure to 138mgRDX/kg, more sensitive than maize[45]		
Wheat (*Triticum aestivum*)	M			Bleaching and necrosis in 42 days exposure to 138mgRDX/kg, more sensitive than maize[45]		
Maize (*Zea mays*)	M			RDX sensitive plant in 15 grass screen test[48]		
				Tolerating concentrations up to 903 mg RDX/kg soil in the 28 days exposure[46]		
Sunflower	D			RDX sensitive plant in 15		

Table 2.3 Toxic Effect of Main Explosives on Plants (cont.)

Name	Type[a]	TNT		RDX	GTN	
		Threshold conc.[b]	Lab description	Lab description	Threshold conc.	Lab description
(*Helianthus annuus*)				grass screen test[48]		
Lettuce (*Lactuca sativa*)	D	20 mg/kg	LOEC[4]	RDX tolerant plant in 15 grass screen test[48]		
Radish (*Raphanus sativus*)	D	7 to 19 mg/kg	LOEC[28]			
Turnip (*Brassica rapa*)	D	50 mg/kg	LOEC[28]			
Garden Cress (*Lepidium sativum*)	D	50 mg/kg	LOEC[28]			
White Mustard (*Sinapis alba*)	D				200mg/L	Inhibit primary root growth by 80%[20]
Cucumber (*Cucumis sativus*)	D	7 to 19 mg/kg	LOEC[28]	Threshold conc.100 mg/kg, LOEC[42]		
Alfalfa (*Medicago sativa*)	D	43mg/kg in freshly amended soil; 1.4mg/kg in aged soil	16 days exposure, EC20- shoot dry mass[29]	Tolerating concentrations up to 1540 mg RDX/kg soil in the 55 days exposure[42]	74mg/kg for freshly amended soil; 83mg/kg for aging soil	16 days exposure, EC20- shoot dry mass[20]
Soybean (*Glycine max*)	D			Bleaching and necrosis in 42 days exposure to 138mgRDX/kg, more sensitive than maize[45]		

Table 2.3 Toxic Effect of Main Explosives on Plants (cont.)

Name	Type[a]	TNT		RDX	GTN	
		Threshold conc.[b]	Lab description	Lab description	Threshold conc.	Lab description
Sainfoin (*Onobrychis viciifolia*)	D			RDX sensitive plant in 15 grass screen test[48]		
Common bean (*Phaseolus vulgaris*)	D	30mg/kg	LOEC[27]			
White Clover (*Trifolium repens*)	D			RDX tolerant plant in 15 grass screen test[48]		
Hybrid Poplar (Populus sp. *deltoides * nigra*, DN34)	D	5 mg/L	21 d hydroponic study, Transpiration and biomass decrease, leaf chlorosis and abscission[43]			
Tobacco (*Nicotiana tabacum*)	D				113.5 mg/L	Failure to germinate[20]

[a]: Type: M-monocots; D-eudicots

[b]: LOEC: the lowest observable effect concentration;EC20: the effective concentrations causing a 20% decrease in biomass.

Table 2.4 Tissue Concentration in Plants

Name	Type[a]	RDX				HMX			
		Exposure period (day)	Exposure conc.	Leaves conc (mg/kg)	BCF[b]	Exposure period (day)	Exposure conc.	Leaves conc (mg/kg)	BCF
Yellow Nutsedge (*Cyperus esculentus*)	M	45	50.3 mg/kg	62.46	1.2[38]				
Woolgrass Bulrush (*Scirpus atrovirens*)	M	112	0.5-3 mg/l (dosing solution)	29-60 (FW)	20-58 l/kg FW[55]				
Perennial Ryegrass (*Lolium perenne*)	M	55	59.2-154 mg/kg	2948-5217	19-88[27]	55	7-17.2 mg/kg	62-102	3.6-14.5[27]
		55	645-1540 mg/kg	2055-3886	2-5[42]	55	8.6-41 mg/kg	26-50	1-3[42]
		42	8.1-9780 mg/kg	119-1690	0.2-14.6[40]	42	3.6-9976 mg/kg	39-325	0.03-10.7[40]
						77	32mg/kg	380	11.9[18]
Yellow Indiangrass (*Sorghastrum nutans*)	M	92	30.3-85 mg/kg	3982-4835	56.9-131.4[16]				
Sudan Grass (*Sorghum bicolor*)	M	28	6-21 mg/l; 25-903 mg/kg	72-436; 314-1414	12-20.8l/kg; 1.3-14.1[46]				
Wheat (*Triticum aestivum*)	M	28	6-21 mg/l; 25-903 mg/kg	65-408; 888-2828	11-19.4l/kg; 2.1-35.5[46]	77	32mg/kg	200	6.3[18]
Maize (*Zea mays*)	M	28	6-21 mg/L; 25-903 mg/kg	95-300; 300-1210	15l/kg; 0.7-12.1[46]				
Lettuce (*Lactuca*	D	45	50.3 mg/kg	1172	23.3[38]				

Table 2.4 Tissue Concentration in Plants (cont.)

Name	Type[a]	RDX				HMX			
		Exposure period (day)	Exposure conc.	Leaves conc (mg/kg)	BCF[b]	Exposure period (day)	Exposure conc.	Leaves conc (mg/kg)	BCF
sativa)									
Radish (*Raphanus sativus*)	D	42	3.43mg/kg	159	46.4[38]				
Canola (*Brassica rapa*)	D					77	32mg/kg	95	3.0[18]
Alfalfa (*Medicago sativa*)	D	55	645-1540 mg/kg	3997-4355	3-6[42]	77	32mg/kg	220	6.9[18]
						55	8.6-41 mg/kg	65-84	2-7[42]
Soybean (*Glycine max*)	D	28	6-21 mg/l; 25-903 mg/kg	76-168; 181-492	8-12.7l/kg; 0.3-7.2[46]				
Common bean (*Phaseolus vulgaris*)	D					77	32mg/kg	50	1.6[18]
Hybrid Poplar (Populus sp. *deltoides * nigra*, DN34)	D	2-7	7.9-26 mg/l	354-723; 46-121 (stem)	27-45 L/kg; 2.9-5.8 L/kg (stem)[50]				
Tomato (*Solanum lycopersicum*)	D	77	2.12mg/kg	15.1	7.1[38]				

[a]: Type: M-monocots; D-eudicots

[b]: BCF: bioconcentration factors = tissue concentration/exposure concentration.

The first group is empirical models[60-63]. This kind of regression-based models is derived from experimental bioconcentration factors. The steady-state concentrations in the root and aerial parts of the plant relative to the chemical concentration in the surrounding soil were related to a simple chemical property, such as K_{ow}. These models do not consider individual plant properties or environmental conditions. The advantage of empirical models is ease of application as only a parameter is required. Also such models are maybe quite good in the predictive power in a certain range of conditions, plants and pollutants. The disadvantage is that empirical models are unable to explain the underlying processes and the application is only for a limited scope. McKone et al suggested that a difference of 10-fold higher or 10- fold lower from a regression model without additional information regarding plant species or without plant and site-specific measurements[64].

The second group is simple compartment mechanistic models [65-69]. This type of models considered diffusion exchange, mass flow in xylem and phloem, metabolism and chemical equilibrium in the plants by a mechanistic manner. The diffusive flux across membranes between soil and root or air and leaves was described by Fick's 1st Law of Diffusion. Metabolism was described by the 1st order kinetics (mostly) or the Michaelis-Menten Equation (few). The chemicals were assumed at equilibrium with biomass (solid), sap (liquid) and air in the plant interior. However, this type model does not incorporate plant physiological basis. For example, transpiration steam concentration factor (TSCF), were applied to avoid detailed simulation of the apoplast and symplast transport processes in plant root. Most developed plant uptake models fell into this category as this kind of models best balanced the theoretical and practical requirements and were applicable to different chemicals and plant species. The input data required

including chemical properties, plant properties and environmental conditions were much more than the empirical modes, however much less than the third group of models described below.

The third group is physiology-based mechanistic models[70-73]. These models are based on the plant physiology and consider plant anatomical characteristics in detail. Mechanisms are broken down to plant compartments of multiple tissue and are parameter-intensive, including plant physiological features (xylem/phloem connections, cuticle thickness, membranes permeability, apoplast, symplast, and vascular sap pH, transpiration rate, sap velocity, etc.), xenobiotic physicochemical properties (molar volume, partition coefficient, etc.), and environmental conditions. Satchivi et al used 7 compartments and 7 processes to describe the transfer through the cuticle, movement into the leaf mesophyll symplast and phloem loading of foliar-applied xenobiotics[71]. Trapp et al divided root into 3 sub-compartments, the apparent free space, the root cortex and the central cylinder, to simulate xylem loading process [73].The complex physiological processes in different plant species are not completely understood for many plant types and many input parameters are difficult to obtain. Tissue-specific data on contaminant concentrations are also lacking, thereby limiting the development and application of this type of model.

The second and third groups of models were series of 1st order differential equations in mathematical form, which were difficult to be solved analytically and the finite difference method was usually applied to obtain numerical solution. This numerical solution described the dynamic uptake from soil, solution, or atmosphere, and the metabolism and accumulation of xenobiotic chemicals in roots, stems, leaves, and fruits,

called as dynamic models. When only steady state was considered, some 1st order differential equations were transformed into linear equations, which were easy to be solved analytically. This analytic solution only described the plant tissue concentration in steady state, called as steady-state model.

Although the steady-state models are simpler and often adopted, the question whether steady-state is reached within one growth season or the life-span of a tree has been put forward[67, 74]. The variability of plant –contaminant interactions on a seasonal and even diurnal level brings to question on steady state assumption[6, 7].

Most models were concerned on neutral organic chemicals, as the 'ion trap' occurring for the ions and dissociating compounds incur more complication and more unknowns relative to specific plant transport processes. Two main pathways for chemicals entering plants were identified. Most recent models included both pathways. One was the soil-plant (root) pathway. Root uptook the chemicals followed by xylem loading, transport in the xylem, xylem unloading to leaves and phloem loading. The other was the air-plant (foliar) pathway which was for more volatile compounds. Chemical diffused into foliar via the stomatal and cuticular, followed by phloem loading, transport in the phloem, phloem unloading to sink regions, and potential xylem/ phloem exchange.

All plant models are compartments modeling, as a series of distinct compartments linked by mass flows. In one compartment, the concentrations of contaminants are same in anywhere. However the real measurements objected to this assumption. Concentrations were observed in gradient distribution along the height of the trunk for volatile compounds. The tree “donut ring” model”[75, 76] was developed to stimulated the diffusion of VOCs from xylem into air while chemicals were transported along the tree

trunk, which resulted in the “loss” of the chemicals. Trapp also improved his tree model in his newest publication to reflect the concentration change along tree trunk[67].

In plant uptake models, some important parameters, surrounding concentration, transpiration flow rate, plant growth (biomass) are simplified greatly and considered as constant or exponential. The complex interrelationship between environmental conditions and plant properties are not accounted for. These deviations to reality affect the accurate prediction of models. An improved approach is to integrate the plant uptake models with the contaminant underground transport models and the models for simulation of water, heat, C and N dynamics in soil-plant-atmosphere systems[77]. Thus the combination of several related mature models is a developing trend to improve the prediction accuracy of the environmental fate of contaminants.

3. MATERIALS AND METHODS

3.1. CHEMICALS

Explosive standards were purchased from SPEX CertiPrep (Metuchen, NJ). Explosives used in dosing solution including RDX, HMX, TNT and PETN were from munitions supply at Missouri University of Science and Technology. The solid explosives were dissolved in acetonitrile and further diluted into Milli-Q (MQ) water or Hoagland solution. Ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO). Methanol and acetonitrile were purchased from Fisher Scientific. Solvents used were of LC or LC-MS grade. MQ (resistance >18 MΩ/cm) quality water was used for all aqueous solutions except for large tree dosing. Deionized (DI) water was used for prepare dosing solution in large tree experiment. The modified 10% strength Hoagland solution was prepared by mixing 0.6ml of 1M potassium nitrate, 0.4ml of 1M calcium nitrate, 0.1ml of 1M monoammonium phosphate, 0.2ml of 1M magnesium sulfate, 0.1ml of micronutrient solution (boron, manganese, zinc, copper and molybdenum), 0.2ml iron chelate solution, 0.25ml of 1M sodium hydroxide in 1 L of MQ water. The pH of 10% Hoagland solution was 6.5-7.0.

3.2. PLANTS, SOIL AND SAND

Poplar (*Poplus deltoides x nigra*, clone 34) cuttings and laurel leaf willow (*Salix pentandra*) cuttings were harvested from the coppice growth in collaboration with Ecolotree® North (Liberty, Iowa). Big bluestem (*Andropogon gerardii*) and ryegrass (*Lolium perenne L*) seed were purchased locally. Plants were grown in the rooftop Baker

Greenhouse Facility with temperature of 10-30ºC and humidity of 30-90% based on the recording of whole year. The ambient light was augmented to a 16-hour photoperiod with 400 W metal halide growth lamps. The cuttings were trimmed to about 24cm for small tree and 48cm for large trees in length, grown in tap water and then transplanted in soil or sand reactors after active growths were observed. The grasses were grown from seeds germinated in the reactors. Seeds were sowed evenly on the surface in each reactor and surface watered.

Silt loam soil (40% sand, 45% silt and 15% clay) was used in all experiments except for large trees. The tree and shrub soil (Sta-green®) was used in large tree experiment. The organic matter was determined by ashing a 2 gram sieved dry sample at 550ºC for 1 hour in a muffle furnace. The loss by weight of the sample during this ignition was calculated as the organic matter. The determined organic content was 8.4±0.3% for silt loam and 50.3±1.6% for Sta-green® soil. K_d was determined by batch sorption test as described in the next section.

3.3. BATCH SORPTION TEST

This experiment is to obtain partition coefficient of different explosives on two kinds of soil, sand and four plant tissues investigated in this research.

The tree stem and grass were cut into 3cm length sections and dried at 70°C for at least 12hr. The soil and sand were dried at 100°Cfor at least 12hr and sieved through a 2mm sieve. The sorption test was done by equilibrating 1 gram dried soil, sand, tree stem, or 0.5 gram dried grass with 20ml 1g/L sodium azide solution spiked with different levels

of explosive at 75rpm agitation for 10 days. Sodium azide served as an antiseptic. The same treatments without soil/sand/stem/grass were done as controls. The contaminant mass lost from solution between samples and controls was calculated as the sorption into dry planting media or plant tissue, based on a mass balance. The isotherms were plotted from the sorbed concentrations and the aqueous concentrations. The Freudlich isotherm was applied to fit the data over 4 log units.

3.4. SHORT-TERM HYDROPONIC PLANTING EXPERIMENT

This experiment was designed as preliminary tests to assess the fate of four explosives in young willow trees. The tests were to make sure the plants dosed with low and medium levels of explosives with reasonable growth and the high dosing level did not kill the trees (Table 3.1). Also the tests produced the short term exposure data to compare with longer exposure in soil planting experiments described in the next section.

The tree cuttings were transferred to 250ml brown glass bottles with 150ml dosing solution (target explosives in 10% Hoagland nutrient solutions). The reactors were wrapped in aluminum foil to inhibit photolysis. Every reactor had one tree cutting. Evaporation from the reactors was prevented by filling the opening with hole-cap and foil. Transpiration was measured by weighing individual reactors with a digital balance every three days. Once the cuttings extracted almost all dosing solution, the experiment ended and the trees were harvested, the remaining solution volumes were measured and sampled for concentration analysis. The whole process cost 12-30days based on different transpiration rate. Dosing solution was a mixture of explosives in 10% Hoagland nutrient

solution with five treatment levels showed in Table 3.1. All tests were run in triplicate and with blanks (Reactors without tree).

Table 3.1 The Different Levels of Target Concentration Applied to Dose Plants (mg/L)

	Control	Low level	Medium level	Half High level[a]	High level[a]
RDX	0	0.2	2	10	20
HMX	0	0.02	0.2	1	2
TNT	0	0.05	0.5	2.5	5
PETN	0	0.03	0.3	1.5	3

[a] The dosing solution was sparged with N_2 for 8hr in the fume hood to volatilize acetonitrile (carrier solvent), which was toxic to plants in preliminary studies.

3.5. SMALL SCALE TREE AND GRASS EXPERIMENTS

The arrangement of the experiment mimicking the groundwater-soil-plant continuum of field settings allowed for investigating the complex relationship of dosing concentration, pore water concentration, sap concentration and tissue extraction concentration and understanding phytoforensics potential as well as validating novel sap sampling method. Two rooting media, soil and quartz sand, were investigated to assess different levels of diverse biological activity and sorption processes related to the higher organic content soil.

2L and 1L I-chem glass wide mouth bottle (Fisher) were applied as tree reactor and grass reactor respectively. Each tree reactor included 3 tree cuttings. The experiment included each a total of 32 test units (4 explosive treatments * 4 species * 2 substrates).Four dosing levels included control, low, medium and high (Table 3.1).For soil planting dosing solution was a mixture of explosives in MQ water; while for sand planting dosing solution is mixture in 10% Hoagland nutrient solutions.

Once trees were showing active growth and at least 15 cm of new shoot growth (4-6 weeks), or grass was dense and at least 15 cm tall (6-8 weeks), dosing began. Dosing was done with a bottom feed tube linking to a dosing bottle (250 ml or 500 ml based on transpiration ratio) to maintain a constant water level and even dosing (Figure 3.1). The reactors and dosing bottles were wrapped in aluminum foil to inhibit photolysis. The dosing bottles were checked and refilled daily, and the refilled volumes were recorded as transpiration rate. The real dosing concentration was determined by sampling dosing bottles weekly. The pore water concentration was determined by drawing liquid out using syringe from bottom of each reactor biweekly.

The whole dosing process continued for 90 days for trees and 54days for grasses to ensure contaminants throughout the plants and steady-state reached. The dosing process was extended to 110 days for willows and 84 days for grasses dosed by high level mixtures of explosives as the high concentration of explosives inhibited the growth of plants resulting in the low transpiration rate. Thus longer dosing term made the cumulative transpiration rate in these slow growing plants comparable to other normal growing plants.

In this experiment, only tree stems were analyzed for contaminant concentration because stems (the transient vascular tissue) were expected to keep relative steady concentration. At the termination tree stems were cut into three sections with each approximately 6cm in length to assess the impact of sampling height. Section 'Low' was the stem below soil/ sand; section 'Mid' was the stem above soil/sand, but below the lowest shoot; and section 'High' was the stem above the lowest shoot. Bark (phloem) was removed from xylem-wood of stems. Grasses were collected about 1cm above the substrate surface without advanced segmentation. All samples were split for sap analysis and tissue concentration analysis.

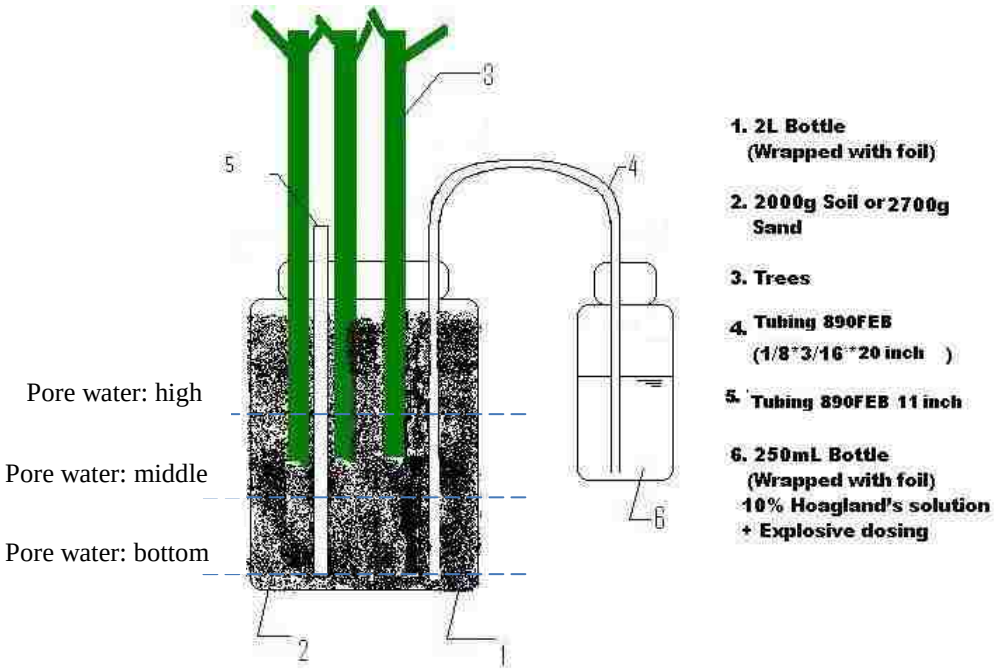

Figure 3.1 The Schematic Diagram ofthe Small Scale Tree Experiments

After harvest of plants, the substrate contaminant distributions were assessed by sampling pore water at other 2 higher levels of reactors (Figure 3.1). The high layer was 1/3 height below the surface, the height of trees inserted, standing for the dense root zone, or about 3 cm below the surface for grasses. The middle layer was 2/3 height below the surface for trees or 1/2 height below the surface for grasses. The sampling method was to centrifuge the sand or soil in 1.5ml centrifuge tube with filter (Figure 3.3). The centrifugation conditions are 11,050 rcf (10,000 rpm) in 30 minutes.

3.6. TIME-SERIES GRASS EXPERIMENTS

Based on initial results in small scale experiment, time-series grass experiments were undertaken to investigate time dependent *in-planta* concentrations. A steady state of tissue concentration was expected to be reached after certain exposure times. Furthermore, the partition relationships between sap and tissue were compared from different harvests to reveal if the partitions were consistent during whole exposure periods.

Aluminum foil pan with size of28cm*23cm*6.4cm was utilized to allow for multiple harvests as enough biomass planted in each reactor. Only quartz sand was investigated to focus on the extraction of plants. The experiment included each a total of 8 test units (4 explosive treatments * 2 species). Four dosing levels included control, low, medium and half high (Table 3.1).

Once grass was dense and at least 15 cm tall (about 8 weeks), dosing started. Because the pan reactors were much shallower than 1- or 2-L bottle reactors, dosing

bottle methods were inapplicable. In order to prevent dosing solution from touching the aerial parts of the plants, two 7.6cm-long glass tubes (ID=1.3cm) were inserted to the bottom of the reactor and two 60ml glass short-stem funnels were placed on the top of glass tubing (Figure 3.2). The dosing solution was added into the funnel daily until the sand surface was moist, and the added volumes were recorded as transpiration rate. The pore water concentration was determined by drawing liquid out using syringe from bottom of reactor biweekly.

The harvest started after 4 weeks' dosing, then continued every other week for 16 weeks or until all plants were gone. Each reactor was separated into 20 equal grids. At each sample period grasses were harvested from 3 random grids for triplication. All samples were split for sap analysis and tissue concentration analysis. At experiment termination, the reactors were spiked with explosives mixture to assess the sand biodegradation potential.

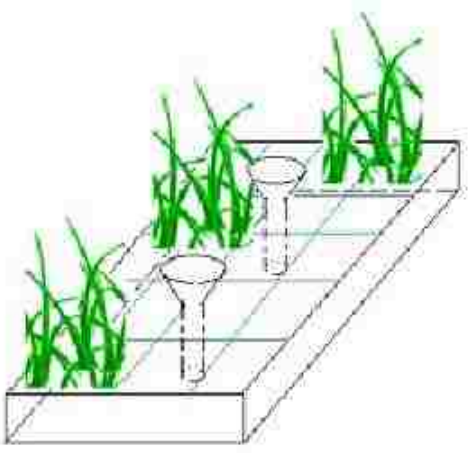

Figure 3.2 The Schematic Diagram of the Time-Series Grass Experiments

3.7. LARGE SCALE TREE AND LONG TERM EXPERIMENTS

This experiment was to study time and compartment dependent *in-planta* concentrations in larger, older trees with heartwood and sapwood. The results were compared with those from small scale tree experiment. This experiment was critical to improve the understandings of the explosives fate in the old trees present in the fields as almost all previous lab data came from young small cuttings. The use of older trees is important as the translocation in the xylem tissues changes with age of the trees. Older tissues do not maintain their conductivity as trees become lignified with age, and recent research by Burken demonstrated that lignin and lipid content changes the binding of organic contaminants in phytoforensic studies[78]. This scale tree has been used in seminal phytoforensic work [7].

20-L and 4-L buckets were applied as reactor and dosing tank respectively. Only willow was investigated in this experiment. These willows were grown from 3- to 5-year old cuttings, approximately 5 to 10 cm in diameter in order to offer ample sampling mass for tree coring. Because of the large soil demand in this experiment, a commercial potting soil mix, Sta-green® tree and shrub soil, was used. The experiment included each a total of 6 test units (2 explosive treatments*3 triplicates).

A dosing tank was linked to each reactor by "bottom to bottom" tubing. Reactors (1#, 2#, and 3#) were dosed at low level initially (72 days) and changed to high level for the remaining period due to high sorption in the Sta-green® soil used. Reactors (4#, 5#, and 6#) were dosed at medium level for the whole experiment period of 240 days. The dosing levels were same as the small scale tree experiment (Table 3.1). The dosing tanks were checked and refilled daily and the refilled volumes were recorded as transpiration

rate. The reactors and the dosing tanks were sampled biweekly to assess pore water concentration and dosing concentration by the method same as small scale experiment.

Total 6 harvests occurred on 63rd day, 112th day, 144th day, 175th day, 194th day, and 240th day respectively. Leaves were sampled in every harvest and randomly from 2 active growing branches exclude the newest and oldest leaves. Leaves from one branch were divided into high and low samples. Tree cores using standard sampling tool were sampled at every harvest besides 5th harvest. Two tree cores were sampled from different height of the trees to access the concentration difference in same plant compartment. The main branches were sampled in the 3rd and in the last harvest besides reactor 1#, where the only main branch was sampled in the last harvest. The samples were acquired every other 50-70cm along the main branch from bottom to the top to assess the distribution of contaminant. 2-4 samples were collected from each branch according to the different length of the branches. Bark (phloem) was removed from xylem-wood. All samples were split for sap analysis and tissue concentration analysis. At experiment termination, contaminant distribution in the soil was assessed by sampling pore water from soil cores taken from approximate middle height of the reactors. Three soil cores were taken by standard soil core sampling tool from each reactor.

3.8. PLANT TISSUE AND SAP ANALYSIS

3.8.1. Solvent Extraction Method. The plant tissue extraction procedure was carried out as previously published [17]. Briefly, plants were cut into small pieces and ground in liquid nitrogen using mortar and pestle. Homogenized tissues (0.8-1.0 gram for

tree stems and 0.5-0.7 gram for grasses) were put into 20- or 40- ml amber glass vials sealed with Teflon-lined septa and mixed with 10ml acetonitrile for tree stems or 20ml methanol for grasses. The extracts were vortexed for 15 seconds, and sonicated for 6hr. The supernatant was taken, filtered through 0.22 µm nylon filter into 1.5ml vial, and diluted at least 2- fold before injection into LC-MS/MS for analysis.

3.8.2. Centrifuge Extraction Method. Fresh plants were wrapped with aluminum foil and put in the freezer, and thawed for half of an hour at room temperature. The thawed samples were cut into small pieces (2 – 2.5 cm in length) to fit the 1.5ml centrifuge tubes with caps. The diameters of tree stems prevented the stems from taking the spaces in the bottom of the centrifuge tubes and the untaken space stored separated sap during centrifugation. However, the centrifugal filters (cellulose 30,000MWCO) (Figure 3.3) were needed for grasses to separate sap and grass during centrifugation. Otherwise, after centrifugation the sample became the moist grass at the bottom and dry grass at the top. The samples were centrifuged at 23,940 rcf (15,000rpm) at 15°C.After sap was collected, the samples were dried at 70°C for 12hr. The weight of fresh samples, samples after centrifugation, dried samples were recorded to calculate the extraction efficiency (%, sap out/total sap, g/g).Freezing time and centrifuging duration were two parameters optimized for best sap extraction efficiency. Freezing time was tested for 0-24 hours; and centrifuging time was tested for 10-60 minutes. The optimization tests were done with five replicates.

According to the optimization test results, the experimental samples were frozen overnight and subjected to 30min centrifugation. The collected sap was then diluted 10foldbefore injection into LC-MS/MS for analysis to decrease matrix interference. Early

analysis diluted low fold suggested that high dissolved solids content of plant sap inhibited the ionization process for MS detector.

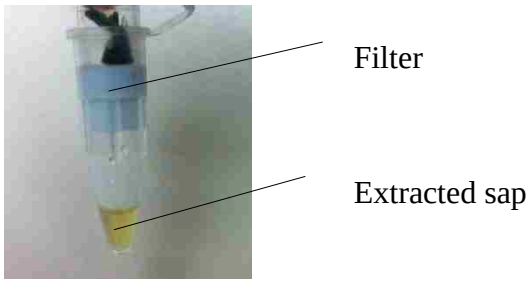

Figure 3.3 The Sap Extracted After Frozen-Centrifugation Treatments for Grass Samples (the 1.5ml centrifuge tube with filter, cellulose 30,000MWCO).

3.8.3. HPLC-MS/MS Analysis. A 4000Q TRAP mass spectrometer (ABSCIEX, Foster City, CA) equipped with an electrospray ionization interface and A Shimadzu UFLC system (Columbia, MD) were used. A knietex C-18 reversed phase column (Phenomenex, 75 mm × 3.0 mm i.d., 2.6 μm) was used to separate explosives with methanol:water (60:40 v/v) containing 1 mM ammonium acetate. The flow rate was 0.25mL/min and the injection volume was 10 μL. Negative electrospray ionization (ESI−) with the multiple-reaction monitoring (MRM) mode was utilized. The ion source temperature was set at 350°C with an ion spray voltage of -4500 V[15].

The blank tissue spiked with predetermined RDX or HMX levels indicated recovery efficiency of above 75% or 60% for all species. The saps were diluted 4 folds and spiked with predetermined RDX or HMX levels, indicating recovery efficiency of

above 70% or 50% for all species. The detection limits based on signal to noise (S/N) ratio at 3-5 for both methods were summarized in Table 3.2. These results were obtained from solvent extracts without any dilution and sap with 4 fold dilution. In all experimental sample analysis besides the earlier small tree stem sap analysis, higher folds dilution was done to decrease interference and increase recovery efficiency.

Table 3.2 The Method Detection Limits (MDL) for Solvent Extraction Method and Centrifuge Extraction Method Based Signal to Noise Ratio (S/N) of 3-5

	Sap (*4 dilution)[a], ng/ml		Determined tissue[b], ng/g FW		Calculated tissue[c], ng/g FW	
	HMX	RDX	HMX	RDX	HMX	RDX
Poplar stem	3	0.4	10	10	162	10
Willow stem	0.8	0.3	5	5	33	7
Ryegrass	0.4	0.4	5	5	4	3
Big bluestem	0.8	0.8	10	1	7	9

[a]: Sap was obtained from centrifuge extraction method. The determined MDLs were multiplied by 4 due to 4 folds dilution.

[b]: Determined tissue was obtained from solvent extraction method.

[c]: Calculated tissue was obtained from the sap MDLs by equation (1) described in the following section and the regression equations from the batch sorption tests to compare with the results from solvent extraction method. Fresh weight (FW)= Dry weight (DW)*4 for grass (assuming the moisture content of grass is 75%) or *2 for stem (assuming the moisture content of stem is 50%)

3.9. DATA TREATMENT AND ANALYSIS

Excel was used to analyze data and plotting tools. Analysis of variance (ANOVA) was conducted with add-on data analysis package in Excel to assess the impact factors. Linear regression analyses were conducted using the least squares method. The p-value of <0.05 indicates a 95% confidence level. The R^2-value of the regression model indicates the proportion of the variance explained by the model.

The method to compare sap concentration and the solvent extracted tissue concentration was based on mass balance: the mass in solvent extraction = the mass absorbed in the biomass + the mass in sap. The calculated tissue concentrations according to the equation (1) were compared with the measured tissue concentration.

$$C_e = Cs + \frac{C_w n}{\rho_w (1-n)} \quad (1)$$

Where:C_e was the tissue concentration by solvent extraction (mg/kg DW), C_s was the explosives absorbed on the dry tissue (mg/kg DW), C_w was sap concentration (mg/L), ρ_w was the density of sap (g/mL) and was assumed as 1.0g/mL , n was the moisture content of tissue. Cs and C_L complied with the partition isotherm determined by batch sorption tests.

3.10. FULL SCALE FIELD ASSESSMENT

Field Site at Longhorn Army Ammunition plant (LHAAP) in Karnack Texas were sampled and analyzed, using tree core collection. Perchlorate in tree core samples was analyzed by the centrifuge sap extract method. The tree core samples were simply frozen, centrifuged to get the tree sap, followed by an ultrafast ion chromatography-tandem mass spectrometry (UFIC-MS/MS) method. Tree cores were collected from 185 trees in site covering 13 species. Figure 3.4 showed the positions and numbers of the sampled wells and trees. Table 3.3 showed the species name, numbers sampled, and numbers sampled in duplication.

The samples was kept in 20ml glass vials with cap and frozen in the -18ºC for at least one day. Then the vials were defrosted at room temperature for 30 minutes. The tree cores were transferred into two 1.5ml centrifugation tubes. Each tube can hold 0.70±15% gram of samples. The samples were then centrifuged at 24,400 rcf for 30 min at 15ºC. After centrifugation the tree cores were taken out and the saps from two tubes were combined into one tube. At last 25µl sample were taken from combined sap and diluted with 50µl mobile phase and 25µl internal standard (5ppb in final diluted solution), then analyzed by UFLC-MS/MS.

Sodium perchlorate standard was purchased from Fischer Scientific (Pittsburg, PA, USA). Ultrapure water used to dilute the solutions was generated using a Mili-Q Advantage A10 and Millipore Elix water purification system (Millipore, MA, USA). The isotope labeled internal standard (IS) perchlorate, $NaCl^{18}O_4$, was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Methylamine was purchased from Sigma-Aldrich, (Saint Louis, MO, USA).

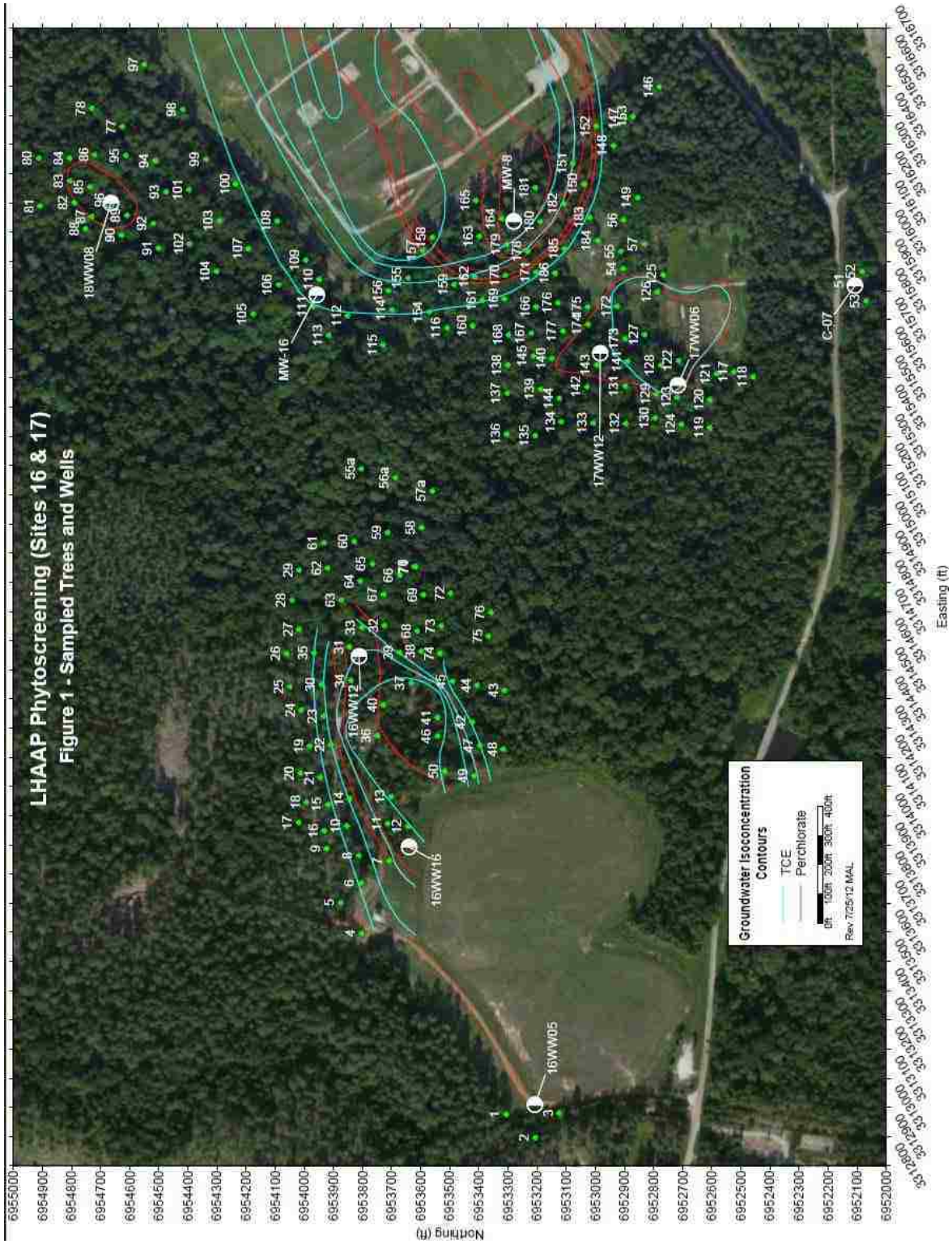

Figure 3.4The LHAAP Field Map of Sampled Trees and Wells

Table 3.3 The List of Sampled Tree Species in LHAAP Field

Tree species	Tree numbers	Tree numbers with duplicate samples
Quercus	90	10
Q. nigra	5	
Q. alba	2	1
Q. laurifolia	1	
L. styraciflua	39	6
Pinus	18	1
Ulmus	13	
U. alata	1	
T. distichum	7	
Carya	4	
Salix	3	
Cornus	1	
M. azedarach	1	
SUM	185	18

Sodium perchlorate is used to prepare perchlorate standard. Isotope labeled perchlorate ($NaCl^{18}O_4$) was used as the internal standard (IS) for quantification. A 2.1 x 250 mm IonPak (Dionex IonPak®AS-21) column and Schimadzu UFLC system were used for the separation. The sample injection volume was 20 µL. Mobile phase is 200 mM methylamine in water and flow rate was 500 µL/min. Total run time was 12 minutes. A 4000Q Trap tandem mass spectrometer (AB SCIEX, Foster City, CA) system was used for detection. The mass spectrometer was operated in a multiple-reaction monitoring (MRM) mode with ESI-negative ionization. The quantification ion pair was m/z 98.7 ($^{35}Cl^{16}O_4^-$) / 82.9 ($^{35}Cl^{16}O_3^-$) amu and confirmation ion pair was m/z 100.9 ($^{37}Cl^{16}O_4^-$

)/84.8 ($^{37}Cl^{16}O_3^-$) aum. Internal standard ion pair was m/z 106.9 ($^{35}Cl^{18}O_4^-$)/89 ($^{35}Cl^{18}O_3^-$) amu.

The estimated instrument detection limit (IDL) was 0.20ppb with a S/N ratio of 3. Spiked recovery was determined by spike standard perchlorate into tree sap samples. Both high (125ppb) and low (2.5ppb) concentration spike were tested. The percent recoveries for the high and low spikes were in the ranges of 99.5-100.9% and 67.29-92.86%, respectively.

4. PLANT MODELING

4.1. CONCEPTUAL MODEL

The developed mechanistic model of soil-plant system was revised from published model approaches[67, 68]. The developed model endeavored in simplifying processes and minimized the required input parameters under the premise of not reducing the predictive accuracy. The model was strictly limited to neutral and non-volatile compounds with only root pathway considered. The chemical phloem transport was not incorporated into the model besides phloem flow from leaf to branch. The phloem system is normally used by plants for downward transport of the photosynthesis products from leaves through stems to fruits and roots. Therefore, overall phloem transport does not significantly affect the most *in-planta* concentration of non-volatile chemicals by root uptake. However, the branch tissue concentration is raised identifiably by phloem flow from leaves with much higher concentration based on the experimental results, thus the phloem flow from leaf to branch cannot be overlooked.

The conceptual diagram of the model is shown in Figure 4.1, consisting of five compartments (soil, root, stem, branch and leaf) for trees and three compartments (soil, root and leaf) for grasses. Chemical is uptaken by root in transpiration flow stream and is transported to the stem xylem. Stem xylem connects to branches xylem and leaves are then supplied by xylem in branches. This model included the branch compartment and phloem flow from leaf to branch because the branch sap concentrations have been observed to be significantly higher from the stem sap concentrations based on the experimental measurements. In addition to the xylem advection transport, the diffusion exchange between root and soil was included in transport processes. The biodegradation

in soil and the metabolism in plant were also considered. An inherent assumption of this model was the equilibrium in the plant compartment interior. The partition of chemicals between biomass (solid) and sap (liquid) was assumed to happen instantaneously. This assumption was applied in all previous plant uptake models if partition was considered.

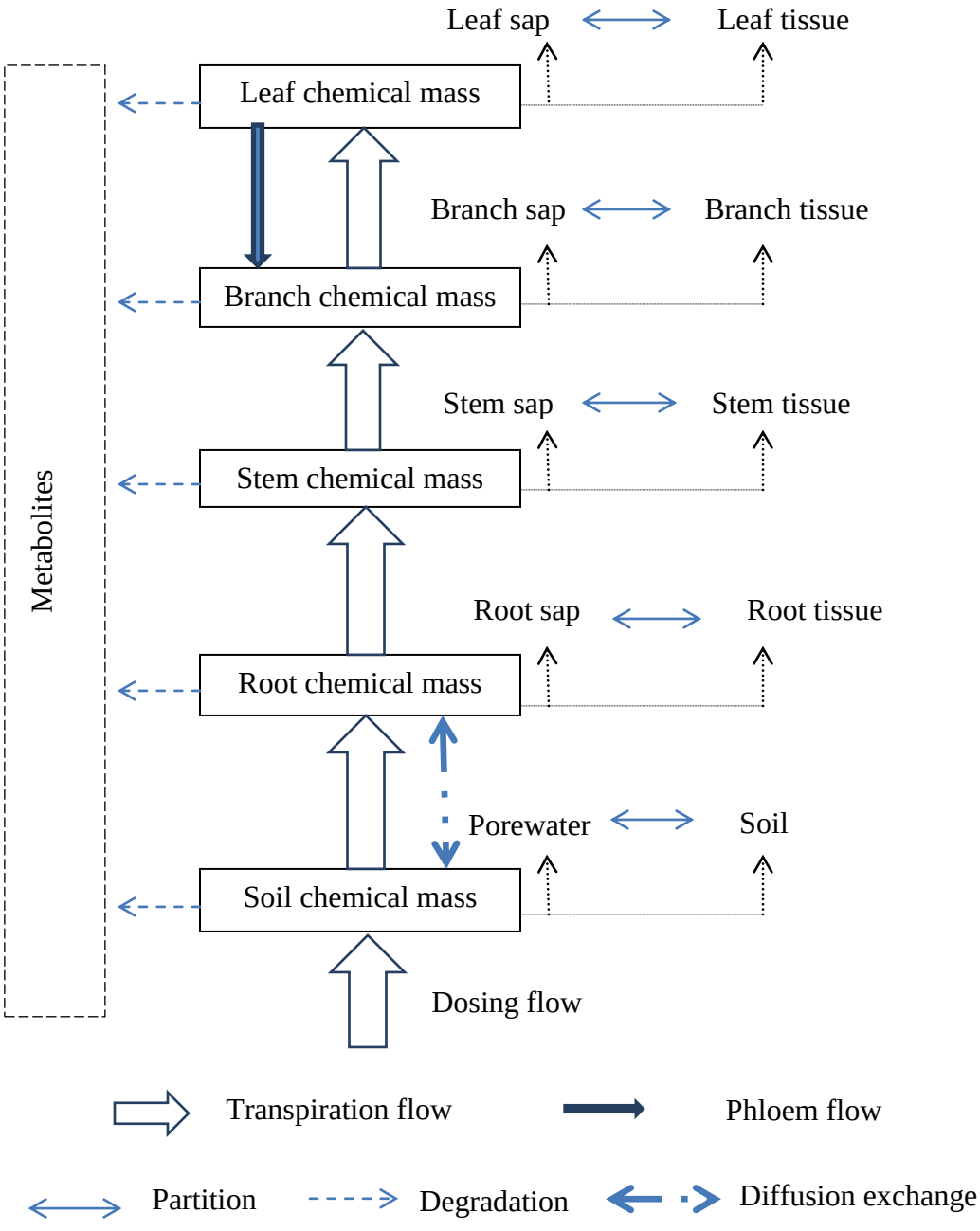

Figure 4.1 The Conceptual Model Showing Processes and Compartments Simulated

4.2. MASS BALANCE EQUATIONS

All compartments were formulated as differential equations based on the fundamentals of mass conservations. Concentrations in every compartment were calculated based on mass balance and partition equilibrium between solid and liquid.

$$m = C_w \frac{Mn}{\rho_w} + K_d C_w M(1-n) \quad (2)$$

$$C_e = \frac{m}{M(1-n)} \quad (3)$$

$$C_w = C_e \frac{\rho_w(1-n)}{K_d \rho_w(1-n)+n} \quad (4)$$

Where:m was the chemical mass in compartment (μg); C_w was the pore water concentration in soil or sap concentration in plant (mg/L); M was the bulk soil mass or fresh tissue biomass (g), n was the water content (W:W), ρ_w was the density of sap and assumed as 1 g/mL; K_d was the partition coefficient (mL/g); C_e was the total soil concentration or tissue concentration (mg/kg DW).

The exponential growth was assumed for the plant growth to calculate the biomass. When the biomass was larger than 10 times than the initial biomass, the exponential growth rate decreased to half of the initial input value to simulate the slower growth for the mature plants and prevent the excessive and unrealistic biomass.

$$\frac{dM}{dt} = Mk \quad (5)$$

Where:k was the growth rate (d^{-1}).

The water movement between compartments was entirely determined by requirements for transpiration providing energy input to drive water movement and water needs for tissue growth. The model estimated transpiration from transpiration conductance multiplied by leaf area and evaporation from evaporation conductance times soil surface area. Water needed for plant tissue growth was calculated from growth rate and water content of various plant tissues, but was minimal compared to transpiration.

$$Q_L = A_L T_c + \frac{k_L n_L}{\rho_w} \quad (6)$$

$$Q_B = Q_L + \frac{k_B n_B}{\rho_w} \quad (7)$$

$$Q_S = Q_B + \frac{k_S n_S}{\rho_w} \quad (8)$$

$$Q_R = Q_S + \frac{k_R n_R}{\rho_w} \quad (9)$$

$$Q = AE_c + Q_R \quad (10)$$

$$A_L = \frac{M_L (A/V)_L}{\rho_L} \quad (11)$$

Where:Q was flow rate (mL/d); A was area (cm^2); T_c was transpiration conductance (cm/d); E_c was evaporation conductance (cm/d); A/V was ratio of area to volume (cm^{-1}), ρ was the density of fresh tissue or bulk soil (g/cm^3).The subscripts for

compartments were root, R, stem, S; branch, B, and leaf, L. No subscript represented soil compartment.

The change of chemical mass in soil was influx with water minus efflux to root minus biodegradation. The efflux to root included both advection transport with transpiration stream and the diffusion exchange described by root surface area multiplied by root permeability multiplied by the concentration gradient between soil and root. The resistance to diffusive exchange came from the root biomembrane and root tissue, and the latter was assumed to be major resistance. The root permeability depended on the molecular weight and K_{ow} of the chemicals has been elaborated in detail in the previous publication [67].

$$\frac{dm}{dt} = QC_d - Q_R C_w - PA_R(C_w - C_{w,R}) - mk_m \tag{12}$$

$$A_R = \frac{M_R(A/V)_R}{\rho_R} \tag{13}$$

Where:C_d was the dosing concentration (mg/L); P was the root permeability (cm/d); k_m was the 1st order degradation (transform) rate (d^{-1}).

The change of chemical mass in root was influx minus efflux to stem minus root metabolism. The transpiration stream concentration factor (TSCF), which was the ratio of the concentration in xylem to that in external solution, such as pore water in soil, was a measure for translocation upwards. TSCF was calculated less than 1.0 especially for polar and high lipophilic compounds in different experiment arrangements[60, 62, 63]. In the companion study the stem sap was measured directly using a novel sap extract method.

The measured TSCF was 0.22 for RDX and 0.15 for HMX for both poplar (*Poplus deltoides x nigra*, clone 34) and willow (*Salix pentandra*) trees rooting in the sand and soil. TSCF of 0.16 for RDX and 0.21 for HMX were also reported by previous publications[19, 50]. Thus the roots resisted the translocation upwards of chemicals. This conclusion also can be explained in physiological basis. Water passes the root and enters xylem by both symplastic and apoplastic pathways, however, non-ion organic chemicals use apoplastic pathway mainly. To simulate the resistance of roots, a reflective coefficient was incorporated for advection transport term. Between roots and stem tissue, if the reflective coefficient was added in the advection transport from soil to root, the root sap concentration would be similar to the stem sap which was almost 10 times lower than pore water concentration by direct measurements. The 10 times difference between root sap and pore water was unrealistic considering the diffusion exchange process. Thus in this model, the reflective coefficient was added in the advection transport from root to stem. This root reflective coefficient depends on the chemical properties and plant species physiology.

$$\frac{dm_R}{dt} = Q_R C_w + PA_R(C_w - C_{w,R}) - \sigma Q_S C_{w,R} - m_R k_{m,R} \quad (14)$$

Where:σ was the root reflective coefficient.

The change of chemical mass in stem was influx minus efflux to branch minus stem metabolism.

$$\frac{dm_S}{dt} = \sigma Q_S C_{w,R} - Q_B C_{w,S} - m_S k_{m,S} \quad (15)$$

The change of chemical mass in branch was influx minus efflux to leaf minus branch metabolism. In the companion study the branch sap was measured directly using a novel sap extract method. The branch sap concentrations were significantly higher than the stem sap concentrations, suggesting transport from leaves to branch, likely in phloem. The pholem flow term only appeared in the tree model and was not included in the grass model.

$$\frac{dm_B}{dt} = Q_B C_{w,S} - Q_L C_{w,B} - \gamma Q_L (C_{w,B} - C_{w,L}) - m_B k_{m,B} \quad (16)$$

Where:γ was the phloem flow rate.

The change of chemical mass in leaf was influx minus leaf metabolism.

$$\frac{dm_L}{dt} = Q_L C_{w,B} + \gamma Q_L (C_{w,B} - C_{w,L}) - m_L k_{m,L} \quad (17)$$

4.3. STELLA MODEL

The models were solved in Stella® (Strongly TypEd Lisp Like LAnguage) modeling software, which is a modeling tool for building a dynamic modeling system by creating a pictorial diagram of a system and then assigning the appropriate values and mathematical functions to the system. Stella® offers a practical way to dynamically visualize and make stimulation of complex systems much easier[79]. The time-dependent mathematical relationships in Stella® are in the format of finite-difference equations that

were evaluated by fourth-order Runge– Kutta methods. Plant models developed by Stella® software have been reported [71, 80] and allow for easy dissemination and application.

The conceptual model and mass balance equations were programmed using Stella® software. The developed soil-plant system model was composed by two sub-modules. The plant growth module was to simulate the dynamic change of biomass and transpiration flow rate to four plant compartments for trees or two plant compartments for grasses. All input parameters in this module were independent of chemicals. The plant uptake module was to simulate the dynamic change of chemical concentration in all four compartments for trees or two compartments for grasses and the abiotic soil compartment. The output of the plant growth module became the input of the plant uptake module. The procedure to develop Stella model was to create a pictorial diagram and then assigning the appropriate values and mathematical equations to the system. The time dependent mathematical relationships were numerically solved by finite-difference method to show a dynamic simulation results. Figure 4.2 and Figure 4.3 showed the pictorial diagram of the plant growth module and the plant uptake module respectively.

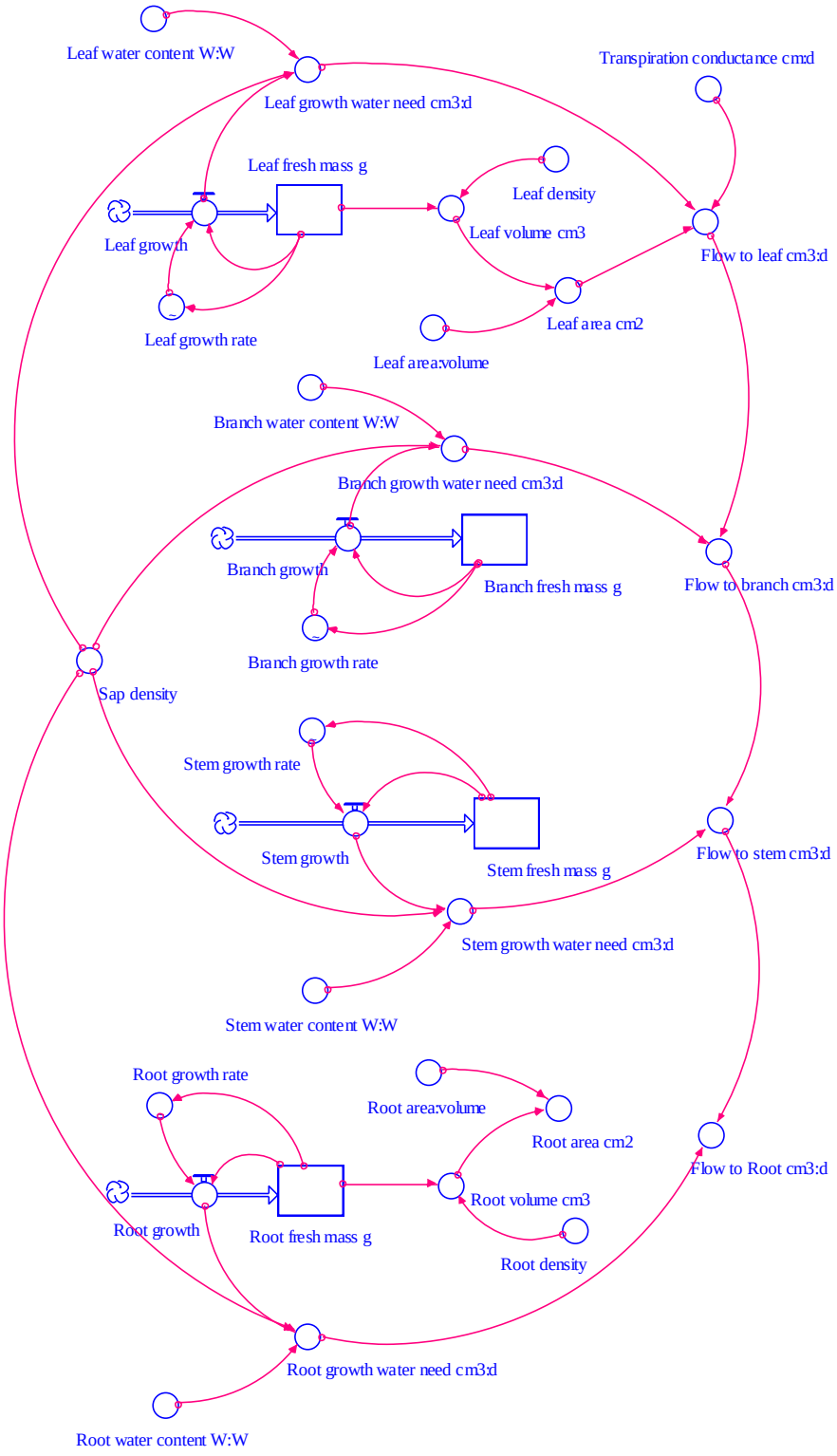

Figure 4.2 The Pictorial Diagram of the Plant Growth Module

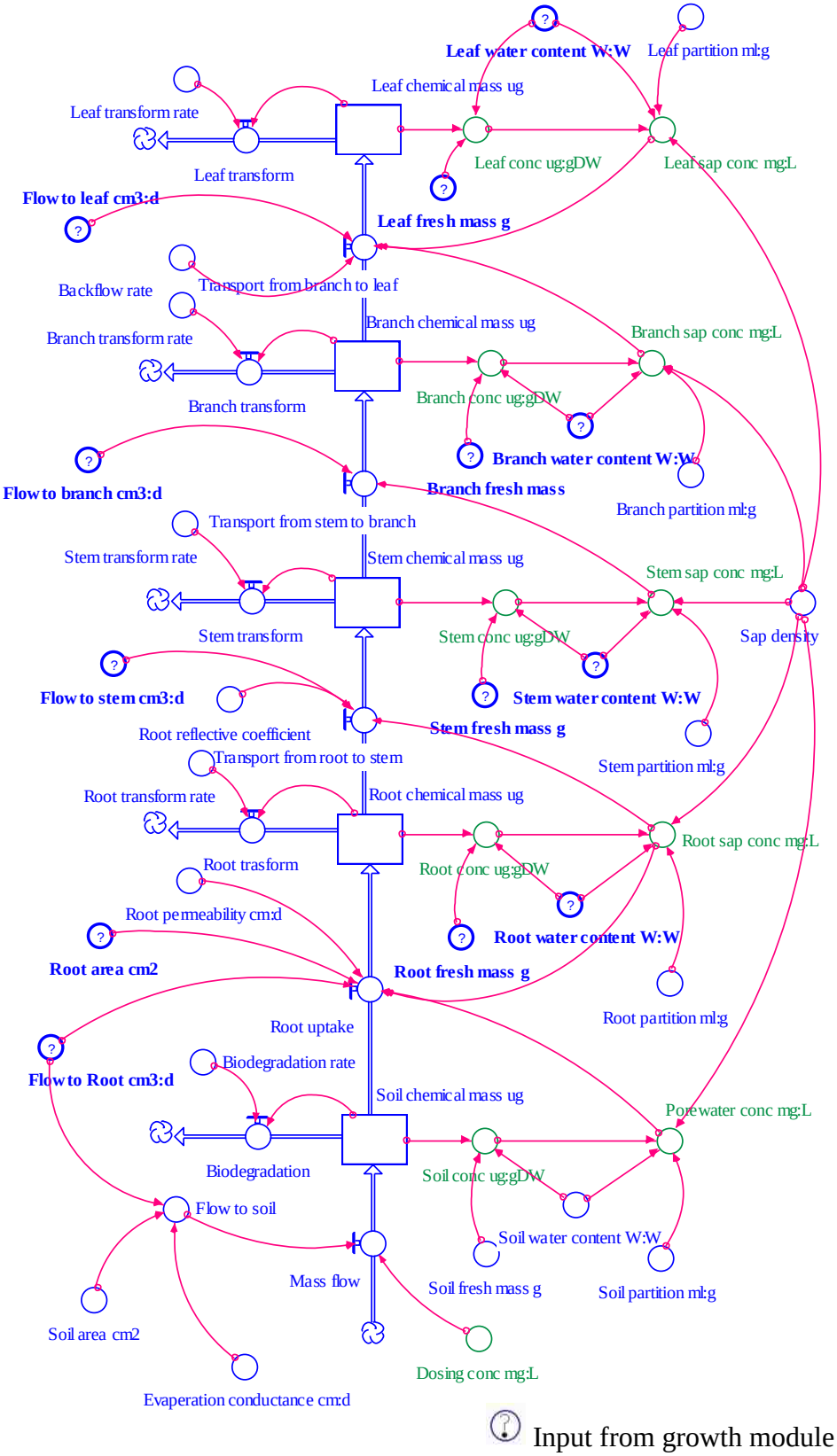

Figure 4.3 The Pictorial Diagram of the Plant Uptake Module

5. RESULTS AND DISCUSSION

5.1. BATCH SORPTION TEST

5.1.1. Sorption on Rooting Media. Batch sorption study is widely used to determine the isotherm type and sorption constants. Nonlinear sorption and higher sorption coefficients at low concentrations were reported and the Freudlich isotherm was recommended by many publications[25, 37, 39, 41]. Because the super sensitive analysis technology LC-MS/MS allowing for samples with ppb level was applied in this research, the tested concentration ranged over from three to five orders of magnitude. Thus the power fit in Excel corresponding to the Freudlich isotherm was used for regression analysis. The essence of power fit in Excel is log-log transform plus linear regression using the least squares method. The isotherms of two kinds soils were shown in Figure 5.1 for RDX, Figure 5.2 for HMX, Figure 5.3 for TNT, and Figure 5.4 for PETN. Quartz sand was tested also and no sorption was found. All determined distribution coefficients were in the range of reported values (Table 2.2). After normalized to the organic content, the $LogK_{oc}$ of all four explosives were similar between 2 kinds of soils (Table 5.1). The two different soils revealed similar Koc value normalized to the organic content, which indicated the organic content, was the most crucial impact fact on soil sorption behavior.

For compounds, TNT showed the highest sorption capability to solids than other compound, which coordinates to its K_{ow} value. To be surprised, PETN and HMX showed moderate sorption behavior which were out of expectation as the high K_{ow} value of PETN and the low K_{ow} value of HMX. RDX indicated the weakest sorption capability to solids, although some higher sorption behavior was expected from its K_{ow} value.

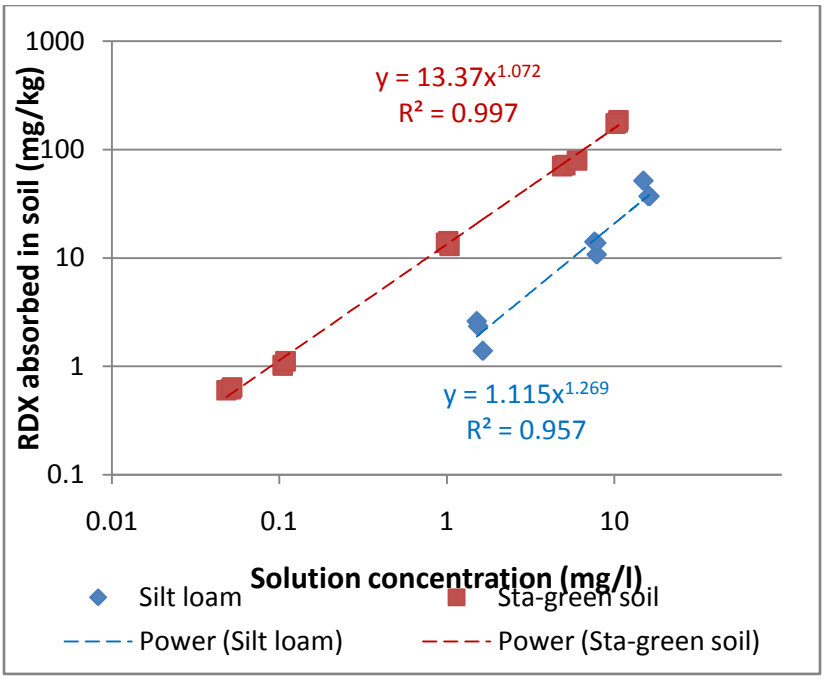

Figure 5.1 RDX Isotherm on Soils

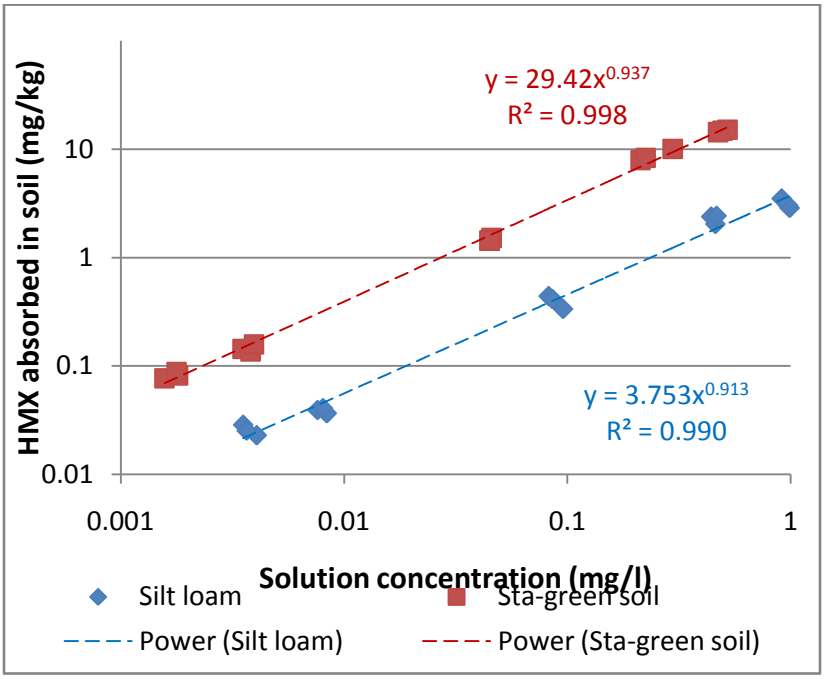

Figure 5.2 HMX Isotherm on Soils

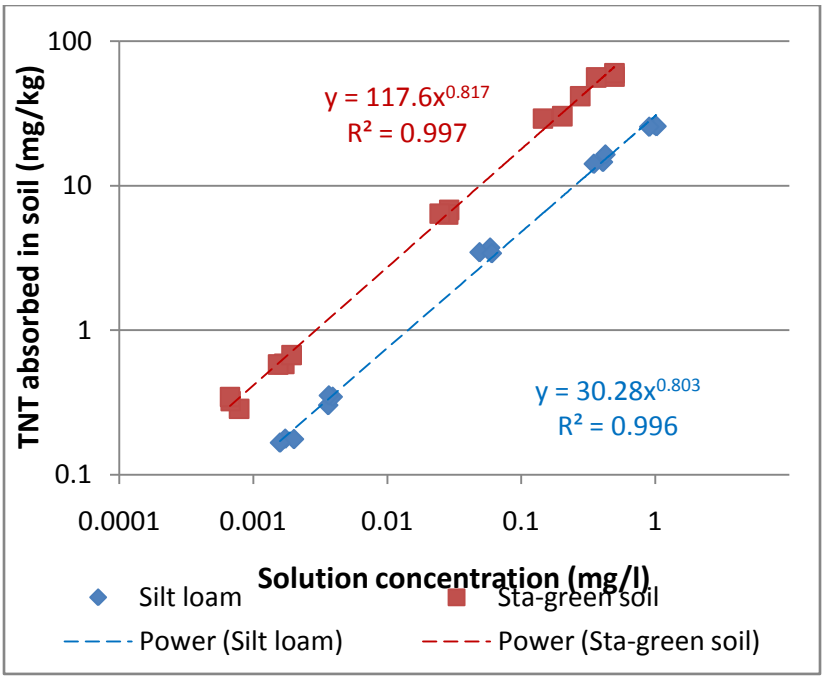

Figure 5.3 TNT Isotherm on Soils

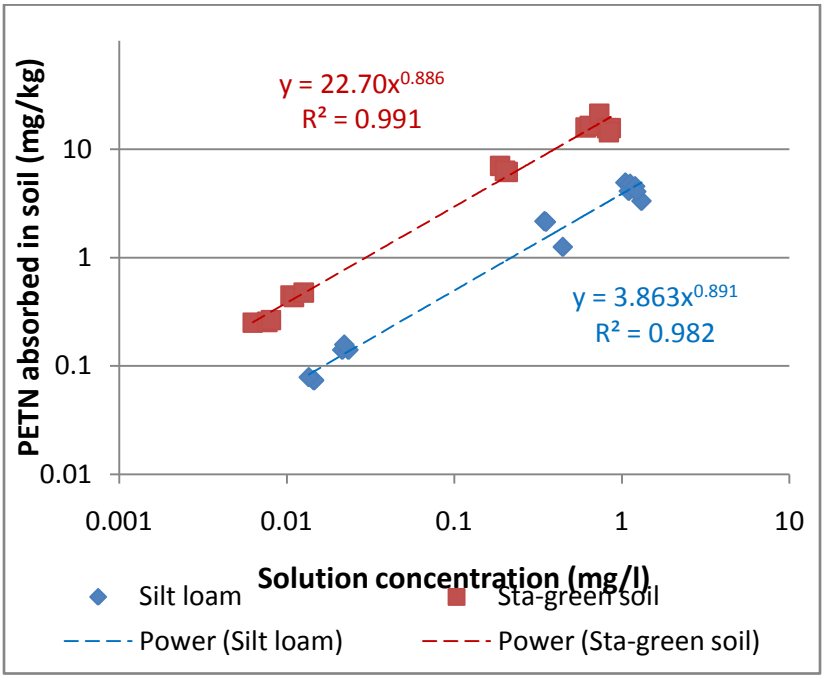

Figure 5.4 PETN Isotherm on Soils

Table 5.1 The Organic Carbon Partition Coefficients Calculated from Determined Soil Distribution Coefficients

Soil		HMX	RDX	TNT	PETN
	Log Kow	0.06-0.19	0.81-0.87	1.6-1.84	2.38 (Est.)
Sta-green soil	Kd (L/kg, C=1)	29.43	13.37	117.65	22.70
f_{oc}=50.28±1.59%	Log Koc	1.77	1.42	2.37	1.65
PAC soil	Kd (L/kg, C=1)	3.75	1.12	30.28	3.86
f_{oc}=8.41±0.30%	Log Koc	1.65	1.12	2.56	1.66

5.1.2. Sorption on Plant Tissues. Similar to sorption tests on soils, four plant tissues including willow stem, poplar stem, ryegrass leaf and big bluestem leaf were tested. However, only two compounds, RDX and HMX were conducted because TNT and PETN were detected rarely in plant aerial tissue in this study. The isotherms were shown in Figure 5.5 for RDX and Figure 5.6 for HMX.

For plant tissues, woody tissue showed clearly higher binding capability than grass especially for HMX. For compounds, the results indicated the partition coefficient of HMX was higher than that of RDX on woody tissues, however quite closing on grasses or leaves. Same as sorption to soil, the binding to plant tissues may not follow the same trend as the Log K_{ow} partitioning relationship, an important finding to understand phytoforensic data.

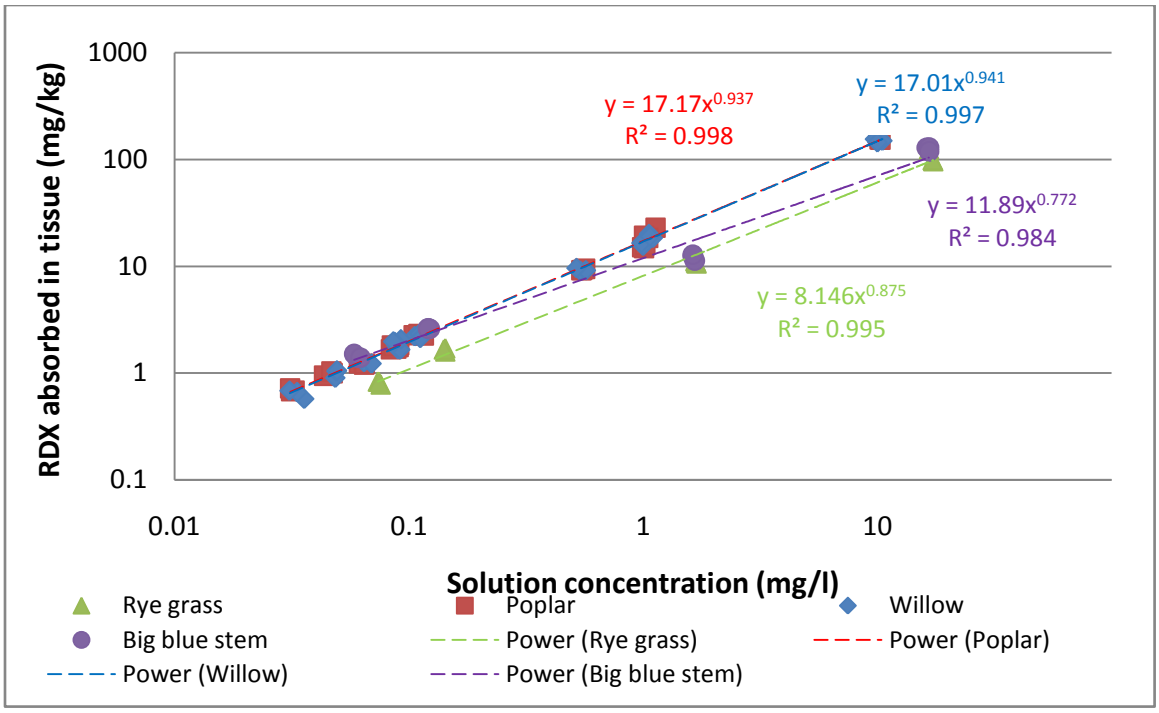

Figure 5.5 RDX Isotherm on Plant Tissues

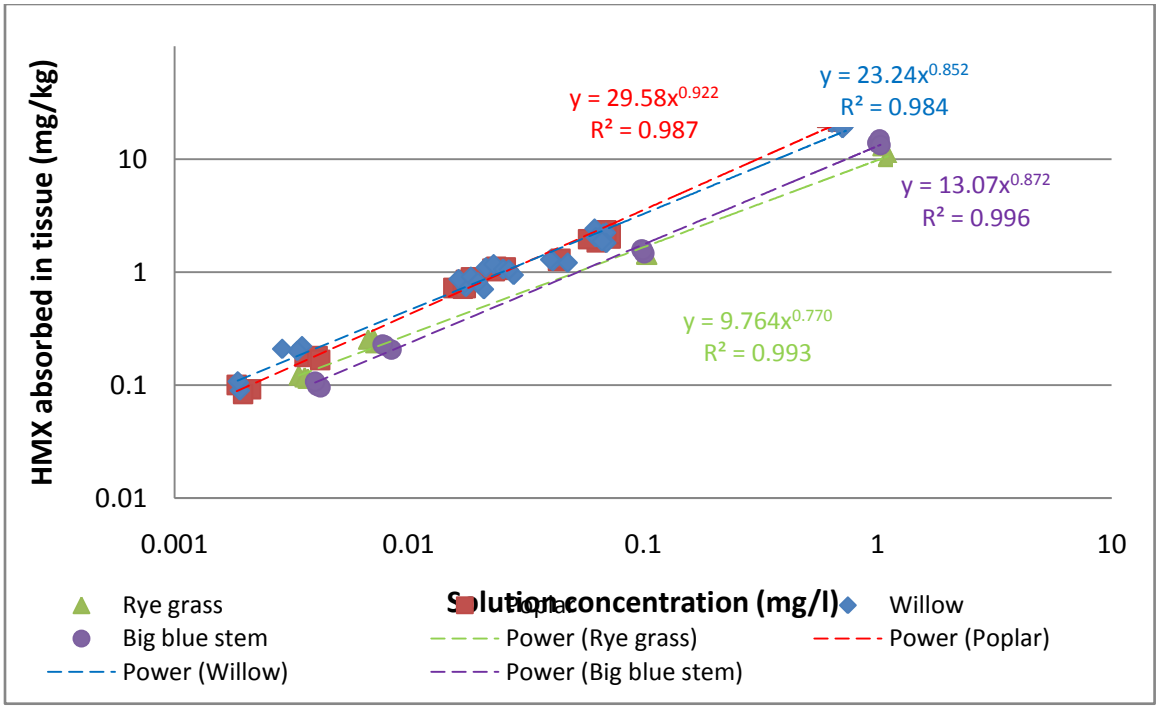

Figure 5.6 HMX Isotherm on Plant Tissues

5.2. DEVELOP THENOVEL PLANT ANALYSISMETHODS

5.2.1. Centrifuge Extraction Method Optimization. Several tests to evaluate the effect of freezing on sap volume centrifuged out from plant tissues were done with the freezing time ranging from 0, 2, 4, 8, 16 and 24 hours. Results indicated that freezing of 2 hours efficiently improved the recovered sap volume: 10 times larger than those from unfrozen tissues for grasses, 2 times larger for poplar and 1.5 times larger for willow. Longer freezing time did not further improved extraction efficiency significantly.

The test to evaluate the optimum centrifuging duration was also done with the centrifugation ranging from 10-60 minutes. Results indicated that optimum volume of the sap centrifuged out was obtained with the centrifuging duration of 40 minutes for tree stems, 15minutesfor ryegrass and 30 minutes for big bluestem.

Based on these test results and consideration of convenience on works arrangement, overnight freezing and 30min centrifugation were applied in all dosed-plant experimental samples.

5.2.2. Centrifuge Extraction Efficiency. The sap extraction efficiency was expressed as weight of extracted sap/ total moisture weight (%). The sap extraction efficiency depended on species. The volumes of sap recovered per fresh weight from grasses were much larger than those from tree stems. The following statistical data stemmed from the dosed-plant experimental samples. The centrifuge extraction efficiency was 18.8±9.7% for willow stem with moisture content of 42.1±5.8%, 26.2±11.5% for poplar stem with moisture content of 47.6±6.3%, 51.1±12.9% for ryegrass with moisture content of 79.1±2.8%, as well as 53.4±10.2% for big bluestem with moisture content of 71.4±3.5% (mean±SD). The tissue structure and moisture content (Figure 5.7) were

considered to be two main affect factors on sap extraction efficiency. A general linear model was applied to the data in order to determine if moisture content had a statistically significance on centrifugation extraction efficiency. The results showed that moisture content was statistically significant (P-value < 0.05) in the improvement of sap extraction ratio for ryegrass and big bluestem, while moisture content had no significance for willow and poplar stems. Large tree cores with moisture content less than 30% showed difficulty to extract liquid from samples.

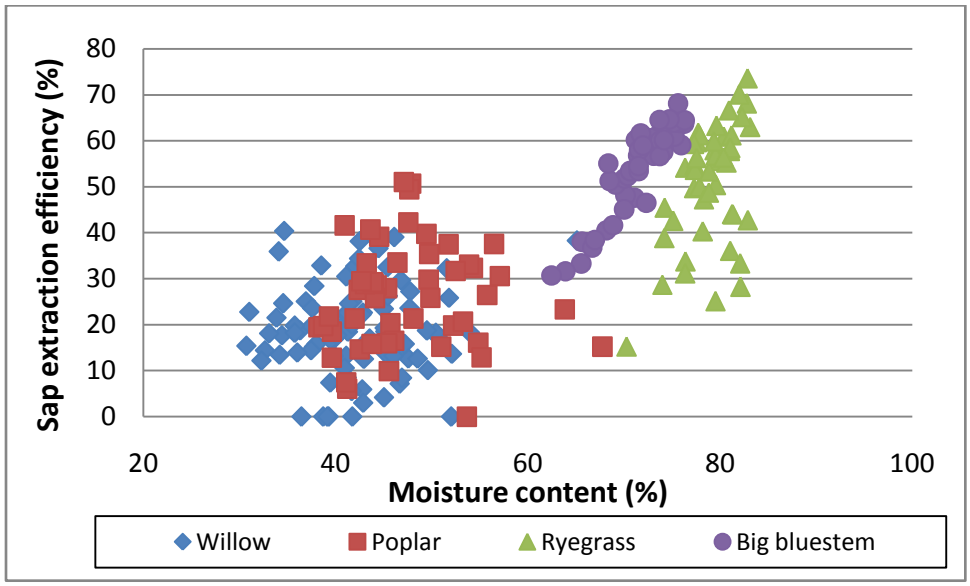

Figure 5.7 The Sap Extract Efficiency Related to Moisture Content

5.2.3. Comparison of Extracted Sap Concentration to Tissue Concentration.

The proposed methods do not extract all chemical from plant tissue as do traditional methods. Only part of sap is extracted. Equilibrium is assumed for non-exhaustive extract

methods, as extractions performed under nonequilibrium situation inevitably sustain poor precision. The amount of compounds adsorbed by the tissue biomass (the solid phase)and in the tissue sap was based on the distribution constant (partition coefficient) between the tissue and the sap. The partition coefficients depend on the properties of both tissue and compounds.

In order to validate the freeze- centrifuge extraction, all plants samples from dosed-plant experiments were split to determine sap concentration by centrifuge extraction method and tissue concentration by solvent extract method. The conformity between novel method and traditional method was evaluated by the ratio of calculated tissue concentrations to measured values. The calculated values stemmed from extracted sap concentration and partitioning relationship determined by batch sorption tests. For RDX, the ratios of calculated/measured tissue concentration were 0.46±0.20 (mean ±SD) for willow, 0.45±0.17 for poplar, 0.42±0.29 for ryegrass and 0.70±0.56 for big bluestem. For HMX they were 0.43±0.24 for willow, 0.35±0.09 for poplar, 0.53±0.39 for ryegrass and 0.66±0.61 for big bluestem.

The calculated tissue concentrations were consistently lower than the measures values. Several reasons were speculated to explain the discrepancies. Firstly, the sap concentrations are not homogeneous within the tissue so that the extracted sap fails to stand for the total sap concentration. Also the sap concentrations of small tree stems may be underestimated as low dilution fold (2-6) were applied for these samples. The high dissolved solids content in plant sap inhibited the ionization process for MS detector and produced smaller results. For all grass samples from dosed-plant experiments, more than

10 fold dilutions were applied, which may explain the calculated/measured ratios were higher for grasses than those of tree stems.

On the other hand, the partitioning relationship from the batch sorption tests may not be identical to the partitioning in the live tissue interior. The different solid/liquid ratio was a possible reason for the differences between real samples and dead samples. Another possibility was to the different the ionic strength in the sap and in the DI water used in the sorption tests. The higher ionic strength in plant sap (0.5M, [73]) increased partitioning to tissue biomass. The future batch sorption test for plants is suggested to use the solution adjusted to similar ionic strength as plant fluid to get more accurate values.

5.3. THE ROLE OF BACTERIA DEGRADATION AND SOIL SORPTION

As mentioned before, TNT and PETN were detected rarely in plant aerial tissue in this study. Thus only RDX and HMX were discussed here and in the following all chapters. Previous studies concluded that TNT was located in the roots and highly metabolized to bound residues and more polar products. The parent compound of TNT was rarely recovered from plants[30, 32, 36, 45, 52, 53]. Still no solid conclusion was made for PETN as much less studies on this compound. Two researches mentioned that no GTN, a similar compound to PETN, was determined in yellow foxtail tissues after 5 days of hydroponic exposure[22] and in ryegrass after a 35 days' soil exposure[20]. Thus, the results from this research were consistent to the previous publications. Given the lack of detection, there is little potential for phytoforensic applications for TNT and PETN.

Figure 5.8a showed the average RDX pore water concentration during the whole exposure periods for each test unit in small scale tree and grass experiment. In sand reactors, the pore water concentrations in all 4 species and 3 treatments were closing to dosing concentrations. The results corresponded well with the preliminary sorption and degradation tests. The lack of sorption capability has been exhibited on the sand by sorption tests described in the method section. Another preliminary test carried out was the degradation test, which spiked explosives to sand. RDX and HMX remained steady in the whole test period of 4 weeks.

Measured pore water concentrations were consistently lower than dosing concentrations in all soil reactors, likely due to sorption and biodegradation. Initially low concentration in soil reactor is largely due to initial sorption. The consistently lower pore water concentration indicated biodegradation. Biodegradation of RDX and HMX have been reported in previous studies[3, 37]. This also explained the difference of pore water concentration in the different species rooting in soil. Temperature directly affected the biodegradation rate. The trees were cultivated in spring while the grasses in summer. The willows treated with high dosing level were also planted in summer as the previous tress planted in spring with others were all dead due to phyto toxicity of acetonitrile. From then on, all high dosing solutions were sparged with N_2 for 8hr in the fume hood to volatilize acetonitrile (carrier solvent) before being used for dosing. Thus the grasses rooting in soil with all treatments and willow rooting in soil treated with high level had lower pore water concentration. Besides temperature, transpiration ratio (Appendix C) also made difference. The low transpiration ratio extended the retention time of solution in reactors resulting in higher biodegradation potentials and thus lower pore water

concentrations. 3 test units with highest transpiration ratio, more than 120ml/d/tree, were willow in soil dosed by medium level, poplar in soil dosed by medium and high levels. These reactors indicated lest difference in pore water concentration between soil reactors and corresponding sand reactors. In low level dosed soil reactors, the transpiration ratio of willow was 72ml/d/tree, higher than transpiration ratio of poplar, 40ml/d/tree. Thus higher concentration was determined in willow reactor than poplar reactor. The transpiration ratio in grasses reactors were in the range of 12-51ml/d/reactor with the average of 23ml/d/reactor, much lower than trees reactors. Thus the low pore water concentrations were consistently determined in grasses soil reactors.

Figure 5.8b showed the average HMX pore water concentration during the whole exposure periods for each test unit in small scale tree and grass experiment. Overall, HMX results were similar to RDXs. However, the ratios of pore water concentration to dosing concentration were larger than those of RDX. For example, in sand reactors the pore water concentrations were above the corresponding dosing concentrations especially for high transpiration trees. This difference can be explained by three reasons. Firstly, HMX is more persistent and less amenable to biodegradation than RDX[18, 35-37]. Secondly, HMX has stronger sorption ability to soil than RDX shown in the batch sorption tests. At last, RDX is preferentially uptaken by the plants than HMX with higher TSCF[63, 73]. The preliminary hydroponic tests showed the capability of selective uptake of the trees, which resulted in the increase of contaminant concentration in root area accompanying the extract by trees. The initial dosing solution volume was 150ml. After the willows extracted 100±16ml solution, the solution concentration increased from initial 2.03±0.04mg/L to 4.1±1.1mg/L for HMX and from initial 18.4±1.5mg/L to

29.6±9.1mg/L for RDX (mean±SD, n=6). HMX appeared more resistant by plants due to the low K_{ow} value and larger molecule than RDX. The concentration increases in hydroponic solution also were reported in the previous researches[50]. Kim et al reported the higher concentrations of TNT in the rhizosphere than in the bulk soil and concluded that mass transported from the surrounding soil into the rhizosphere was higher than that by root uptake[81].

5.4. TIME DEPENDENT TISSUE CONCENTRATION

Tissue concentration reaches steady state levels when chemical influx equals to efflux and reactions. In the strict sense, the steady state in plants may be unrealistic due to complex physiological processes and the constantly changing environment conditions in the field, thus a relative stable tissue concentration, such as in the range of ≈ 30% fluctuation or a very slow concentration change, is acceptable in phytoforensics. Different plant species maybe need longer or shorter term to reach steady state due to different plant physiology. In this research, time-series grass experiments were aimed to determine time dependent tissue concentration for grasses; and large scale tree and long term experiments were aimed to determine time dependent tissue concentration for trees.

5.4.1. Time Dependent Tissue Concentration on Grasses. The grass concentrations were shown in Figure 5.9 and Figure 5.10 for RDX; Figure 5.11 and Figure 5.12 for HMX. 'S' shape curve, instead of ideal exponential curve were observed. Two possible reasons were put forth. The first reason was the imperfect mixing and uneven uptake. The added dosing solution was extracted by plant before it mixed well

with the “clean” water in reactors, resulting in the earlier high concentration in plant. The uptake into plants is also not perfect flow as modeled. Plants have some transport tissues that are very rapid in uptake and others that are not as fast. Basically, the soil and plant tissues are modeled as ideally (complete mixture in one compartment and plug flow for transport between compartments) and neither is the actual case. The differences are observable in the transition of input conditions. Another possible reason was grass frond with a lower metabolism rate in the earlier term. Plants and associated microbes may need time for metabolic processes to respond and adjust to transform the xenobiotics more efficiently or respond to the compound exposure. Similarly, the tree leave analysis also showed early high concentrations. Although some fluctuations were observed at the different sampling time, the change trends over time were clearly identified. The tissue concentration increased at the beginning, then approached a relatively steady state after 10 weeks exposure in all dosing treatments. The compounds accumulate in glass frond continually until the transformation rates catch up to uptake rate as the frond tissues without efflux. Therefore, it takes long period for grass to reach steady state.

Table 5.2 and Table 5.3 compared the results of small scale experiment and time series experiment for both tissue concentration and sap concentration. The averages of all concentrations after 10 weeks were used to represent the results of time series experiment. The steady tissue concentrations of ryegrass from both small scale experiment and time series experiment were similar for low and medium dosing treatments although the shorter exposure time of 8 weeks in the small scale experiment, 26.1±5.6 and 386.2±23.9 mg RDX/kg DW in the time series experiment compared to 32.3±7.7 and 436.4±60.3 mg RDX/kg DW in the small scale experiment for low and

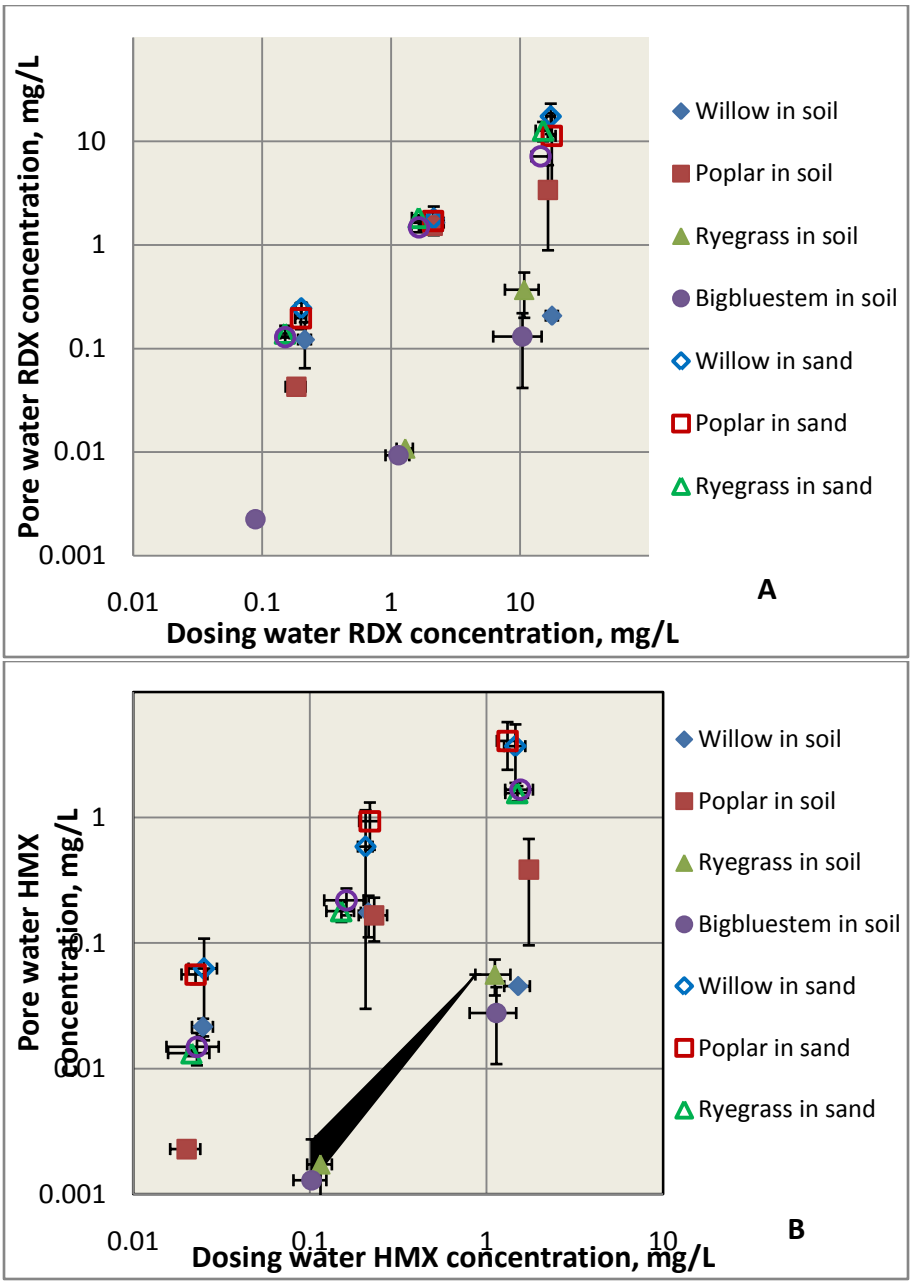

Figure 5.8 Average Pore Water Concentration and Dosing Concentration

Data from the small scale tree and grass experiment during the whole exposure period.In soil reactors, the much smaller values of pore water concentration in the beginning of exposure were removed from the statistics. The error bars represented the standard deviation. No data for ryegrass and big bluestem in the soil with low dosing treatment as the pore water concentration was below than detention limit. A showed RDX; B showed HMX.

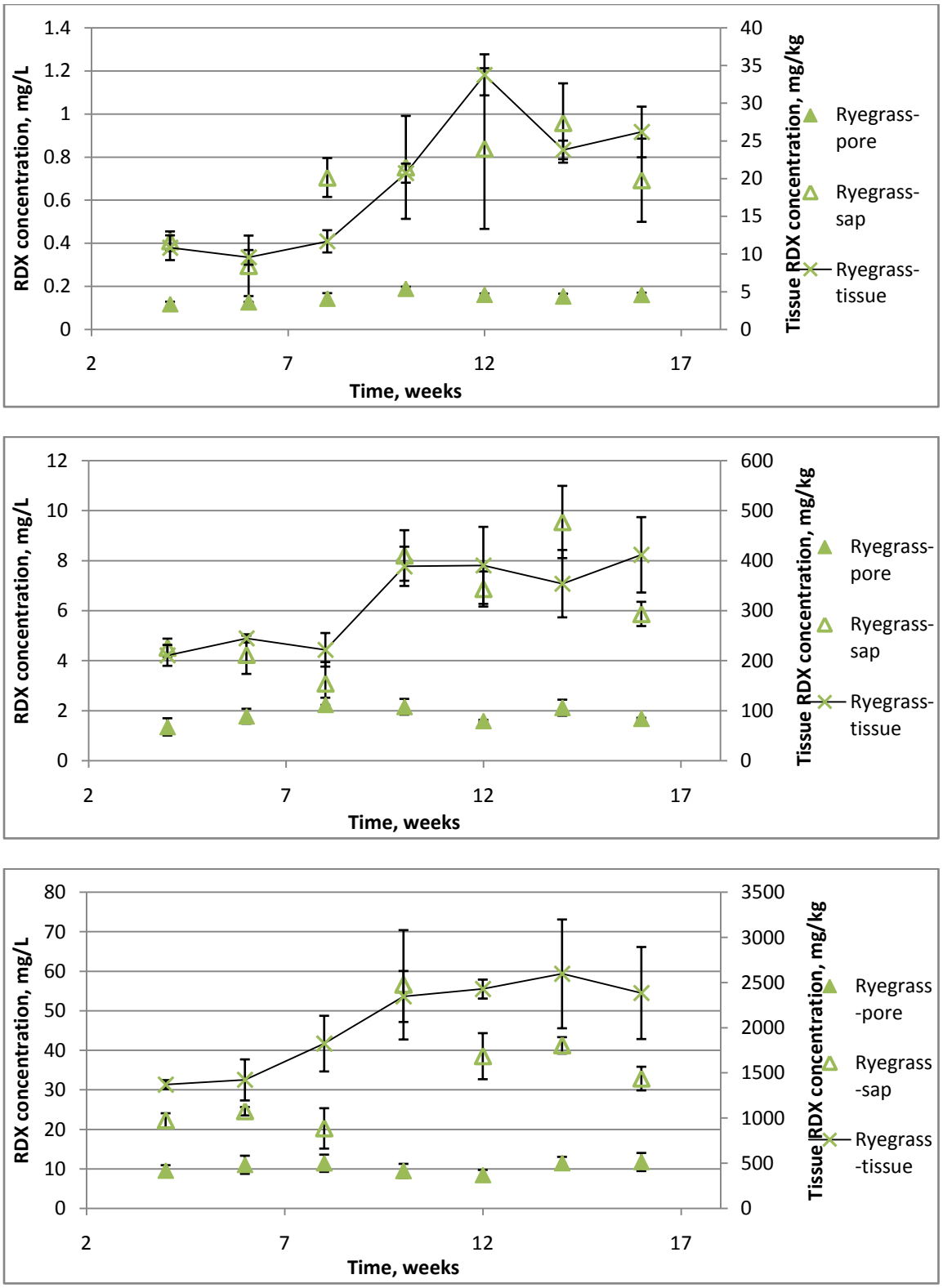

Figure 5.9 Ryegrass Tissue RDX Concentration (Based on DW), Low/Med/High

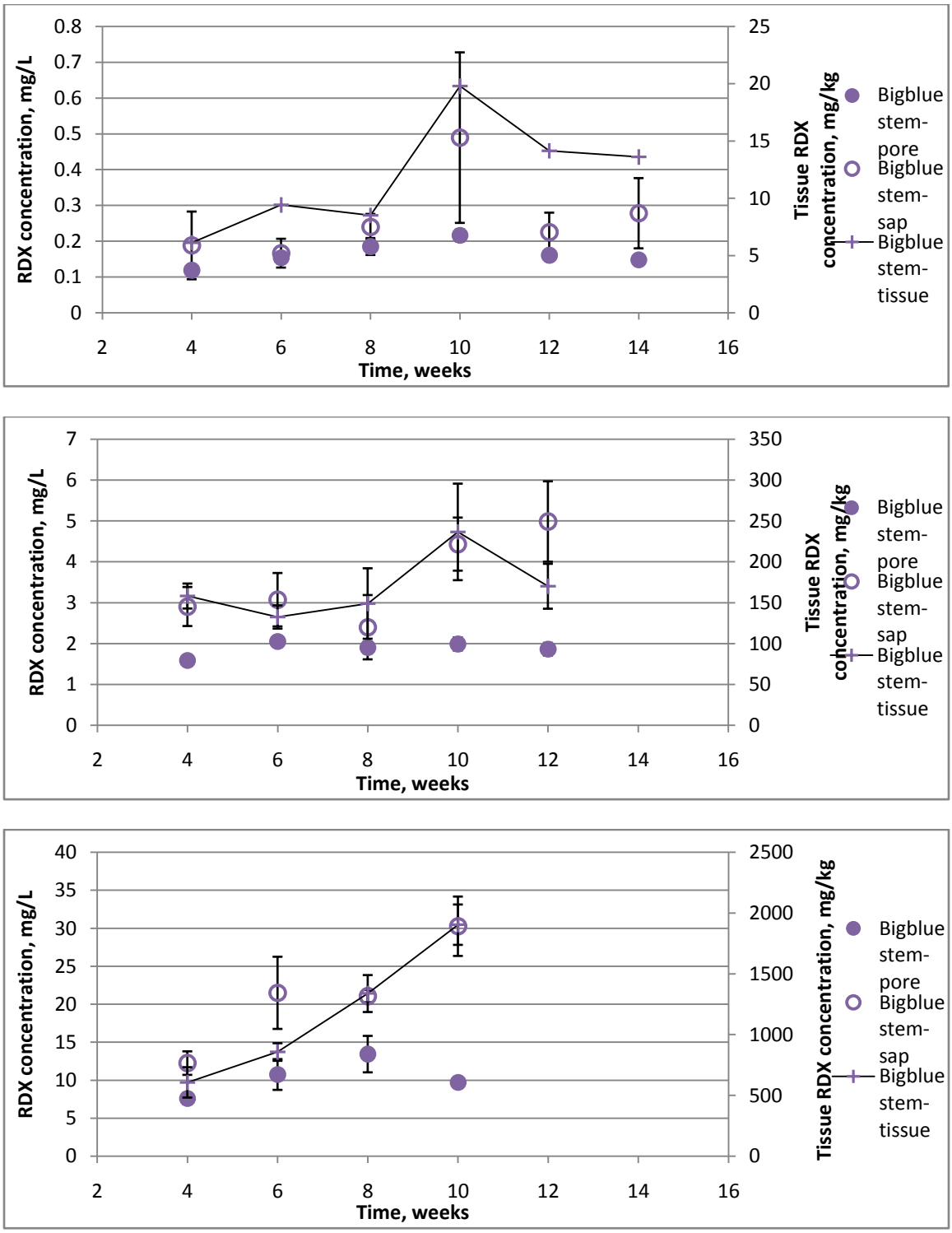

Figure 5.10 Big Bluestem Tissue RDX Concentration (Based on DW)

Less biomass available for big blue stem resulted in no data after 10 weeks for high dosing, 12 weeks for medium dosing and 14 weeks for low dosing.

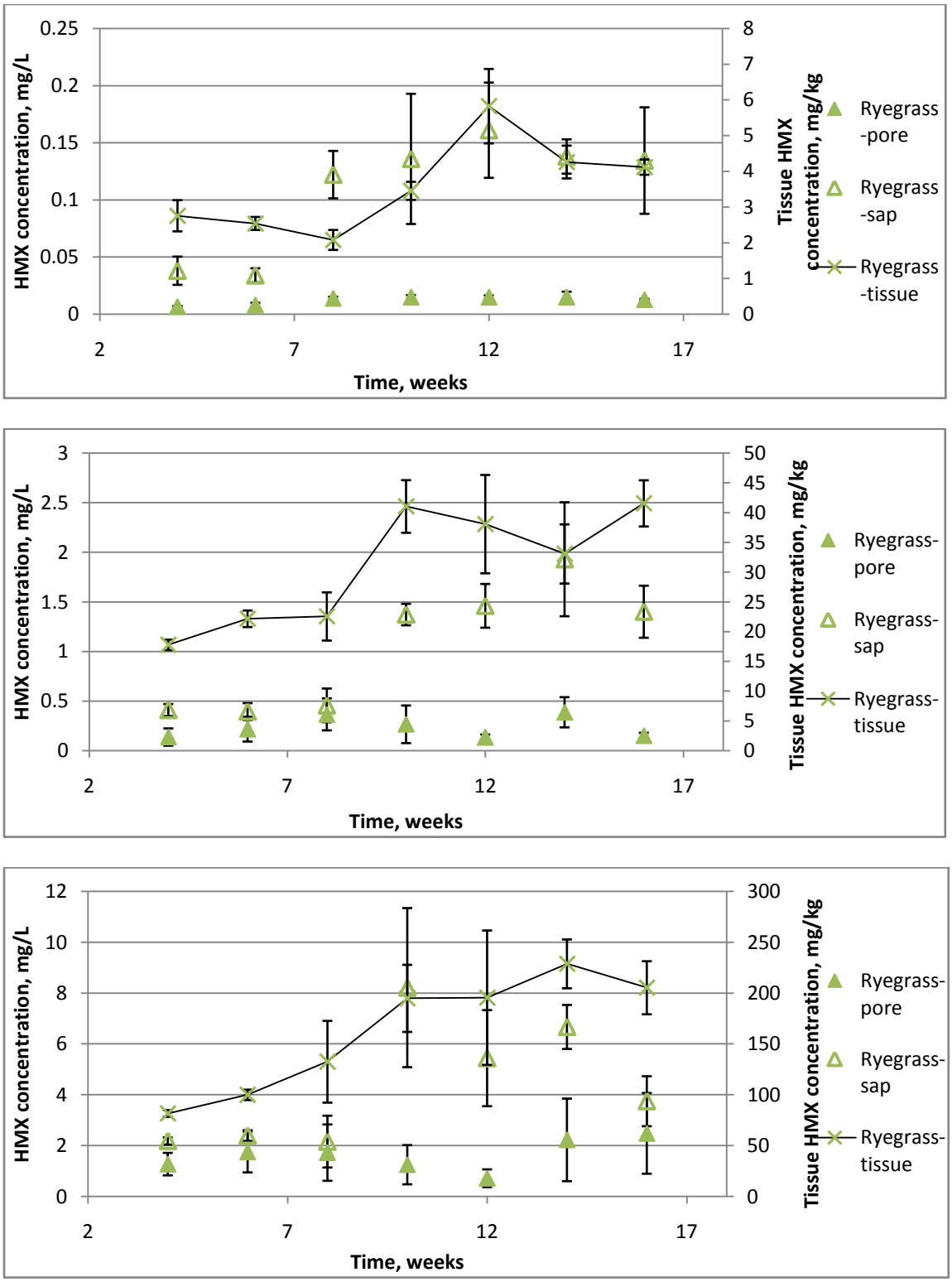

Figure 5.11 Ryegrass Tissue HMX Concentration (Based on DW), Low/Med/High

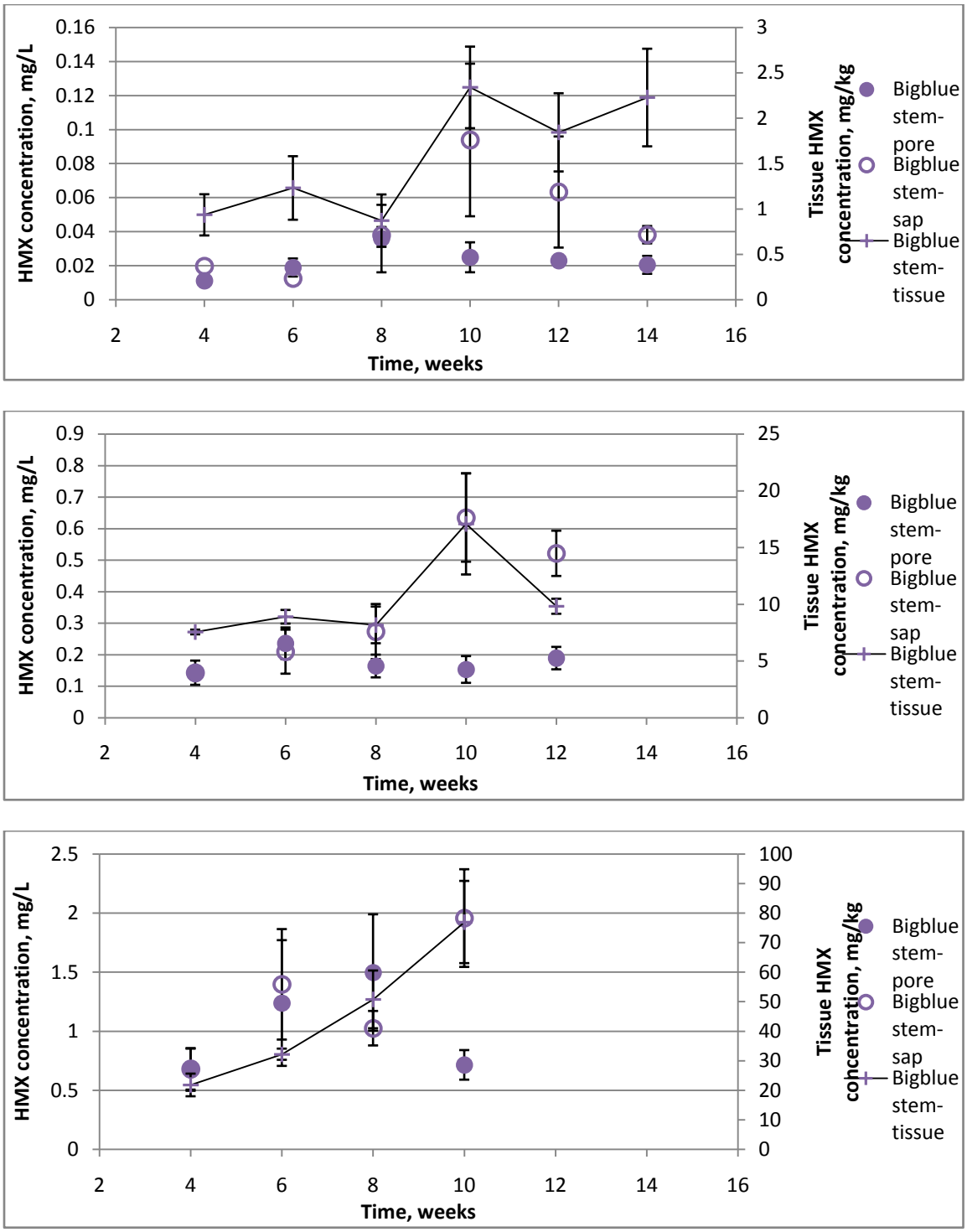

Figure 5.12 Big Bluestem Tissue HMX Concentration (Based on DW)

Less biomass available for big blue stem resulted in no data after 10 weeks for high dosing, 12 weeks for medium dosing and 14 weeks for low dosing.

Table 5.2 Grass RDX Tissue Concentration Comparison between 2 Tests

Reactor		Sap concentration, mg/L		Tissue concentration, mg/g	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
Ryegrass	L	0.70±0.19	0.81±0.12	32.34±7.66	26.11±5.56
	M	5.87±0.50	7.62±1.60	436.42±60.31	386.21±23.88
	H	40.40/2	42.32±10.14	2649.01/2	2439.55±110.30
Bigbluestem	L	0.17±0.032	0.33±0.14	5.43±1.02	15.85±3.43
	M	2.30±0.37	4.71±0.39	129.69±82.00	203.35±47.03
	H	21.20/2	30.27	807.75/2	1904.58

Experiment 1: Smallscale experiment (8 week exposure);

Experiment 2: Time series experiment (≥10 week exposure);

The concentrations in high dosing solution used in experiment 2 were half of those in experiment 1 to decrease the phyto toxicity. Thus the results were divided by2.

Table 5.3 Grass HMX Tissue Concentration Comparison between 2 Tests

Reactor		Sap concentration, mg/L		Tissue concentration, mg/g	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
Ryegrass	L	0.077±0.028	0.14±0.013	4.31±0.71	4.41±1.00
	M	0.90±0.067	1.54±0.26	57.12±10.23	38.44±3.90
	H	4.48/2	6.02±1.89	247.05/2	206.19±15.83
Bigbluestem	L	0.017±0.006	0.065±0.028	0.97±0.18	2.14±0.26
	M	0.20±0.069	0.58±0.080	13.43±3.40	13.45±5.13
	H	2.14/2	1.96	123.01/2	76.96

Experiment 1: Small scale experiment (8 week exposure);

Experiment 2: Time series experiment (≥10 week exposure);

The concentrations in high dosing solution used in experiment 2 were half of those in experiment 1 to decrease the phyto toxicity. Thus the results were divided by2.

medium dosing level respectively. Similarly, 4.3±0.7 and 57.1±10.2 mg/kg DW were HMX concentration in the small scale experiment, while 4.4±1.0 and 38.4±3.9 mg/kg DW were HMX steady concentration in the time series experiment for low and medium dosing level respectively.

However, for big bluestem, the tissue concentration differences between 2 experiments were clear, showing steady state was not reached in the small scale experiment, 15.9±3.4 and 203.4±47.0 mg RDX/kg DW in the time series experiment compared to 5.4±1.0 and 129.7±82.0 mg RDX/kg DW in the small scale experiment for low and medium dosing level respectively. The HMX tissue difference between the two experiments reduced. Transpiration ratios were 2 times higher in ryegrass (48 ml/day/reactor) than big bluestem (22 ml/day/reactor) in small scale experiment, which may explained the differences between two species. Higher transpiration ratio possibly shorted the time necessary to reach steady state. For high dosed level, it was clear that the concentrations from the time series experiment were higher than those from the small scale experiment for both species. The high dosing treatment in the small scale experiment exhibited potential sublethal toxic effect on both species as the low transpiration ratio of 13 ml/day/reactor.

In summary, the relative steady state in grass aerial tissue can be reached for RDX and HMX. 2 month is a conservative estimation for young grasses applied in the experiments to reach steady state for RDX.

5.4.2. Time Dependent Tissue Concentration on Trees. Trees have more tissue compartments than grasses. The time periods needed to reach steady state for individual tissue compartment was based on transport routine. Roots reach steady state

firstly, and then followed by stem and branch. Leaves reach steady state at last. Further the transport vascular tissue, root, stem and branch, with both influx and efflux are assumed to reach steady state quickly than terminal tissue (leaves). However, the relatively large biomass of stem provides storage for compounds, which extends the time required to reach steady state. Thus even the question whether steady-state is reached within one growth season or the life-span of a tree has been put forward. Trapp et al proposed a formula to estimate the time to achieve 95% of steady state for stem [74]:

$$t\,(95\%) = \frac{\ln(0.05)}{Q/(M * K_d) + k} \tag{18}$$

Where Q is the flow rate; M is the stem woodmass; K_d is wood-water partition coefficient; k is the sum of the growth rate and the metabolism rate. Based on this equation, the stem tissue of the small trees used in the small scale experiment needed about 20 days to reach steady state for RDX due to the partition, while the stem tissue of the large trees used in the big scale experiment needed about 100 days. These estimations still did not include the time required for pore water and root to reach steady state because of soil sorption and root partition. HMX, based on Trapp's model, requires longer times because of greater wood-water partition coefficient.

The results from the large scale tree experiment were showed in Figure 5.13for RDX and Figure 5.14for HMX. The change of pore water concentration during the experiment period (Appendix E) concealed the change trends of tissue concentration. For stem, tissue RDX and HMX concentration continuously increased until 175 days and the results of 240 days were similar to the values of 175 days. However, leaves RDX and

HMX showed different trends between different dosing level treatments. For medium dosing level, the leaves concentration increased along with the whole experiment period. For high doing level, the concussive trends were observed possibly due to the sublethal toxic effect resulted from high level of explosives indicated by the lower transpiration rate (average 1.8L/d for medium treatments and 1.2L/d for high treatment) and higher leaves drop rate (average 13% for medium treatments and 26% for high treatments). These data were obtained in the middle term (about 144 days) of the entire exposure term (240 days). Leaf analysis showed very early high concentration especially for high treatments suggested the possible lower metabolic content during the earlier experiment period. The physiological reactions in plants may be adjusted to transform the xenobiotics more efficiently.

In summary, the time required to attain steady state increases in the range of root<stem<branch<leaves in trees. If the pore water concentration is steady, the small tree stems need about one month and leaves need about two months for RDX and longer for HMX to reach approximate steady state. Larger scale trees cost longer.

5.5. SPATIAL DEPENDENT TISSUE CONCENTRATION

The analysis results (Figure 5.13 and Figure 5.14) clearly showed the highest tissue concentration of the whole tree was located in leaves due to the accumulation of the compounds at the point of water evaporation. The experimental results also showed that branch tissue concentrations were higher than stem tissue concentrations consistently

in all trees at the both full analysis harvests, suggesting the potential phloem flow from leaves to branch, which increased the branch concentration.

The intra- and inter- variance of tissue concentrations were assessed by the relative standard deviation from different samples (Table 5.4 for RDX and Table 5.5 for HMX). The intra- variance derived from single tree, determined by sampling different positions in the stem, branch, and leaf. In the small scale experiment, the tree stems were cut into three sections as described in the method chapter. In large scale tree and long term experiment, 2 tree cores were taken from different height. For branches, 2-4 samples were taken every other 50-70cm along the main branches. For leaf, after removing all newest and oldest leaves, samples were divided into 2 sections, the section closing to top (high) and the section closing to bottom (low). The intra- variances were similar between small scale trees and big scale trees.

Compared with stem and branch, leaves showed higher variance as expected. The differences came mainly from the different branches. The leaves in the same branches showed closer concentrations and the concentration differences decreased with respect to time. The leaves closing to bottom (older) showed higher concentration than those closing to top (newer) especially at the earlier harvests. (Figure 5.13 and Figure 5.14) These results were in accordance with the publication by Brentner et al., in which Phosphor imager autoradiography showed higher concentration of ^{14}C labels in the edge of leaves and older leaves when poplar was exposed to ^{14}C-RDX for 5 days[54]. Although the length of main branches reached 2-3m, the concentration along the branches showed high consistency in both harvests. Similar to branches, the differences of stem from different height were not significant besides the earlier harvests.

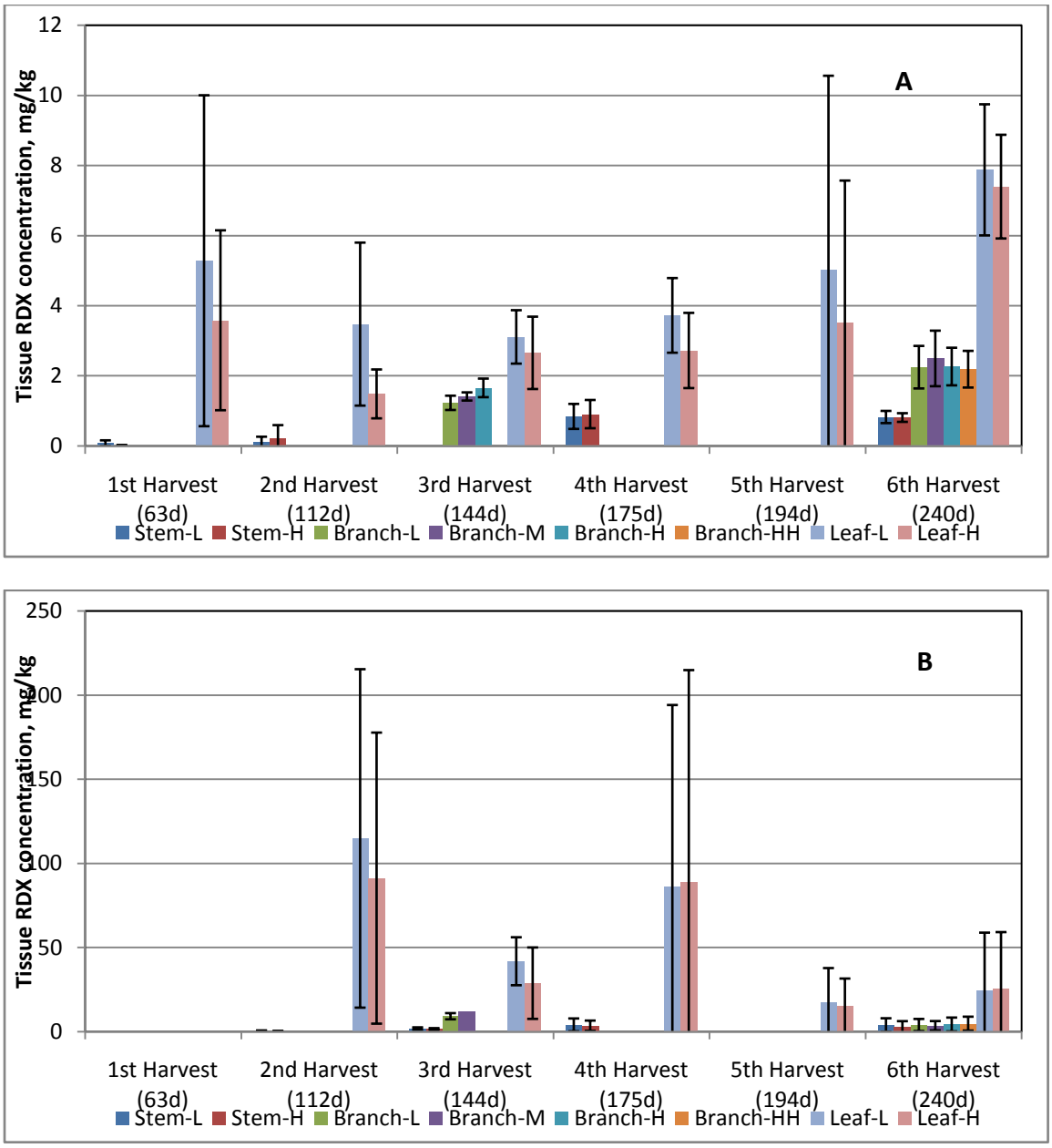

Figure 5.13 Tissue RDX Concentration (Based on DW) of Each Sampling Position

L, M, H,HH represented low, middle, high, more higher position respectively. Low closed to bottom; High closed to top. Stems were not sampled in the 5th harvest. No data also for stem concentrations from all medium dosed reactors in the 3rd harvest as the RDX results were questionable. Branches were samples only in the 3rd time and the last time. 1# tree had only one main branch, which was harvest in the last time. The 2# tree in the 5th harvest suffered from the effect of the surface dosing at that time due to the blocking of bottom dosing pathway and had absolutely abnormal higher concentrations, thus was not included in the statistics. The 6# tree had high concentration due to dying in the last harvest and was not included also. The error bar represented the standard deviation from triplication planting. A showed the test units dosed by medium level; B showed the test units dosed by high levels.

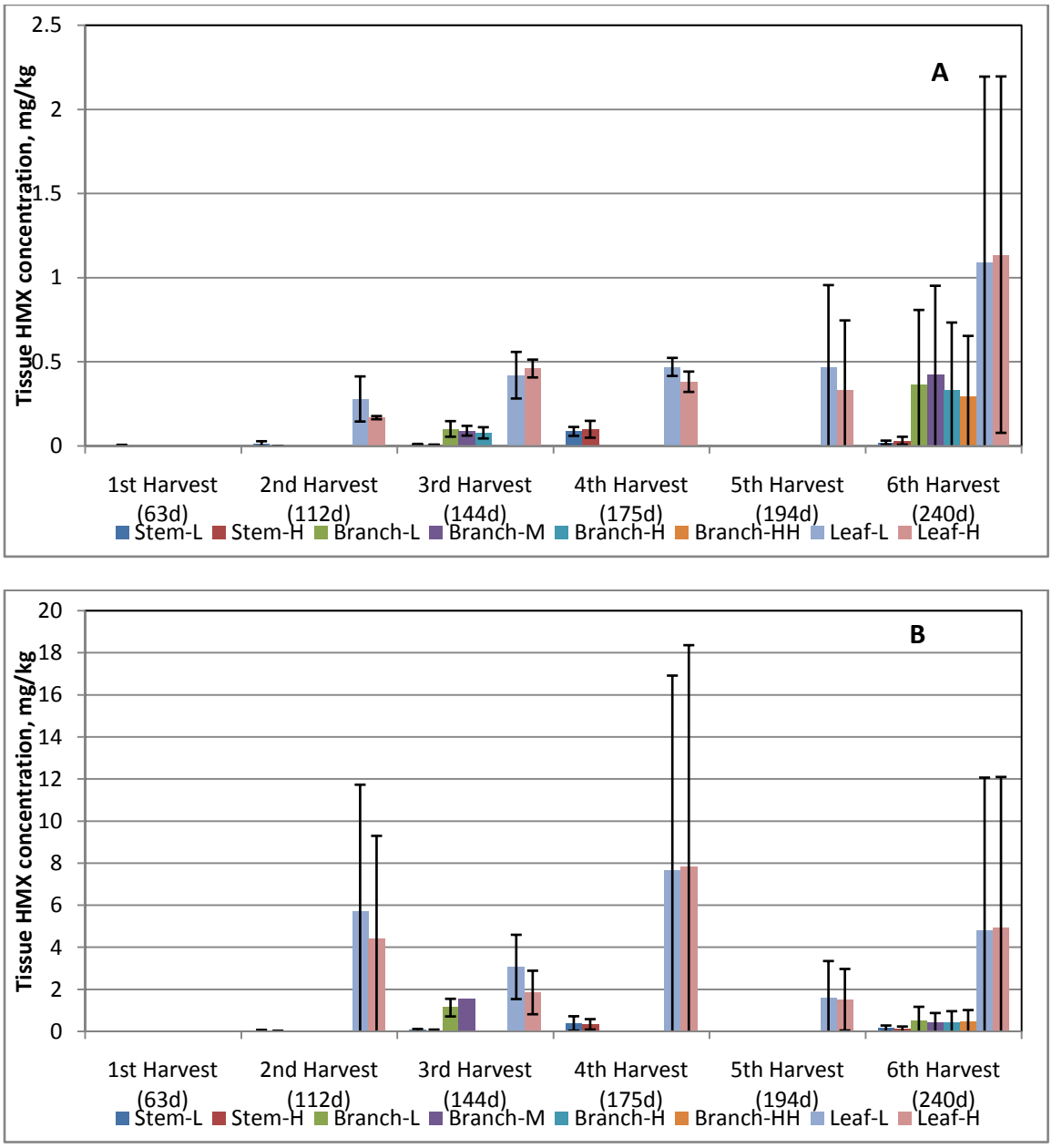

Figure 5.14 Tissue HMX Concentration (Based on DW) of Each Sampling Position

L, M, H,HH represented low, middle, high, more higher position respectively. Low closed to bottom; High closed to top. Stems were not sampled in the 5th harvest. Branches were samples only in the 3rd time and the last time. 1# tree had only one main branch, which was harvest in the last time. The 2# tree in the 5th harvest suffered from the effect of the surface dosing at that time due to the blocking of bottom dosing pathway and had absolutely abnormal higher concentrations, thus was not included in the statistics. The 6# tree had high concentration due to dying in the last harvest and was not included also. The error bar represented the standard deviation from triplication planting. A showed the test units dosed by medium level; B showed the test units dosed by high levels.

In contrast to the intra-variance of single tree, the inter-variances of different trees were clearly enlarged in large scale trees. The inter-variances were similar to the intra-valiances in the small scale tree experiments, however, much greater in the large scale tree experiment for both RDX (Table 5.4) and HMX (Table 5.5). Therefore, treating stem, branch, or leaf as one compartment is reasonable for explosives in phytoforensic testing. The RSDs of stem, branch, and leaf concentration were larger than those of pore water concentration, especially for high dosing treatment, and the RSDs were in same magnitude among stem, branch and leaf. These results suggested the probability of greater differences among the same plant species in field, which must be considered in site assessment applying phytoforensics.

5.6. EXPOSURE CONCENTRATION DEPENDENT TISSUE CONCENTRATION

The foundation of phytoforensics is that the plant tissue concentrations depend primarily on exposure concentrations. Exposure concentrations refer to bioavailable concentration and were represented by pore water concentration in this research. The tissue concentrations determined by solvent extraction method in all experiments were plotted to the corresponding pore water concentrations in Figure 5.15 for RDX and Figure 5.16 for HMX. Similarly the sap concentrations determined by centrifuge extraction method in all experiments were plotted in Figure 5.17 for RDX and Figure 5.18 for HMX. Several data from the initial harvests in the time series grass experiments and the large scale tree experiments were not included due to short term exposure much less than necessary for steady state. As the tested concentration ranging over from three

Table 5.4 RDX Concentration Intra- and Inter- Variance Determined in Experiments

Average RSD (range) (%) of concentration	Large tree experiment (inter-)	Large treeexperiment (intra-)[a]	Small tree & grass experiment	Time series grass experiment
Dosing tank	4 (1-20)			
Pore water	53(3-97)			
Stem	76 (18-133)	***19 (2-66)***		
Branch	38 (10-89)	***12 (3-20)***		
Branch-sap	45, 80			
Leaf	79(22-136)	***31 (6-86)***		
Leaf-sap	76(32-137)			
Willow-stem			19(4-42) ***19 (0-37)[a]***	
Willow-sap			17(2-31)	
Poplar-stem			12(8-17) ***17 (8-32)[a]***	
Poplar-sap			21(6-62)	
Ryegrass			23(13-42)	13(3-23)
Ryegrass-sap			22 (9-44)	18 (4-48)
Big bluestem			36(17-63)	19(8-38)
Big blue-sap			13 (4-20)	24 (4-50)

[a] intra- variance. The difference expressed as the range of the relative standard deviation (RSD).

"Sap" meant to sap concentration determined by centrifuge extraction method; others meant tissue concentration determined by solvent extraction method.

Table 5.5 HMX Concentration Intra- and Inter- Variance Determined in Experiments

RSD (%) of concentration	Large tree experiment (inter-)	Large tree experiment (intra-)[a]	Small tree & grass experiment	Time series grass experiment
Dosing tank	8(1-27)			
Pore water	44(2-87)			
Stem	92(22-149)	***29 (2-91)***		
Branch	80 (36-123)	***14 (5-23)***		
Branch-sap	116			
Leaf	82(11-150)	***31 (2-84)***		
Leaf-sap	80 (47-98)			
Willow-stem			19 (6-34) ***13 (2-33)[a]***	
Willow-sap			28(12-63)	
Poplar-stem			20(11-32) ***20 (5-31)[a]***	
Poplar-sap			12(9-14)	
Ryegrass			21 (7-44)	13 (4-34)
Ryegrass-sap			26 (7-46)	24 (6-47)
Big bluestem			26 (18-35)	18 (3-33)
Big blue-sap			32 (24-36)	27 (13-52)

[a] intra- variance. The difference expressed as the range of the relative standard deviation (RSD).

"Sap" meant to sap concentration determined by centrifuge extraction method; others meant tissue concentration determined by solvent extraction method.

to five orders of magnitude, the power fit in Excel was used for regression analysis. The essence of power fit in Excel is log-log transform plus linear regression using the least squares method.

The results were promising. The R^2-values of the regression model were larger than 0.9 for all four species and two compounds in both small scale experiment and time series grass experiment. Although in the small scale experiment the big bluestems did not reach steady state at harvest, their tissue concentration still correlated exposure concentration well. For large trees the R^2-values of the regression model were not as well as small trees, however, still in acceptable level, larger than 0.7. The rationale for the correlation is explained as follows: Firstly, the pore water sampled from the bottom of all reactors, which did not stand for the pore water concentrations in the whole reactors. The concentration of pore water in the whole reactor was not consistent spatially. The big reactors were expected with greater heterogeneity than the small reactors. Secondly, the tissue concentrations of large scale trees were more varied and did not reach expected steady states. Thus the lower correlation in big scale experiment was to be expected.

The regressions equations from different experiments were compared for all species. Ryegrass showed higher plant: soil ratio in small scale experiment (217 L/kg for RDX and 184 L/kg for HMX) than in time series experiment (190 L/kg for RDX and 135 L/kg for HMX). The soil reactors indicated higher plant: soil ratio than sand reactors. Plants extraction and bacteria biodegradation occurred concurrently and long retention time and high degradation potential in soil resulted in the low concentration of pore water at the time of sampling, thus resulted in the higher plant: soil ratio. Big bluestem showed lower plant: soil ratio in small scale experiment (85 L/kg for RDX and 64 L/kg for HMX)

than in time series experiment (113 L/kg for RDX and 87 L/kg for HMX) because steady state was not reached in the 8 weeks exposure. In this study, the ryegrass tissue concentrations were determined from 3.80 mg/kg DW to 2649.01 mg/kg DW for RDX and 0.86mg/kg DW to 247.05 mg/kg DW for HMX, in the range of 119-5217 mg/kg for RDX and 26-380mg/kg for HMX from the previous publications[18, 27, 40, 42].

Willow showed lower plant: soil ratios in large trees. The large trees did not reach steady state completely so that the tissue concentrations were lower. Another larger reason was that the pore water concentrations were overestimated in big reactors discussed above. Thompson et al reported the plant: soil ratio of 2.9-5.8 L/kg for small poplar stems after 7 days' hydroponic exposure. The leaves had much higher plant: soil ratio of 27-45 L/kg[50]. These results were in good accordance with the results in this study if the difference of exposure time was considered.

Although sap concentration did not indicate the whole tissue concentration as calculated tissue concentration from sap measure smaller than the measured values, sap concentration indicated underground exposure concentration very well, which validated the novel centrifuge extraction methods for phytoforensic field assessment. Sap concentration in aerial tissue revealed a clear correlation with exposure concentration ranging over 4 log units. The R^2-values of the regression model were larger than 0.85 for all four species and two compounds in both soil and sand reactors. The sap concentrations in the grasses were demonstrated higher than the pore water concentration, showing the increased potential to delineate plumes and soil contamination using phytoforensic methods. The novel methods save time, labor, and solvent and are thusly

very attractive to screen a vast area and analyze a great deal of samples fast, simply, cheaply.

In summary, the concentration in aerial tissue revealed a clear correlation with exposure concentration represented by pore water concentration in rooting media, which validated the phytoforensics on RDX and HMX. Grasses are quite good sampling objects as they have a noticeably higher tissue sap concentration than pore water concentration. Also they are quicker to reach steady state because they do not have woody tissue transport process. However, grasses also have two disadvantages. Firstly, the plant: soil ratios are quite varied in different species, which impede the quantitative prediction. Secondly, the roots of grass are not deep enough to catch the deeper contaminants. On the contrary, trees have deeper roots and less varied relationship as stems (trunk) have best correlation to exposure concentrations. The problems for trunks were much lower xylem sap concentrations which need very sensitive analysis methods and the long time required to reach steady state especially for larger trees. The leaves had higher tissue concentration although not as high as grass. However, the leaves concentrations are varied and have worst correlations to exposure concentration. Thus a 2 steps approach is proposed for field energetics phytoforensics. Sample grasses and leaves firstly for the qualitative screening, then followed by tree core sampling for the quantitative prediction.

5.7. SMALL SCALE TREE AND GRASS EXPERIMENTS SIMULATION

5.7.1. Input Parameters Determination. The developed model was conducted to simulate the 24 test units of the small scale tree and grass experiments. The input parameters successively derived from measurements in the experiment studies, literature data, and calibration by experiment data if necessary. The calibrations were accomplished by adjusting the values until the model output matched the experimental measured data. The test units of medium dosing level were used for parameter calibration. The calibrated parameters were then applied to simulate the low and high dosing level test units. Table 5.6 listed the values of input parameters independent of experiment settings and chemical properties. The parameters of leaf area:volume and transpiration conductance were derived from the literature, however, also were validated by experiment data. The tree leaf areas of each of six test units were measured for once in the middle of the large scale tree experiment. The average calculated value was 0.18±0.02 cm/d based on the added dosing solution volume per day divided by leaf area. This value included both evaporation and transpiration. Therefore, the transpiration conductance value of 0.17 from the literature[68] was in close agreement. Once validating the parameter of transpiration conductance, the parameter of leaf area:volume can be validated by the measured biomass and ET flow rate from experiments. Simulated ET flow rates were close to the measured values for both grasses and trees at the beginning of dosing, which validated the parameter value of leaf area:volume. In Table 5.6, only one parameter was calibrated from the experiment data. The parameter of phloem flow rate was adjusted to comply with the measured ratio of branch sap to leaf sap.

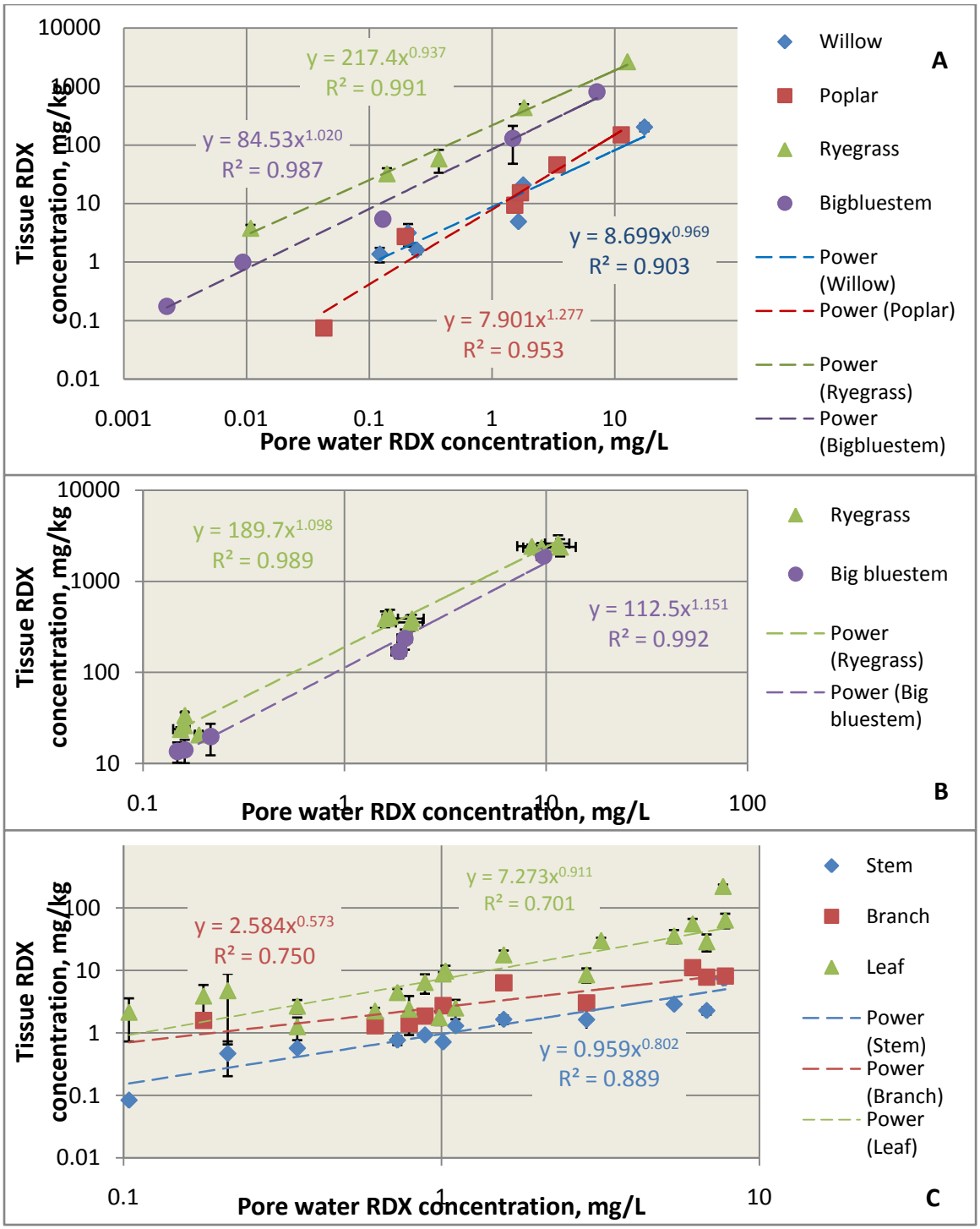

Figure 5.15 Tissue RDX Concentration Related to Pore Water Concentration

A: Small scale experiments; B: Time series Experiments (The first 3 harvests, $4^{th}/6^{th}/8^{th}$ week, were not included); C: Big scale experiment (The first and second harvest was not included). The error bars represented the standard deviation. Regression analysis was carried out by Excel.

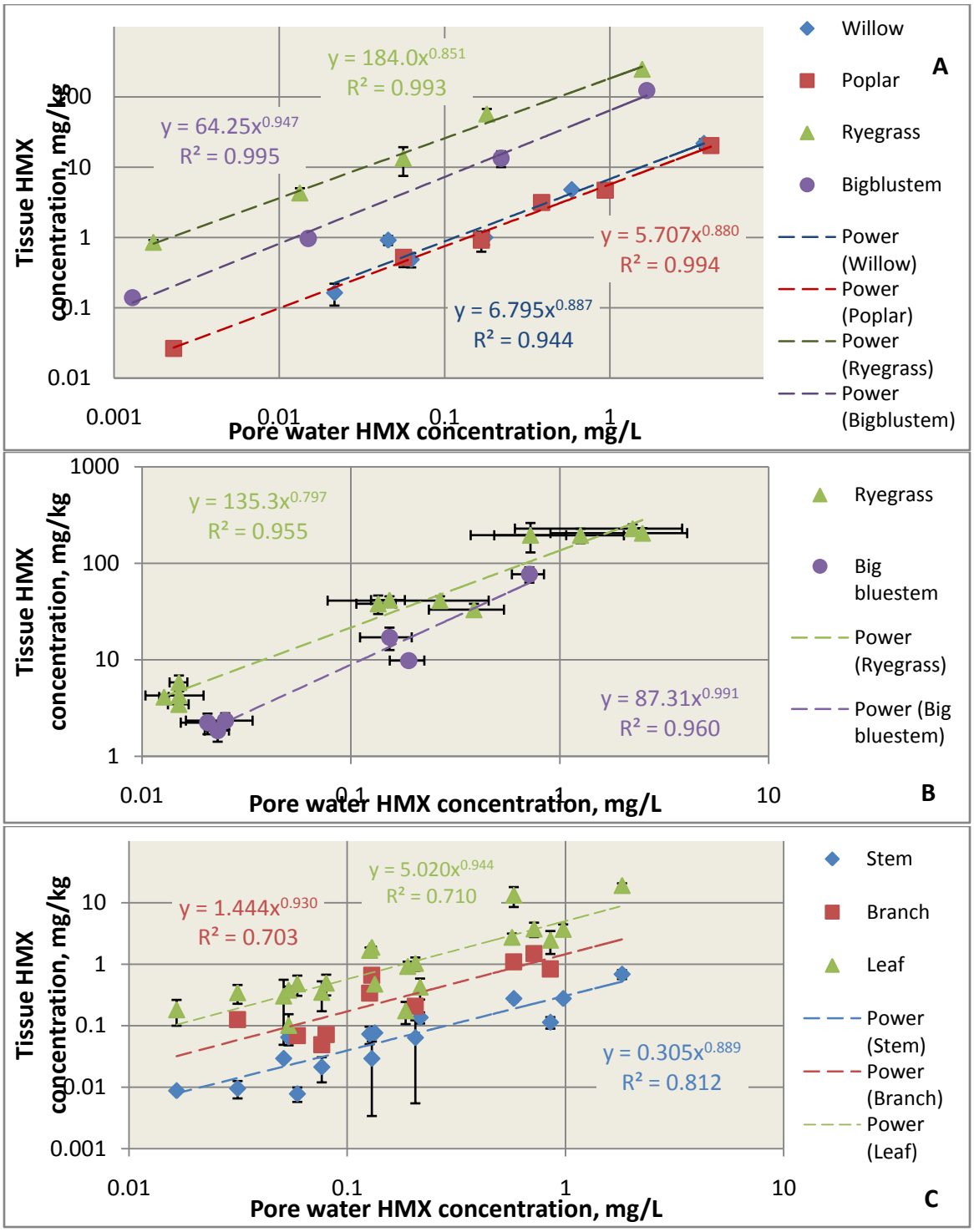

Figure 5.16 Tissue HMX Concentration Related to Pore Water Concentration

A: Small scale experiments; B: Time series Experiments (The first 3 harvests, 4th/6th/8th week, were not included); C: Big scale experiment (The first and second harvest was not included). The error bars represented the standard deviation. Regression analysis was carried out by Excel.

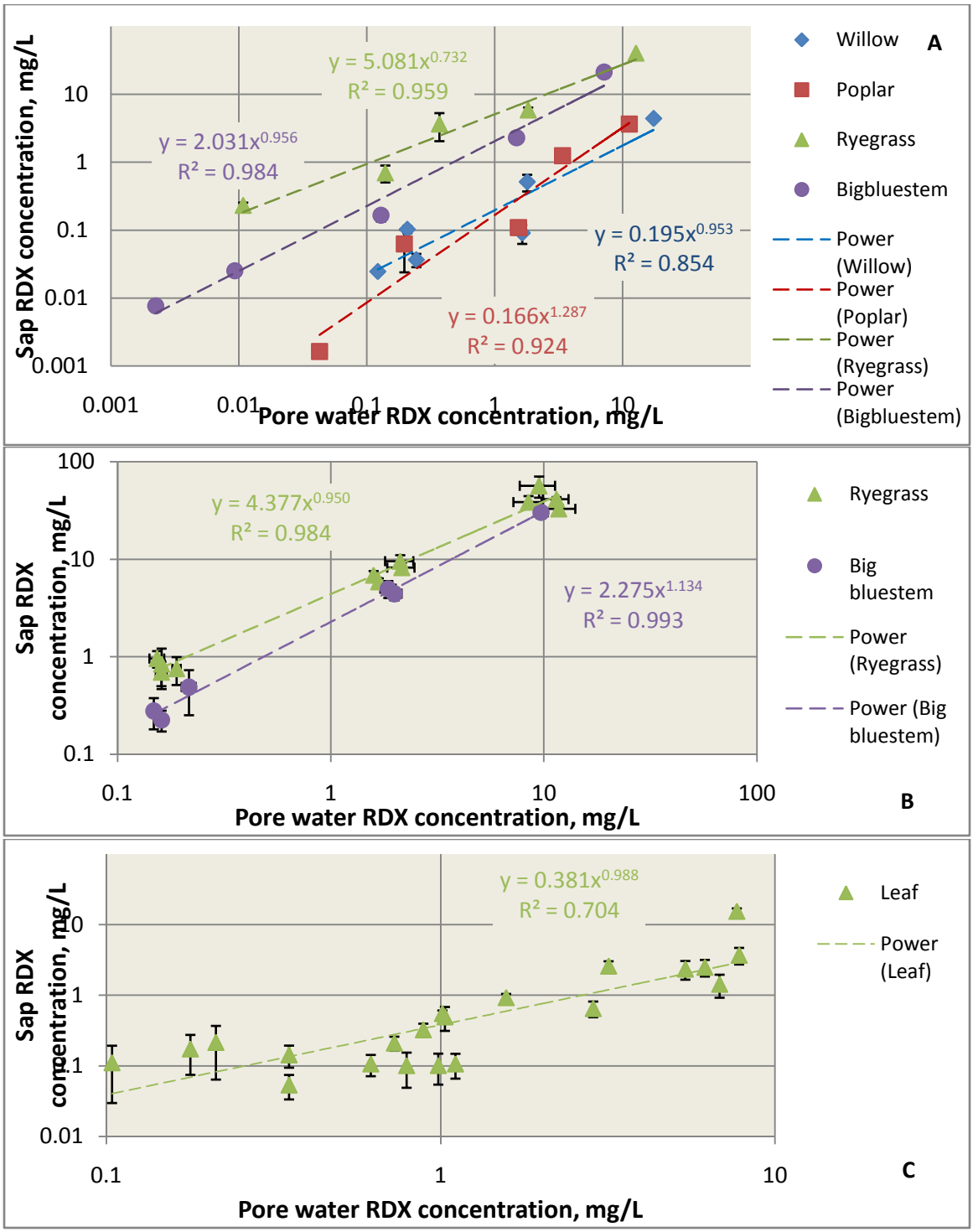

Figure 5.17 Sap RDX Concentration Related to Pore Water Concentration

A: Small scale experiments; B: Time series Experiments (The first 3 harvests, $4^{th}/6^{th}/8^{th}$ week, were not included); C: Big scale experiment (The first and second harvest was not included). The error bars represented the standard deviation. Regression analysis was carried out by Excel.

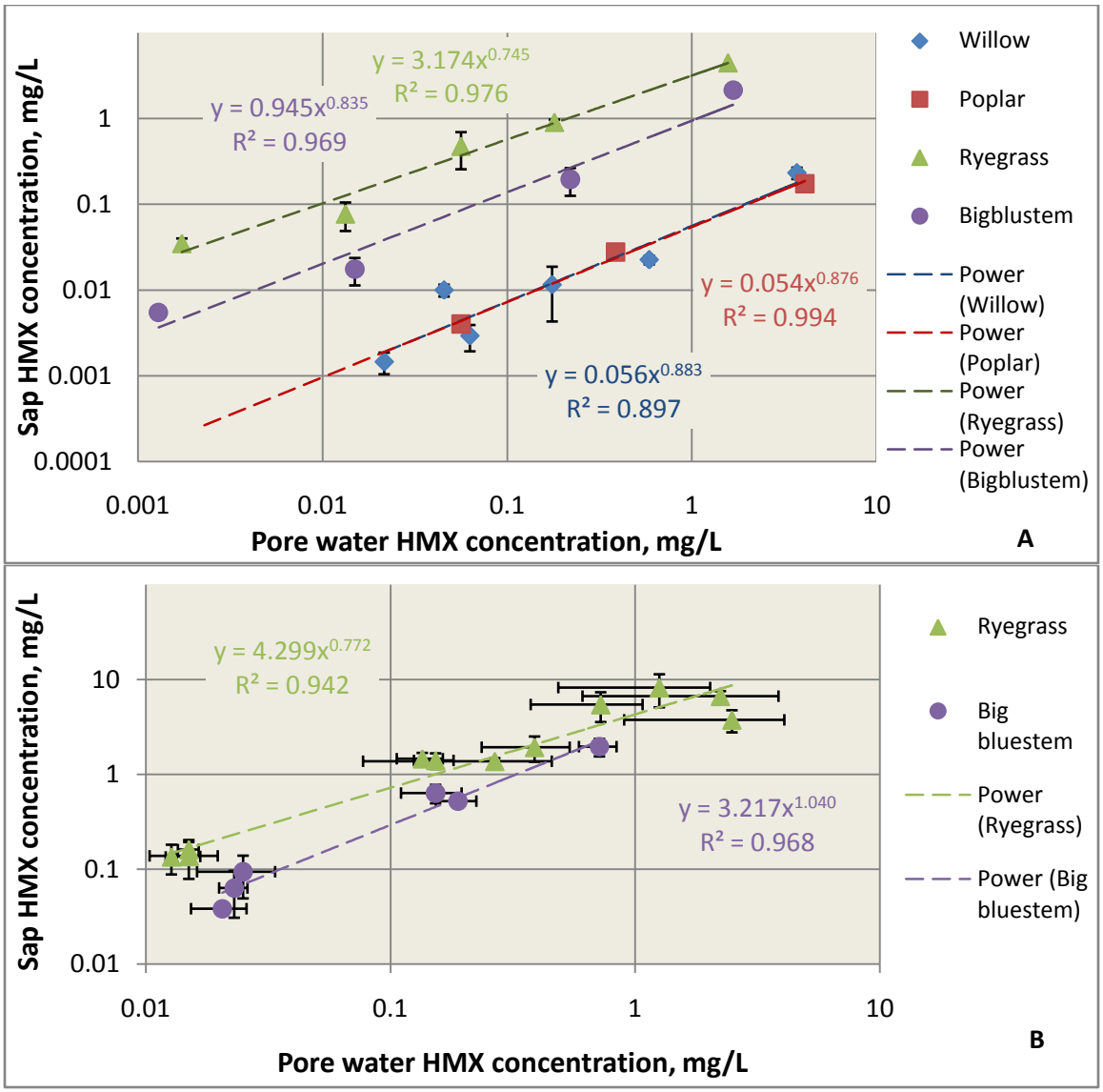

Figure 5.18 SapHMX Concentration Related to Pore Water Concentration

A: Small scale experiments; B: Time series Experiments (The first 3 harvests, $4^{th}/6^{th}/8^{th}$ week, were not included). The error bars represented the standard deviation. Regression analysis was carried out by Excel.

Many sap samples from large tree experiment could not be accurately determined due to the interference peak coinciding with HMX peak. The low HMX concentration made the problem worse. Thus the number of sap samples with reliable HMX measures was not big enough to match with pore water samples for regression analysis. There is no quantification problem for RDX. However, most stem samples were too dry to extract sap and the number of branch samples was limited.

Figure 5.7 listed the values of input parameters dependent properties of chemicals and also characteristics of plant species and soil. The reference values could not be acquired for many from the literature. The uncertainty in sorption parameters was solved as the sap concentration can be determined directly by the novel sap extract method (Yuan, 2012), however, uncertainty in degradation parameters still existed. Degradation rates and pathways of xenobiotics in wood are currently not well defined. While not known metabolism in xylem can occurs as xylem is not entirely void of metabolic process as well as the possible presence of endophytic bacteria. However, the metabolism rate in wood is thought to be lower than that in leaf and root. Thus the model assumed the transform rate of branch and stem were negligible and thereby limit the number of calibration on fitting parameters. The root partition value was assumed based on the measure values of other tissue because of lacking experimental measurements on root. In Figure 5.7, four parameters were calibrated from the experiment data. Firstly three parameters related to plant, leaf and root transform rate as well as root reflective coefficient, were calibrated from the sand reactors of medium dosing level. Lastly the biodegradation rate was adjusted to comply with the measured pore water concentration in the corresponding soil reactors. In the case of grass, leaf transform rate, root transform rate and root reflective coefficient had to be calibrated at the same time, which greatly increased the uncertainty. Among these three parameters, leaf transform rate was not zero obviously base on the previous studies[16, 34, 36, 40, 42, 55], and root reflective coefficient was also not zero because the higher pore water concentrations than dosing concentration were observed in the sand reactors. Root transform rate was assumed to be negligible in grass. This assumption had supporting data. If the root transform was not

equal to zero, the dynamic simulation would show the leaf concentration decreased after 70 days of exposure, which was not observed in the time series grass experiments. In the case of tree, only root transform rate and root reflective coefficient were required to be calibrated at the same time. The root transformation rate was not negligible because of the quite low root reflective coefficient derived from the measured TSCF in trees [19, 50], which were different from grass. In grass, chemicals were translocated upward much more efficiently and no great accumulation occurred in the roots and thus indicating limited metabolism of chemicals in the root. In summary, all calibration results were consistent with the understandings on RDX and HMX from previous studies. HMX with crown structure is chemically more stable and therefore less amenable to biodegradation than RDX with chair structure[35]. The biodegradation of RDX and HMX has been reported [3, 37]. In plants HMX was concluded to be more recalcitrant than RDX[18, 19, 36, 40]. The metabolism of RDX in leaf has been reported[16, 36, 40, 42, 55].

Table 5.8 listed the values of input parameter dependent of experiment settings, which mainly derived from the experiment measurements. The initial biomass, growth rate and soil fresh mass were not identical between any two reactors. The average values from similar reactors were determined as the input parameters for these reactors. No accurate measurements of root biomass could be acquired so that the biomass of root was estimated from leaf biomass[67, 82]. The same treatment was conducted on root growth rate. The leaf growth rate was calibrated by the flow rate and leaf biomass at the time of harvest. The dynamic change during experiment period was not considered. The leaf biomass and the branch biomass were measured together in the experiment and half to half was allocated between them.

Table 5.6 Input Parameters, Independent of Experiment Settings and Chemical Properties

Parameter	Symbol	Value	Unit	Reference
Leaf area:volume	$(A/V)_L$	40	cm^{-1}	[68]
Leaf density	ρ_L	0.85	g/cm^3	[82]
Leaf water content	n_L	0.74 ryegrass 0.67 big bluestem 0.64 tree	W/W	Measured
Branch water content	n_B	0.56 small tree 0.48 large tree	W/W	Measured
Stem water content	n_S	0.48 small tree 0.35 large tree	W/W	Measured
Root area:volume	$(A/V)_R$	40	cm^{-1}	[68]
Root density	ρ_R	1.0	g/cm^3	[73]
Root water content	n_R	0.9	W/W	[67]
Sap density	ρ_w	1.0	g/cm^3	
Transpiration conductance	T_c	0.17	cm/d	[68]
Evaporation conductance	E_c	0.17	cm/d	Typical value: 0.1-0.5 cm/d
Phloem flow rate	γ	0.1		Calibrated

Table 5.7 Input Parameters, Dependent of Chemical Properties

Parameter	Symbol	Value, RDX	Value, HMX	Unit	Reference
Leaf partition	$K_{d,L}$	30 grass 15 tree	30 grass 15 tree	ml/g	Measured
Leaf transform rate	$k_{m,L}$	0.1 ryegrass 0.25 bigbluestem 0.1 tree	0.08 ryegrass 0.22 bigbluestem 0.015 tree	d^{-1}	Calibrated
Branch partition	$K_{d,B}$	25	40	ml/g	Measured
Branch transform rate	$k_{m,B}$	0	0	d^{-1}	Assumed
Stem partition	$K_{d,S}$	30	50	ml/g	Measured
Stem transform rate	$k_{m,S}$	0	0	d^{-1}	Assumed
Root partition	$K_{d,R}$	30	30	ml/g	Assumed
Root transform rate	$k_{m,R}$	0 grass 0.8 tree	0 grass 0.14 tree	d^{-1}	Calibrated
Root permeability	P	0.09	0.06	cm/d	Calculated from [67]
Root reflective coefficient	σ	0.8 grass 0.2 tree	0.5 grass 0.07 tree		Calibrated
Biodegradation rate	k_m	0/0.01 sand 0.08/1 soil1 [a] 0.4 soil2	0/0.01 sand 0.02/0.14 soil1 0.2 soil2	d^{-1}	Calibrated
Soil partition	K_d	0 sand 1 soil1 15 soil2	0 sand 5 soil1 30 soil2	ml/g	Measured

[a]: Soil1 referred to the silt Loam used in small scale tree and grass experiment; soil2 referred to the commercial Sta-green tree and shrub soil used in large scale tree experiment.

Table 5.8 Input Parameters, Dependent of Experiment Settings

Parameter	Symbol	Value	Unit
INIT Leaf fresh mass	M_L	1.2 grass; 15 willow; 10 poplar; 30 big willow	g
INIT Branch fresh mass	M_B	15 willow; 10 poplar; 30 big willow	g
INIT Stem fresh mass	M_S	35/70 willow; 35 poplar; 2500 big willow	g
INIT Root fresh mass	M_R	2.4 grass; 20 willow; 15 poplar; 40 big willow	g
leaf growth rate [a]	k_L	0/0.017/0.035 grass; 0/0.011 willow 0.008 poplar; 0.008/0.014 big willow	d^{-1}
Branch growth rate [a]	k_B	Same as leaf	d^{-1}
Stem growth rate [a]	k_S	0	d^{-1}
Root growth rate [a]	k_R	0/0.025/0.05 grass; 0/0.012 willow 0.01 poplar; 0.01/0.016 big willow	d^{-1}
Dosing conc	C_d	Varied	mg/L
Bare soil area	A	0 small reactor; 500 big reactor	cm^2
Soil fresh mass	M	1460/3250 sand (grass/tree) 1100/2500 soil1 (grass/tree) 11000 soil2	g
Soil water content	n	0.18/0.17 sand (grass/tree) 0.42/0.33 soil1 (grass/tree) 0.6 soil2	W:W

[a]: All grow rates reduce to half if the biomass reached to 10 times of original biomass.

5.7.2. Simulation Results and Discussion. 48 simulations were conducted for 24 test units (4 plant species * 2 rooting media * 3 explosive treatments) on both RDX and HMX. Only 7 test units' experimental results were used in the parameter calibration, and other 17 test units were not involved in the parameter calibration. The 4 medium dosed sand reactors of each species were used to calibrate plant relative parameters, leaf/ root transform rate and root reflective coefficient. Then medium dosed soil reactors of willow and ryegrass was used to calibrate the biodegradation rate of soil with low and high biodegradation potential respectively. Temperature and transpiration rate impacted the potential of biodegradation discussed above. The high dosed ryegrass reactor was used to calibrate the biodegradation rate of analytes in sand. This parameter was only used in 4 high dosed sand reactors where biodegradation was suggested by the observed lower pore water concentration, while in other 8 medium and low dosed sand reactors biodegradation appeared to be negligible. A time step of 1day was used and the integration method was fourth-order Runge– Kutta methods.

The conformity between simulated and experimental data was evaluated by the ratio of simulated values to measured values, Table 5.9and Figure 5.19 for a visually intuitive comparison. The ratios of simulated/measured pore water RDX and HMX concentration were 1.36±0.95 (mean ±SD) and 1.15±0.74 for trees and 1.25±0.51 and 1.40±0.62 for grasses, respectively. The ratios of simulated /measured sap RDX and HMX concentration were 1.48±0.85 and 2.80±0.80 for trees and 1.31±0.71 and 1.39±0.98 for grasses, respectively. The ratios of simulated /measured tissue RDX and HMX concentration were 1.01±0.49 and 1.08±0.36 for trees and 1.13±0.44 and 0.96±0.47 for grasses, respectively. In general, simulated values for pore water

concentration, sap concentration, and tissue concentration were close to measured values. The deviations between the simulation and experimental measurements could be explained by the following reasons.

Firstly, limitations of the model derived from underlying assumptions, process consideration and formulation, as well as parameter selection. In this model phloem transport was neglected except for leaf-branch back flow. Partition in plant and soil compartment interior was assumed constant and in equilibrium. The concentration difference among the same plant or soil compartment was not considered also. At steady state conditions this assumption appears valid; however, at the beginning of exposure the difference in the same compartment, such as stem, was evident, which has been proven by experimental measurements. The heterogeneity in rooting media was also observed. More critically, the complex interrelationship between environmental conditions and plant properties were not automatically accounted in this model. The parameters were assumed constant over the period of simulation. However, these conductance coefficient and kinetic growth and reaction constant were highly correlated with light intensity, humidity, temperature and plant health status and possibly changed over time[67, 69].

Secondly, all 48 simulations were conducted in conformity with the uniform parameter values. For example, although several reactors, such as high dosing willow in soil, high dosing poplar in soil and low dosing big bluestem in sand, showed consistently higher simulated values in pore water, sap and tissue, suggesting higher biodegradation potential in these reactors, the parameter value of biodegradation rate, for example $0.08day^{-1}$ for trees in soil and $1.0day^{-1}$ for grasses in soil for RDX as well as $0.02day^{-1}$ for

trees in soil and $0.14day^{-1}$ for grasses in soil for HMX, was held constant for all soil and sand reactors.

Finally, Novel measure techniques may also contribute. One evident example was small tree sap measurement. High salts content of plant sap inhibited the ionization process for MS detector especially for HMX. 10 dilution fold was required to minimize the sample matrix interferences. Early samples were not noted and 2-6 dilution folds were applied for small trees sap analysis possibly resulting in the underestimated measured results.

The simulation and experimental results showed good correlation between *in-planta* concentrations and exposure concentrations (Figure 5.19), which validated the phytoforensic approach for these two compounds. These results demonstrate the utility of this new computer simulation model in predicting non-volatile xenobiotic behavior in plants and the potential use of such models in the site assessment with phytoforensics. The developed model can be applied for other non-volatile compounds and different conditions with only minor adaptions and might be served as the base of furthermore improvement of soil-plant system model and integrated with contaminant underground transport models. The finds have considerable benefit when paired sampling for phytoforensics applications and monitoring efficiency of phytoremediation applications.

5.7.3. Parameter Influence and Sensitivity Analysis. Sensitivity analysis was conducted on the calibrated parameters including leaf transform rate, root transform rate, root reflective coefficient and biodegradation rate. Another important parameter, transpiration conductance, also was selected for sensitivity analysis because it directly determines the transpiration flow rate. The simulations were carried out on one test unit,

Table 5.9 Comparisons between Model Prediction and Experiment Measurement

The comparisons were expressed as ratio of predicted value to measured value, RDX (HMX); The tree results came from the stem sections of the trees; the grass results came from the sections 1cm above the surface.

Reactors (Species, rooting media, treatment)		Pore water concentration	Sap concentration	Tissue concentration
Willow in soil	Low	1.15 (0.93)	1.27 (2.07)	0.70 (0.98)
	Medium	0.87 (0.97)	3.55 (2.35)	2.03 (1.37)
	High	3.95 (3.08)	1.96 (2.41)	1.94 (1.32)
Willow in sand	Low	0.90 (0.96)	1.43 (3.40)	1.00 (1.03)
	Medium	1.33 (0.87)	1.08 (3.69)	0.82 (0.88)
	High	1.08 (0.88)	1.04 (2.46)	0.69 (1.33)
Poplar in soil	Low	0.26 (1.00)	N.A.	0.94 (0.60)
	Medium	0.61 (0.66)	N.A.	0.65 (0.87)
	High	2.17 (2.23)	1.23 (4.32)	1.03 (1.93)
Poplar in sand	Low	1.07 (0.98)	0.72 (2.02)	0.51 (0.79)
	Medium	1.30 (0.57)	N.A.	0.96 (0.84)
	High	1.58 (0.71)	1.04 (2.47)	0.79 (1.07)
Ryegrass in soil	Low	N.A.	0.44 (0.24)	0.66 (0.29)
	Medium	2.50 (2.60)	0.69 (0.69)	1.42 (0.93)
	High	1.03 (1.35)	0.61 (0.84)	1.26 (0.97)
Ryegrass in sand	Low	1.15 (2.11)	1.31 (1.69)	0.92 (1.00)
	Medium	0.97 (1.11)	1.69 (1.04)	0.75 (0.54)
	High	0.98 (0.91)	2.53 (2.58)	1.27 (1.54)
Bigbluestem in soil	Low	N.A.	0.56 (0.25)	0.80 (0.40)
	Medium	1.06 (1.47)	1.07 (0.84)	0.87 (1.07)
	High	0.69 (0.76)	N.A.	N.A.
Bigbluestem in sand	Low	1.24 (1.94)	2.23 (3.32)	2.21 (1.91)
	Medium	1.16 (0.91)	1.78 (2.05)	1.01 (0.96)
	High	1.67 (0.88)	1.51 (1.70)	1.27 (0.95)

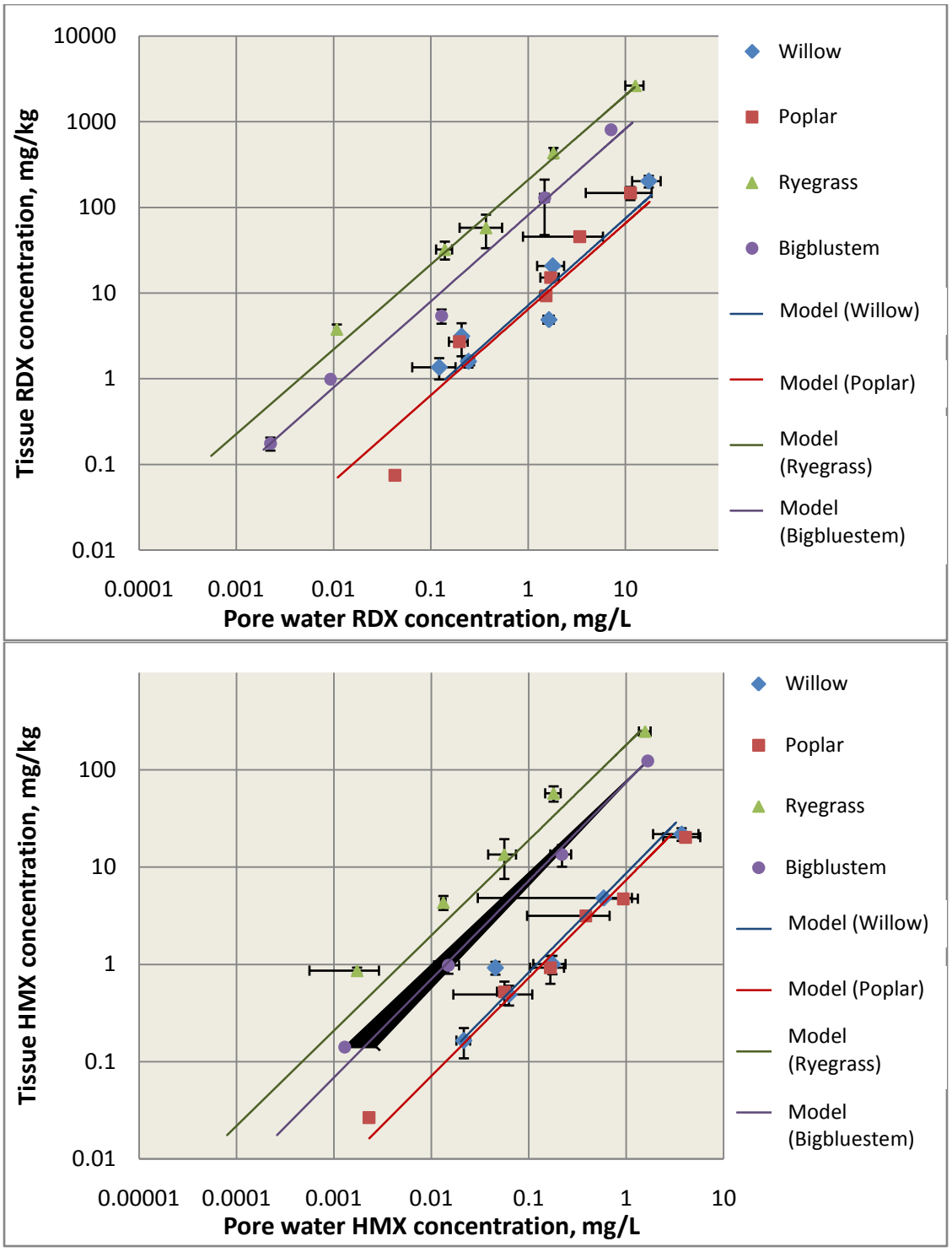

Figure 5.19 Model Prediction and Experiment Measurement Comparisons
(The model lines of each species were obtained by fitting six simulation results.)

willow tree in soil reactor dosed by medium level. The tested parameters were adjusted ±50% based on the values applied in the reactor simulation. Each time only one parameter was changed, the corresponding results on leaf sap concentration, stem sap concentration and pore water concentration were compared with the results using unadjusted parameter and the change ratios,(adjusted result - original result) / original result, were calculated. The simulation period was 90 days, same as the simulations done in previous section. The sensitivity analysis results were shown in Figure 5.20. Leaf sap concentration was most sensitive model output to the parameter change and its change ratios were at the range of -78 ~ 131%. The high sensitivity can be related to the high variability observed in the measured data (Table 5.4). The pore water concentration was least sensitive model output as the change ratios ranging -38 ~ 26%, smaller than the change ratios of parameters, again as observed in the variance among measured data (Table 5.4). The stem sap concentration was between them, changing over -53 ~ 53%. Among tested parameters, leaf transform rate only impacted on leaf concentration and did not affect stem and pore water concentration as one-way flow included in model. The root transform rate affected leaf and root concentration to a limited degree. Similar results were revealed on root reflective coefficient, other than less impact on pore water concentration. The biodegradation rate changed the pore water concentration greatly. The transpiration conductance appeared to be the most impactful parameter, indicating the transpiration flow rate affecting *in-planta* concentration and surrounding concentration to a large degree, which has been revealed by experimental results. The environmental conditions, including air humidity and temperature, directly impact the transpiration conductance. The average fluctuation in a season should be small; however, the

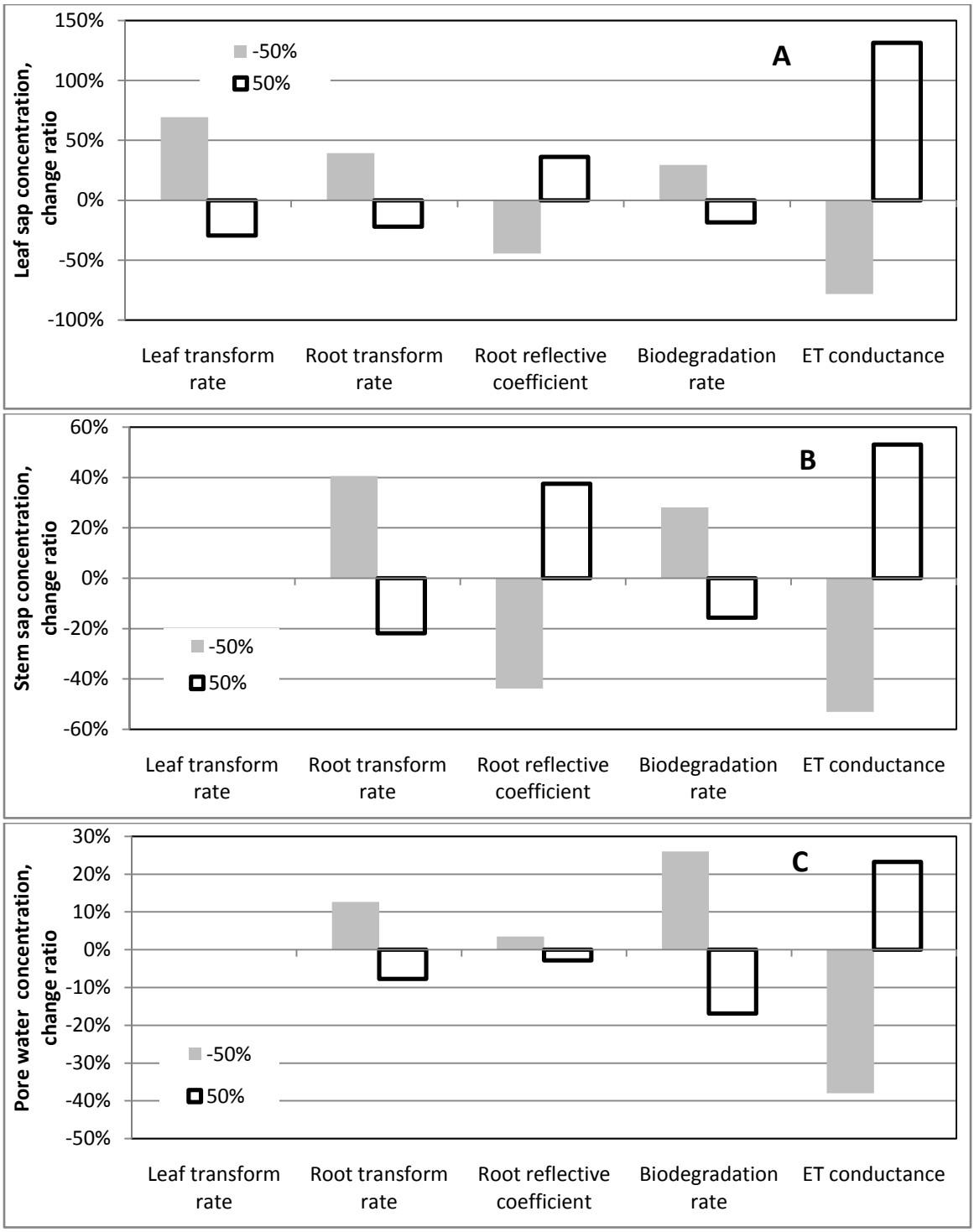

Figure 5.20 Sensitivity Analysis on Calibrated Parameters

The results were expressed by change ratio (results) to change ratio (parameters). A showed leaf sap concentration change; B showed stem sap concentration change; and C showed pore water concentration change.

difference among different seasons may be large and resulted in great discrepancy of *in-planta* concentration, which must be considered in applying phytoforensics. Results from other ongoing research at S&T have recently revealed a much greater than anticipated seasonal variation for *in-planta* chlorinated solvent concentrations for full scale trees (Limmer, Data not shown). The observed variation may be described in part from the model presented here.

Chemical fate in plant is based on plant physiology and chemistry. Grass and tree have evident different physiology and chemistry. Grasses do not have lignin tissue, and xylem in frond is linked to root xylem directly. Thus the upward transport routine is much shorter than that in tree. The root resistance appeared to be low resulting chemical accumulated in frond tissue at a high concentration. On the contrary, a more prominent root resistance was observed in the trees, thus resulted in much lower concentration in stem xylem. Although chemical also accumulates in leaf tissue in tree, the leaf concentration is significantly lower than grass frond concentration due to low stem xylem concentration. Trees have separate vascular transport tissue, xylem and phloem in trunk and branch, and the terminal tissue at the point of water evaporation is not directly linked to root. The evaporation of the water leads to concentrating of the solutes (RDX or HMX) that are not volatile. Thus the transport route in trees is much longer and need long time to reach a relative steady state.

Comparing large scale tree and small scale tree, the main differences appear at two aspects. One is the change of chemical composition. Wood is composed mainly of cellulose, hemicellulose, and lignin. Lignin is hydrophobic and is regarded as main adsorbents for organic compounds and impacts the partition relationship between sap and

wood[74].Older tissues do not maintain their conductivity as trees become lignified with age, and recent research demonstrated that lignin and lipid content changes the binding of organic contaminants in phytoforensic studies[78]. However no study on RDX or HMX partitioning to lignin were searched. In this study, the stem (older) showed slightly higher partition coefficient than that of branch (newer) based on sap and tissue concentration measurements. But this conclusion is not completely confirmed due to lack of enough supporting data. The branch sample numbers were limited and some of tree core samples were too dry to get liquid, which further decreased the sample numbers available.

The other is the lager wood biomass in large scale tree serving as chemical container, which slows the upward transport of chemical and evidently increases the time needed to reach steady state in plants.

In order to better understand the impact of tree scale on *in-planta* concentration, two simulations were conducted on small scale tree and large scale tree respectively. The small tree simulations were carried out on willow tree in soil reactor dosed by medium level. The large tree simulation retained the biomass, growth rate, and soil mass applied in large tree experiment, but applied the soil characteristics in small tree experiment to highlight the impact of plant. The simulation results were shown in Figure 5.21. The larger soil volume in large reactor had higher sorption capability resulting in lower soil pore water concentration, thus leading to the lower stem sap concentration. The biomass of large scale tree served as huge container limiting the chemical entering leaves. Thus an early great difference was showed between small tree leaves and large tree leaves based on simulated results. In real field, the difference between them may be lower as early high concentration was measured in larger scale tree leaf. In sum, stem showed only

slight difference between large tree and small tree and also correlated to soil concentration very well for both scales. This simulation suggested the results from laboratory can be applied to site large tree at least for tree stem.

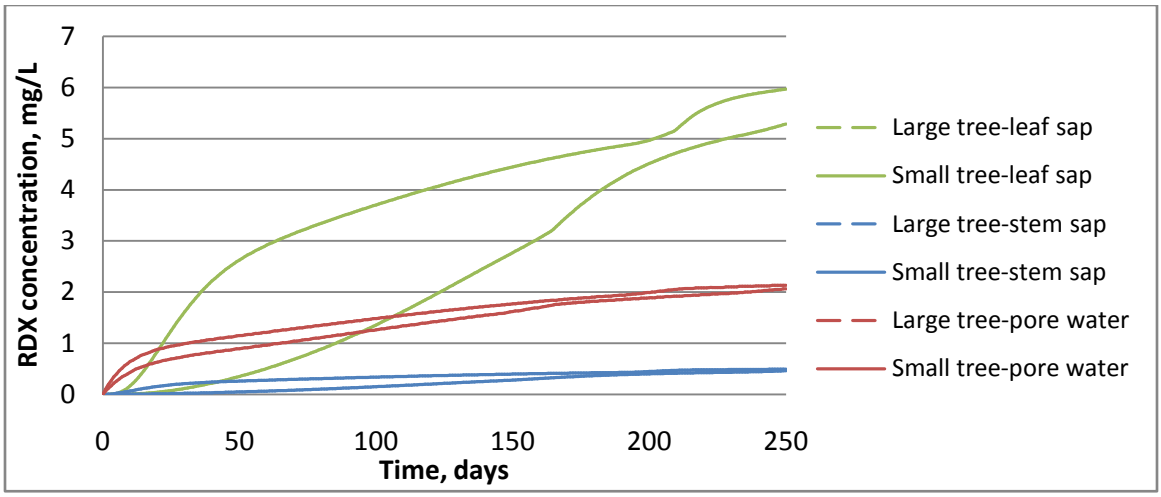

Figure 5.21 Comparison of Large Tree and Small Tree Simulation Using Same Soil

5.8. FULL SCALE FIELD ASSESSMENT

Field Site at Longhorn Army Ammunition plant (LHAAP) in Karnack Texas were sampled and analyzed, using tree core collection. Perchlorate in tree core samples was analyzed by the developed sap extract method with simply frozen, centrifuged to get the tree sap, followed by an ultrafast ion chromatography-tandem mass spectrometry (UFIC-MS/MS) method.

Total 212 samples were centrifuged and the saps were collected from 211 samples. The only tree species of M. azedarach could not get enough sap by

centrifugation to do UFLC-MSMS analyze. The sap extracted was less 5µl.Species impacted the centrifugation efficiency. The efficiency decreased in the order of Pinus>L. styraciflua>Quercus>Ulmus. Only species with tree number lager than 10 were assessed. For Pinus the sap centrifuged out was about 35±20% µL from 0.70±15% g cores (FW), while for Quercus the sap centrifuged out was about 15±20% µL from 0.70±15% g cores (FW).

Figure 5.22 showed the plume maps and the tree and well concentrations. The coordinate system used the NAD83 North Central Texas State Plane. Contours shown represented logarithmic groundwater contours provided by EPA. Groundwater wells shown only included those sampled June 2012. The concentrations were log10 transformed to correct for the large spread of the data. The tree species detected high concentration of perchlorate included all main species, Pinus, L. styraciflua, Quercus and Ulmus, showing wide applicability for different plant species.

The tree detection results were basically in agreement to the results of groundwater monitoring. For example, the groundwater from well MW-8 and well 17WW06 (Figure 3.4) had highest concentration of 73 mg/L and 66 mg/L of perchlorate respectively. The trees with highest xylem sap concentration, 0.22-0.95 mg/L, were 4 trees just besides these two wells. The concentration difference between the tree xylem sap and groundwater was larger than expectation, attaining about 150 times, suggesting the lower concentration in soil pore water. Also from tree data, several possible "hotspots" where no groundwater sampling was available were revealed. In summary, the results from LHAAP field were promising. Plant sap concentration was related to the groundwater concentration and can be used to assess sites inexpensively, rapidly and with

little disturbance. The further studies of the specific quantitative relationship for perchlorate were needed as the partition, metabolism and transport through root were still not fully understood for ion chemicals. The model developed in this study was not applicable for ions also.

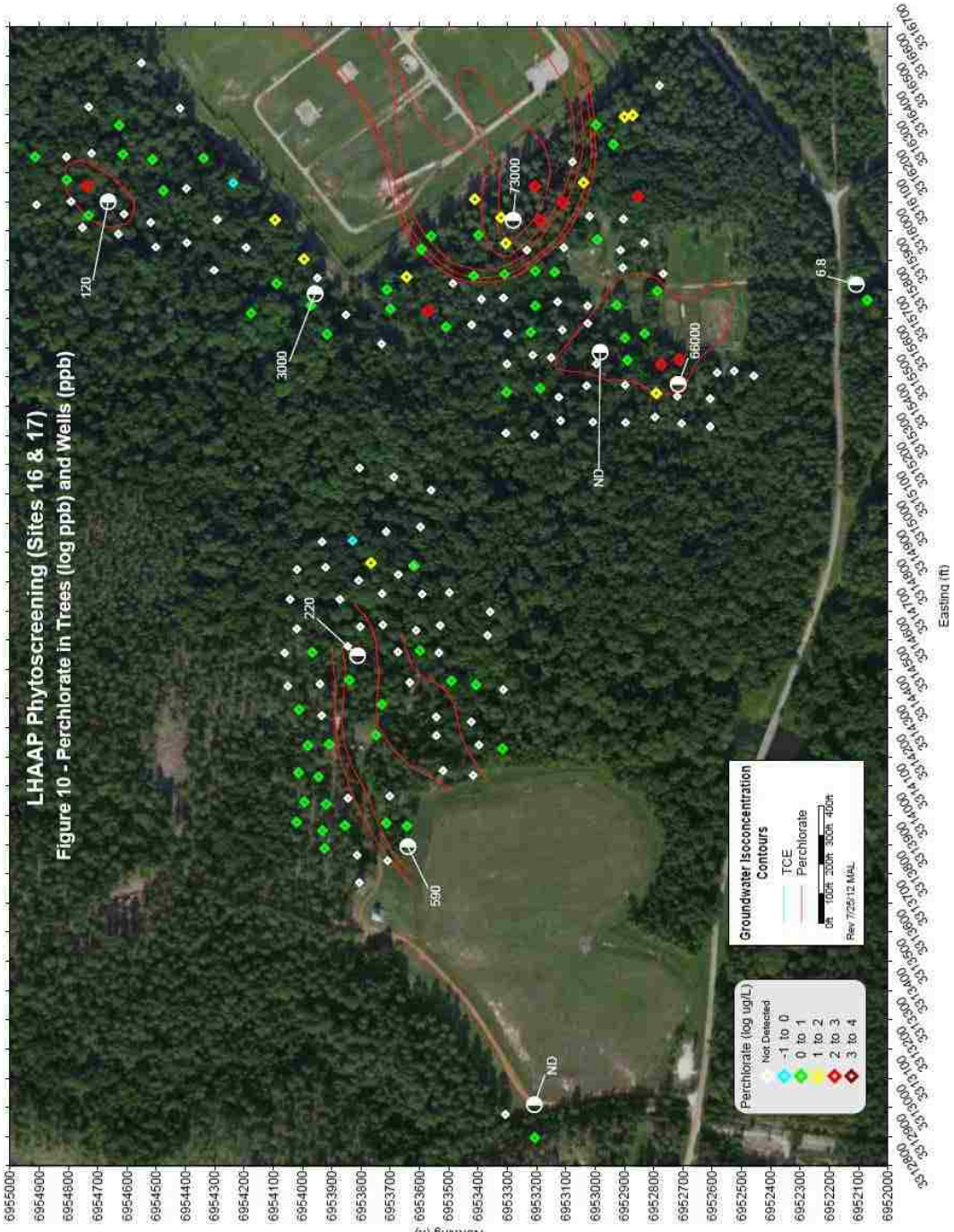

Figure 5.22 The LHAAP Field Concentration Map of Sampled Trees and Wells

6. CONCLUSIONS AND RECOMMENDATIONS

Explosives and energetics are common soil and groundwater pollutions, often dispersed and usually difficult to delineate in subsurface. Traditional sampling methods of contaminant delineation are expensive, time-consuming and invasive. UXO concerns complicate any site investigations, limiting efforts that require any disturbance of the soil, such as soil collection or installing groundwater wells. The combination of UXO concerns and expense of sampling prohibits preliminary screening efforts. This research was to develop novel phytoforensics approaches to delineate energetics in the subsurface. Specific objectives included developing novel plant sampling and analysis methods; better understanding the fate of non-volatile compounds in plants; as well as developing dynamic soil-plant system model to predictively relate plant samples to subsurface concentrations based on plant, soil and environmental properties.

Studies ranged from whole plant studies conducted in the greenhouse (explosives) to full scale field assessment (perchlorate).Four different plants species, including woody perennial trees and monocot grasses, were planted both in soil and sand reactors with continuous exposure to a mixture of explosives in the greenhouse. Time dependent assessments were carried out to determine kinetic mechanisms of uptake, transport and accumulation. Tissue concentrations were analyzed by both traditional solvent extraction and novel methods of extracting sap by high speed centrifugation after frozen-thawed. Solvent extracts and sap were analyzed by a newly developed analytical method of liquid chromatography and tandem mass spectrometry (UFLC-MS/MS).

A dynamic soil-plant system mechanistic model was developed to quantify the relationship between tissue concentration and soil pore water concentration for non-

volatile organic chemicals with root pathway only. The model included processes of diffusion exchange between root and soil, mass flow in xylem, metabolism and chemical equilibrium in soil and within plant tissues. The movement of water was entirely determined by transpiration and growth requirement among tissues. This model was run using Stella® to mathematically simulate laboratory experiments.

6.1. CONCLUSIONS

From the experimental data analysis and modeling completed, the underlying hypothesis that plant tissue analysis can effectively relate to groundwater concentrations were validated. Specific conclusions can be drawn as shown below.

Conclusion 1: The freeze-centrifuge extract method effectively extracted sap from varied plant species including both dicots and monocots. The extraction efficiency, i.e. removal of sap solution, was 18.8±9.7% for willow stem, 26.2±11.5% for poplar stem, 51.1±12.9% for ryegrass, as well as 53.4±10.2% for big bluestem. Efficient liquid extraction allows analysis small tissue samples.

Conclusion 2: The partition coefficients were acquired based on the batch sorption test. Woody tissue showed clearly higher binding capacity than grass for HMX. The partition coefficients in RDX and HMX were found not to correlate directly with the K_{ow} values of compounds.HMX (K_{ow}=0.06-0.19) showed significantly greater partitioning than RDX (K_{ow}=0.81-0.87) for tree woods and similar partitioning for grass. Thus it should be very careful to use partition coefficients derived from K_{ow} values.

Conclusion 3: The novel plant analysis method using freeze-centrifuge and HPLC-MS/MS was validated, showing clear relationship of *in-planta* concentration and groundwater exposure concentration. Sites can be delineated inexpensively, quickly and with little site disturbance if this relationship is valid in many conditions and sites.

Conclusion 4: Soil had significant impact on pore water concentrations and plant tissue concentrations due to biodegradation and sorption being dominant fate processes.

Conclusion 5: Root resistance was observed for all explosives based on the low stem sap concentrations and the enriched pore water concentrations in sand reactors. Thus the higher concentration in rhizosphere is possible in phytoremediation and the soil concentration should not be the only index to assess the effectiveness of phytoremediation. Applying methods developed here, mass removal rates can be calculated for phytoremediation applications.

Conclusion 6: The times required to reach apparent steady state within plant tissue were dependent on the soil characteristics, environmental conditions and chemical properties. At least one month for small tree stems and two months for young grasses and leaves were required respectively to reach the relative steady state for RDX in laboratory study. HMX needed longer time due to higher partitioning and larger scale trees cost much longer due to higher biomass reservoir.

Conclusion 7: The concentration differences in the same plant compartment were smaller than the discrepancies among different trees, which was enlarged in larger scale trees, thus it was reasonable to treat stem, branch, or leaf as one compartment for non-volatile compounds. The tissue concentration decreased in the range of leaves> branch> stem.

Conclusion 8: The soil-plant system model was effective in estimating the concentrations in soil pore water, plant sap and tissue for the experimental results. Model development indicated root resistance and limited xylem loading. The accumulation of non-volatile compounds can lead to highest concentrations in grass and leaves. The developed model may be applicable for other non-volatile compounds and different conditions and serve as the base of furthermore improvement of soil-plant system model and integrated with contaminant underground transport models.

Conclusion 9: The leaf metabolism coefficients were acquired by model parameter calibration. Because of the difficulty of directly measuring the metabolism coefficient in living whole plant, they were rarely reported. For RDX the leaf metabolism rates were estimated in the range 0.1-0.25 d^{-1} in grass and 0.1 d^{-1} in tree; For HMX, they were 0.08-0.22 d^{-1} in grass and 0.015 d^{-1} in tree.

Conclusion 10: The phytoforensic approach was validated on RDX and HMX by both experimental results and simulated results as strong correlation were achieved between plant concentration and exposure concentration. Stems showed best correlation to the pore water concentration. Two steps were proposed for field phytoforensics. Sample grasses and leaves firstly for the qualitative screening, then followed by tree core sampling for the quantitative prediction.

The phytoforensic approach was not feasible on TNT and PETN likely due to the metabolism efficiency and the capability of chemicals passing root tissue to enter xylem. TNT and PETN degrade in rhizosphere as well as plant root and experience greater resistances to enter xylem and go up to the stem. The metabolites were strongly bonded in the root tissue as shown in previous literature.

This research resulted in the substantial advancements in the understanding of energetics transport and fate in plants. The data and plant model were necessary in fully utilizing phytoforensic techniques in site assessment and monitoring. The novel sampling methods are rapid, solvent free, cost effective and labor saving, thereby accessing vast field sampling not available previously. Phytoforensic techniques allow for faster and more accurate site assessment, being less intrusive and much lower costs. A special benefit for explosives is to alleviate UXO concerns in site assessment efforts. The findings of this work also benefit for the development of phytoremediation and protection of environment and human health.

6.2. RECOMMENDATION

The work has been successful in developing phytoforensic approach on RDX and HMX showing application for non-volatile compounds for the first time. However, more efforts are needed on other chemicals and plant species considering the varied chemical-plant interaction. Specific recommendations for further study are listed as follows. These recommendations are based on the phytoforensics application, not including other study field related to plants, such as phytoremediation, where the focuses and considerations are distinguished from phytomonitoring.

Recommendation 1: The calculated tissue concentration form the sap measurements and partitioning obtained from batch sorption tests were consistently smaller the measured values. Several possibilities were speculated. More studies should be done to reveal the reasons.

Recommendation 2: The sap chemical concentrations were in some cases low with high interferences for determination so that UV detector was not applicable. In this study, the MS/MS detection solved this problem, but MS/MS is not readily available. Thus a simple and effective enriching and purifying method should be developed to advance potential applications. After the enriching treatment, the sample may be detected by UV detector. In this way, the freeze-centrifuge sap analysis can be applied widely.

Recommendation 3: The partition, transformation, advection, diffusion processes are crucial for correct prediction of plant concentration from exposure concentration or prediction of exposure concentration from plant concentration. Online database should be built on toxic threshold, plant tissue concentration, tested partition coefficient, metabolism coefficient, diffusion coefficient for different plant species and chemicals stemmed from the worldwide studies. As for now, no online database collects the complete information on plant-chemical interaction, so that the related information search is time and labor consuming and also not exhaustive.

Recommendation4: The root resistances for chemical to enter xylem are dependent on both species and chemicals. The resistance mechanisms are not fully understood[67, 73]. No widely accepted quantitative mathematical expressions were built according to both plant physiological characteristics and chemical properties. The regression relationship of TSCF only derived from K_{ow} value of chemicals is not applicable for all chemicals and does not consider all properties and degradation mechanisms. Thus the more efforts are needed to better understand this process and develop quantitative mathematical expressions.

Recommendation 5: The plant growth module in this developed soil-plant system model was quite simple and not related to the environmental conditions. This model has potential to be improved, such as linking the conductance coefficient and kinetic growth coefficients to environmental conditions; linking the reaction constant with temperature and plant growth (as index of toxicity). Also plant uptake model should be incorporated with the commercial groundwater models. The commercial models should be developed for engineering application and laboratory studies. The metabolism coefficients in plant is difficult to measure directly and are often obtained by model calibration, thus more coefficient values can be reported from experimental studies if the commercial model tools are available.

Recommendation 6: This work was conducted in the laboratory. Field applications are necessary to validate and confirm the conclusions from this work.

APPENDIX A

IN-PLANTA PARTITION BASED ON SAP MEASUREMENT

All plants samples in this study were split to determine sap concentration by centrifuge extraction method and tissue concentration by solvent extract method. The explosives absorbed on the dry tissue (mg/kg DW) (C_s) was calculated according to the equation (1). Then C_s and C_L were used to plot sorption isotherm. The distribution coefficient K_d were calculated by C_s divided by C_L. Due to the tested concentration ranging over from three to five orders of magnitude, the power fit in Excel corresponding to the Freudlich isotherm was used for regression analysis.

Figure A.1 showed the partition relationship on RDX. Figure A.2 showed the partition relationship on HMX. The partition relationships between sap and tissue biomass were in acceptable levels among all four plant species and three compartments in the large scale trees for both RDX and HMX according to the data fit results.

The partition coefficients in large tree wood were smaller than those in small tree wood, suggesting the possible structure change between young tree and old tree. Trees become lignified with age, and recent research by Burken et al demonstrated that lignin and lipid content changed the binding of organic contaminants in phytoforensic studies[78]. However, larger possibility was speculated that the sap concentrations of small trees were underestimated, which resulted in higher partition coefficients. Only 2-6 dilution folds were applied for small tree stems due to the serious matrix interference had not recognized at that time. The high salts content of plant sap inhibited the ionization process for MS detector. Over 10 dilution folds were applied for large trees' sap samples. The partition coefficients of large tree wood were closer to the results from batch sorption tested discussed in the next section.

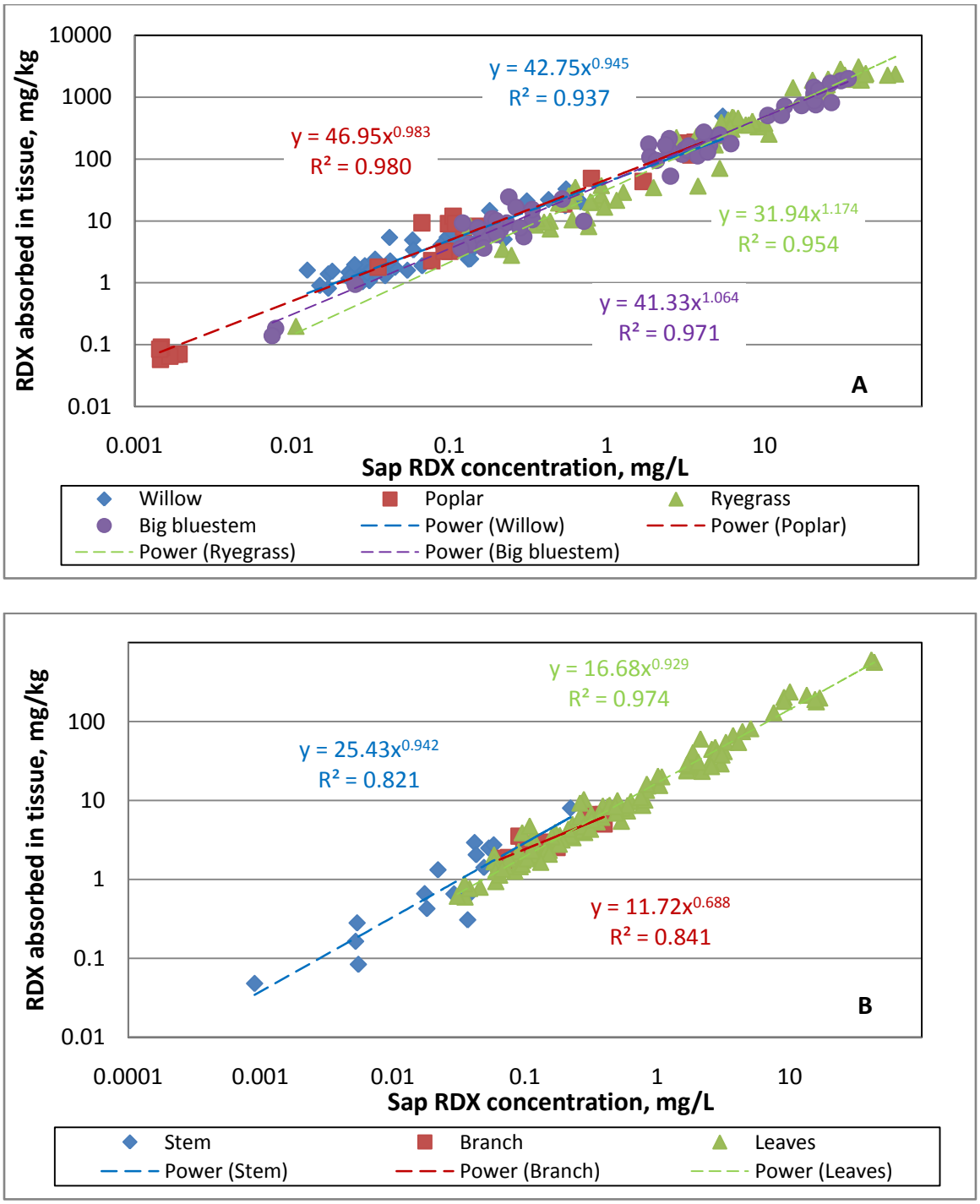

Figure A.1 Isotherms on RDX Partition in Live Plant Samples

Regression analysis was carried out by Excel. “A” showed the results from the stems of small tree and the grasses. “B” showed the results from the tree cores (stem), branches and leaves of large tree.

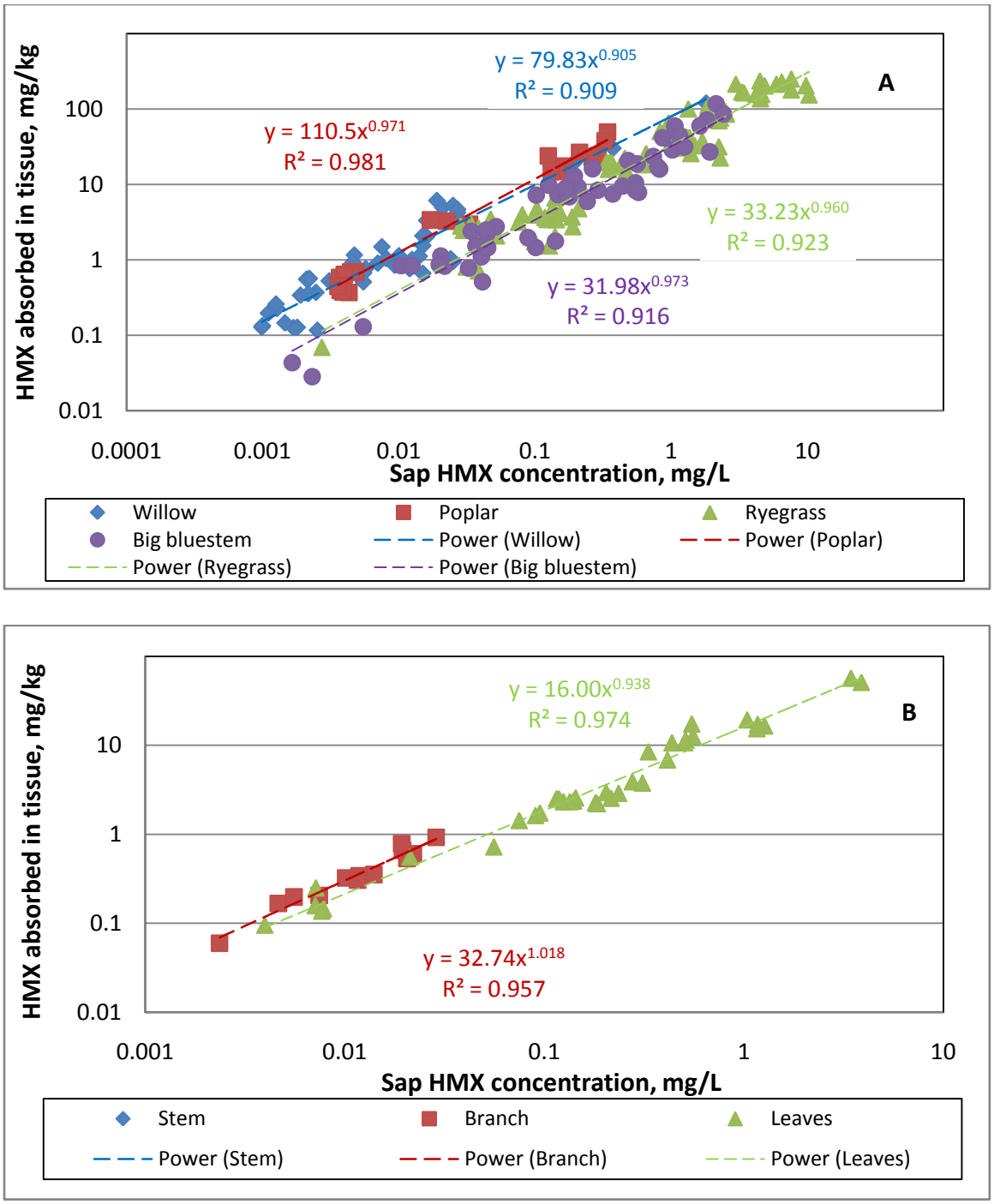

Figure A.2 Isotherms on HMX Partition in Live Plant Samples

Regression analysis was carried out by Excel. "A" showed the results from the stems of small tree and the grasses. "B" showed the results from the branches and leaves of large tree. Most of HMX concentrations in stem sap were below MDLs, making regression analysis infeasible.

To further validate the centrifugation method, two statistical analyses were conducted. Firstly, considering the quite varied centrifugation efficiency even for the same species, the impact of the centrifugation efficiency on partition coefficient was assessed. The partition coefficient of every plant sample was calculated by the equation (2) mentioned above. A general linear model was applied (Appendix B) and showed that the centrifuge extract efficiency had no statistically significance on partition for all 4 species and 2 compounds (P-value > 0.05). This results implicated the concentrations in the sap failed to be extracted was same as those in the sap extracted, which made the proposed method solid even when only a little sap was collected.

Secondly, the effect of exposure time on the partition relationship in the tissue also was assessed. In the time series grass experiment and large tree experiment, the plants were harvested on different exposure periods. The single factor ANOVA analysis was applied (Appendix B). For grass, only ryegrass HMX partitioning was significantly impacted by the exposure time (P-value < 0.05). Ryegrass RDX partitioning was not significantly impacted (P-value > 0.05). Also exposure time had no significance on HMX and RDX partitioning for big bluestem. For large tree, leaf HMX partitioning was significantly impacted by the exposure time (P-value < 0.05). However, it should be noted that the existence of interference peak make quantifying HMX difficult and less accurate. This problem greatly decreased the number of data used to do statistics analysis. Thus the statistic result was biased to a certain extent. Leaf RDX partitioning was not significantly impacted (P-value > 0.05). The P-value of stem RDX partitioning was less than 0.05, but close 0.05 as it was 0.042. Most tree cores were too dry to get sap. This problem also decreased the number of data used to do statistics analysis and maybe

caused smaller P-value. The stem HMX analysis confronted both analysis and centrifugation problems, making the statistics analysis infeasible. Despite some inconsistences, most of results still showed that partition equilibrium was reached much quicker than steady state of *in-planta* concentration reached in the plants and had no evident change in whole experiment periods.

In order to clearly compare the partition relationship obtained fromreal plant samples and batch sorption tests, the regression equations and partition coefficients from the live plant samples were shown in Table A.1 for RDX and Table A.3 for HMX. The results from batch sorption tests using dead dried plants tissues were shown in Table A.2 for RDX and Table A.4 for HMX. Although the difference on specific values, the change tendencies between two tests were similar.

Table A.1 RDX Partition Coefficient (K_d) From Live Plant Samples

Sorbent	Regression Equation (C_s: mg/kg, C_L: mg/L)	R^2	K_d (L/kg)			
			C_L=0.1	C_L = 1	C_L = 10	C_L=50
Poplar	C_s =46.95*C_L^0.98	0.980	48.78	46.95	45.19	44.00
Willow	C_s =42.76*C_L^0.95	0.937	48.47	42.76	37.71	34.55
Big bluestem	C_s =41.34*C_L^1.06	0.971	35.61	41.34	47.99	53.26
Rye grass	C_s =31.94*C_L^1.17	0.955	21.39	31.94	47.70	63.14
Stem[a]	C_s =25.44*C_L^0.94	0.8217	29.06	25.44		
Branch[a]	C_s =11.72*C_L^0.69	0.8411	24.02	11.72		
Leaf[a]	C_s =16.69*C_L^0.93	0.9748	19.61	16.69	14.20	12.69

[a]: these samples were from big willow trees

Table A.2 RDX Partition Coefficient (K_d) From Batch Sorption Test

Sorbent	Regression Equation (C_s: mg/kg, C_L: mg/L)	R^2	K_d (L/kg)		
			C_L=0.1	C_L = 1	C_L = 10
Poplar	C_s =17.17*C_L^0.94	0.998	19.82	17.17	14.88
Willow	C_s =17.01*C_L^0.94	0.997	19.45	17.01	14.88
Big bluestem	C_s =11.89*C_L^0.77	0.984	20.08	11.89	7.04
Rye grass	C_s =8.15*C_L^0.88	0.995	10.86	8.15	6.11
Sta-green soil	C_s =13.37*C_L^1.07	0.998	11.33	13.37	15.79
Slit loam	C_s =1.12*C_L^1.27	0.957	0	1.12	2.07

Table A.3 HMX Partition Coefficient (K_d) From Live Plant Samples

Sorbent	Regression Equation (C_s: mg/kg, C_L: mg/L)	R^2	K_d (L/kg)			
			C_L=0.01	C_L = 0.1	C_L = 1	C_L=10
Poplar	C_s=110.56*C_L^0.97	0.981	126.30	118.17	110.56	103.44
Willow	C_s=79.84*C_L^0.91	0.909	123.20	99.18	79.84	64.27
Big bluestem	C_s=31.99*C_L^0.97	0.917	36.09	33.98	31.99	30.12
Rye grass	C_s=33.24*C_L^0.96	0.924	39.83	36.39	33.24	30.36
Branch[a]	C_s=32.75*C_L^1.02	0.958	30.12	31.40		
Leaf[a]	C_s=16.00*C_L^0.94	0.974	21.23	18.43	16.00	13.89

[a]: these samples were from big willow trees

Table A.4 HMX Partition Coefficient (K_d) From Batch Sorption Test

Sorbent	Regression Equation (C_s: mg/kg, C_L: mg/L)	R^2	K_d (L/kg)		
			C_L=0.01	C_L = 0.1	C_L = 1
Poplar	C_s =29.59*C_L^0.92	0.988	42.22	35.34	29.59
Willow	C_s =23.25*C_L^0.85	0.984	45.92	32.67	23.25
Big bluestem	C_s =13.07*C_L^0.87	0.996	23.48	17.52	13.07
Rye grass	C_s =9.76*C_L^0.77	0.993	28.07	16.56	9.76
Sta-green soil	C_s =29.43*C_L^0.94	0.998	39.24	33.98	29.43
Slit loam	C_s =3.75*C_L^0.91	0.991	5.58	4.58	3.75

APPENDIX B

STATISTICS ANALYSIS REPORTS

LINEAR REGRESSION ANALYSIS:

Centrifugation extraction efficiency (y) to moisture content (x)

Willow:

Regression Statistics	
Multiple R	0.09513338
R Square	0.00905036
Adjusted R Square	-0.002210431
Standard Error	9.722856563
Observations	90

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	75.97744687	75.97744687	0.803705495	0.372433733
Residual	88	8318.986698	94.53393975		
Total	89	8394.964145			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	12.02437414	7.573371573	1.587717443	0.115937012
X Variable 1	0.159656954	0.178089933	0.896496233	0.372433733

Poplar:

Regression Statistics	
Multiple R	0.034348493
R Square	0.001179819
Adjusted R Square	-0.019204266
Standard Error	11.62770844
Observations	51

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7.825505607	7.825505607	0.057879416	0.810883316
Residual	49	6624.976576	135.2036036		
Total	50	6632.802081			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	23.20115928	12.44730506	1.863950403	0.068325835
X Variable 1	0.062365765	0.259229358	0.240581412	0.810883316

Ryegrass:

Regression Statistics	
Multiple R	0.483422369
R Square	0.233697187
Adjusted R Square	0.216668235
Standard Error	11.42382525
Observations	47

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1790.971519	1790.971519	13.72352184	0.000577044
Residual	45	5872.670247	130.5037833		
Total	46	7663.641766			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	-128.5244584	48.50558241	-2.64968385	0.011076737
X Variable 1	2.270888682	0.613003645	3.704527208	0.000577044

Big bluestem:

Regression Statistics	
Multiple R	0.921147732
R Square	0.848513145
Adjusted R Square	0.844906315
Standard Error	4.015278574
Observations	44

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3792.837867	3792.837867	235.2517786	8.11951E-19
Residual	42	677.1434051	16.12246203		
Total	43	4469.981272			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	-137.4132342	12.45185509	-11.0355632	5.46112E-14
X Variable 1	2.673258019	0.174290783	15.33791963	8.11951E-19

Distribution coefficient (y) to centrifugation extraction efficiency (x)

Willow-HMX

Regression Statistics	
Multiple R	0.070441174
R Square	0.004961959
Adjusted R Square	-0.015768
Standard Error	64.6543177
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1000.575341	1000.575341	0.239361738	0.62689579
Residual	48	200648.6783	4180.180798		
Total	49	201649.2536			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	128.9876059	22.72923376	5.674964994	7.83413E-07
X Variable 1	0.50704958	1.036389639	0.489246092	0.62689579

Willow-RDX

Regression Statistics	
Multiple R	0.123293707
R Square	0.015201338
Adjusted R Square	-0.00337977
Standard Error	23.01812869
Observations	55

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	433.4612371	433.4612371	0.818107245	0.369827344
Residual	53	28081.21516	529.8342483		
Total	54	28514.6764			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	46.39304975	7.504126164	6.182338721	9.26943E-08
X Variable 1	0.308551558	0.341132128	0.904492811	0.369827344

Poplar-HMX

Regression Statistics	
Multiple R	0.007502823
R Square	5.62924E-05
Adjusted R Square	-0.05257232
Standard Error	34.39808547
Observations	21

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.265598617	1.265598617	0.001069615	0.974250991
Residual	19	22481.3374	1183.228284		
Total	20	22482.60299			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	126.0848432	18.35733637	6.868362639	1.49262E-06
X Variable 1	0.022440286	0.686143055	0.032704968	0.974250991

Poplar-RDX

Regression Statistics	
Multiple R	0.103641605
R Square	0.010741582
Adjusted R Square	-0.02730682
Standard Error	26.51974403
Observations	28

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	198.5502833	198.5502833	0.282313636	0.599699184
Residual	26	18285.71741	703.2968233		
Total	27	18484.26769			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	59.13959921	12.65969659	4.671486303	8.00029E-05
X Variable 1	-0.2587473	0.486978626	-0.53133195	0.599699184

Ryegrass-HMX

Regression Statistics	
Multiple R	0.120411889
R Square	0.014499023
Adjusted R Square	-0.012876
Standard Error	21.04579396
Observations	38

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	234.5928719	234.5928719	0.529644154	0.471463642
Residual	36	15945.31596	442.9254434		
Total	37	16179.90883			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	35.66887769	14.82420319	2.406124447	0.021382132
X Variable 1	0.202109275	0.277711685	0.727766552	0.471463642

Ryegrass-RDX

Regression Statistics	
Multiple R	0.217823735
R Square	0.04744718
Adjusted R Square	0.021702509
Standard Error	24.3684849
Observations	39

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1094.410156	1094.410156	1.842990339	0.182823736
Residual	37	21971.45308	593.8230562		
Total	38	23065.86324			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	66.6431851	16.04267167	4.154120115	0.000184773
X Variable 1	-0.41213351	0.303582267	-1.3575678	0.182823736

Big bluestem-HMX

Regression Statistics	
Multiple R	0.112994046
R Square	0.012767655
Adjusted R Square	-0.01907855
Standard Error	19.323322
Observations	33

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	149.6983528	149.6983528	0.400916047	0.531260313
Residual	31	11575.11396	373.3907729		
Total	32	11724.81231			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	52.53823072	18.38093259	2.858300603	0.00754747
X Variable 1	-0.21483081	0.339289045	-0.63317932	0.531260313

Big bluestem-RDX

Regression Statistics	
Multiple R	0.156711644
R Square	0.02455854
Adjusted R Square	-0.00331122
Standard Error	17.96994373
Observations	37

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	284.5527609	284.5527609	0.881189613	0.354309416
Residual	35	11302.16072	322.9188777		
Total	36	11586.71348			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	29.38248127	16.84936897	1.743832741	0.089966885
X Variable 1	0.288314815	0.307137097	0.938717004	0.354309416

THE SINGLE FACTOR ANOVA ANALYSIS

Single factor: time; Statistical variables: distribution coefficient

Ryegrass-HMX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
4 weeks	9	447.8451	49.76057	651.6568
6 weeks	7	375.919	53.70271	312.1611
8 weeks	9	376.5352	41.83725	500.7525
10 weeks	9	231.9609	25.77343	80.10746
12 weeks	9	278.4807	30.9423	83.63617
14 weeks	9	228.0942	25.3438	77.28667
16 weeks	9	334.328	37.14755	250.4704

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6312.247	6	1052.041	3.781236	0.003244	2.271989
Within Groups	15024.25	54	278.2268			
Total	21336.49	60				

Ryegrass-RDX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
4 weeks	9	373.3944	41.48827	257.2042
6 weeks	8	386.3803	48.29754	255.542
8 weeks	9	522.0911	58.01013	1184.195
10 weeks	9	336.2806	37.36451	116.938
12 weeks	9	475.0577	52.78419	190.1464
14 weeks	9	355.0674	39.45194	364.2052
16 weeks	9	523.3907	58.15452	432.4393

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4093.526	6	682.2543	1.694099	0.139837	2.268717
Within Groups	22149.82	55	402.724			
Total	26243.34	61				

Big bluestem-HMX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
4 weeks	7	297.8229	42.54613	253.4961
6 weeks	7	262.9034	37.55763	342.2393
8 weeks	9	297.061	33.00678	213.7786
10 weeks	9	274.0854	30.45393	159.1184
12 weeks	6	148.1292	24.68821	210.8034
14 weeks	3	169.2183	56.40609	207.9879

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2673.885	5	534.7769	2.33161	0.062808	2.485143
Within Groups	8027.581	35	229.3595			
Total	10701.47	40				

Big bluestem-RDX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
4 weeks	9	401.8284	44.6476	218.3405
6 weeks	9	414.1453	46.01614	206.1536
8 weeks	9	476.0785	52.89761	411.1381
10 weeks	9	495.3402	55.0378	570.5419
12 weeks	5	216.9574	43.39148	254.1199
14 weeks	3	99.02431	33.0081	139.278

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1576.995	5	315.399	0.955417	0.457066	2.462548
Within Groups	12544.43	38	330.1166			
Total	14121.42	43				

Leaf-HMX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
harvest2	17	353.995	20.82324	24.40675
harvest3	3	50.9287	16.97623	25.37085
harvest4	8	118.0939	14.76173	5.32789
harvest5	5	66.392	13.2784	3.380361
harvest6	3	79.55839	26.51946	22.36566

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	539.5351	4	134.8838	7.78952	0.000182	2.678667
Within Groups	536.7977	31	17.31606			
Total	1076.333	35				

Leaf-RDX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
harvest2	22	384.3787	17.47176	9.171112
harvest3	21	407.7871	19.41843	24.12644
harvest4	24	409.9105	17.07961	16.08991
harvest5	21	360.3728	17.16061	26.3055
harvest6	23	368.4066	16.01768	9.019028

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	133.2818	4	33.32045	1.995779	0.100431	2.45738
Within Groups	1769.719	106	16.69546			
Total	1903	110				

Stem-RDX

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
harvest1	2	84.45037	42.22518	237.3652
harvest2	6	138.8275	23.13792	234.3094
harvest3	2	95.12018	47.56009	0.777117
harvest4	5	252.6523	50.53045	217.595
harvest6	2	46.05132	23.02566	71.47106

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2728.182	4	682.0456	3.480504	0.041556	3.259167
Within Groups	2351.541	12	195.9617			
Total	5079.723	16				

APPENDIX C

WATER TRANSPIRATION IN ALL GREENHOUSE EXPERIMENTS

SMALL SCALE TREE AND GRASS EXPERIMENTS

The transpiration ratio was impacted by species and dosing solution. For species, willow extracted more water (about 60-80 ml/day/tree) than poplar did (about 40-60 ml/day/tree) in both soil and sand reactor dosed by solutions of zero and low concentration. The transpiration ratios of both grasses were much lower than those of trees as expectation. Grasses grew better in sand than soil especially for ryegrass. However, the rooting media did not affect the growth of trees.

Hormetic effects, growth stimulation at low concentrations followed by inhibition at greater concentrations[29], were observed. The medium dosing concentration seemed to stimulate the tree growth as the much higher transpiration ratio was observed in willows planted in the soil and poplars planted in both soil and sand. The high dosing concentration resulted in serious growth inhibition in sand reactors for all four species, which coordinated the finding in hydroponic test. In soil reactor, the toxicity from the high concentration dosing was decreased by the stronger bacteria activity which reducing the pore water concentrations of the reactors. Growth stimulation at low concentrations was not visible for grasses as for trees, but the high concentration inhibitions were clear for grasses.

Figure C.1 showed the transpiration of trees, and Figure C.2showed the transpiration of grasses.

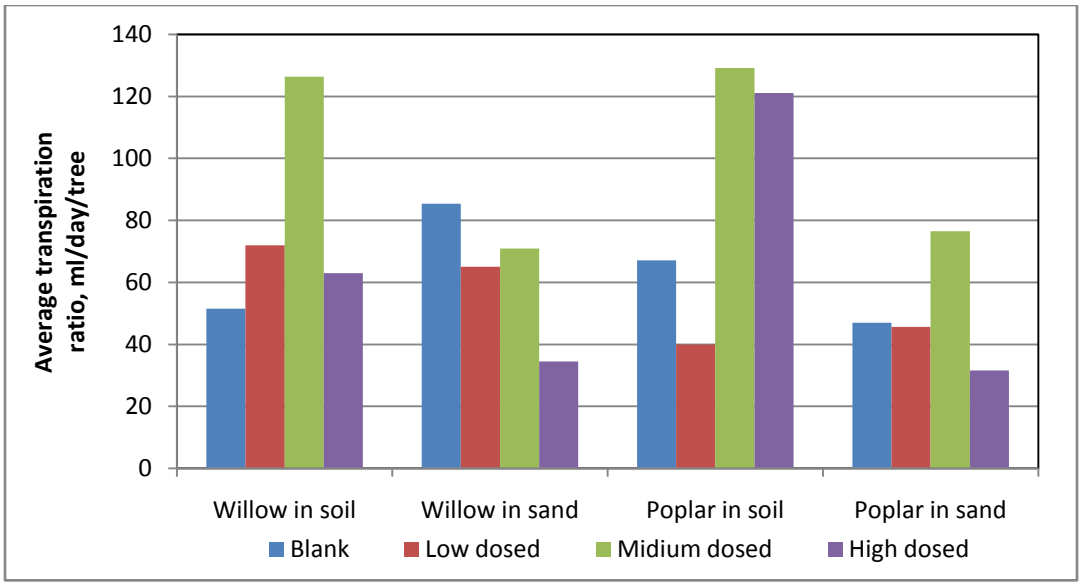

Figure C.1 Transpiration of Trees

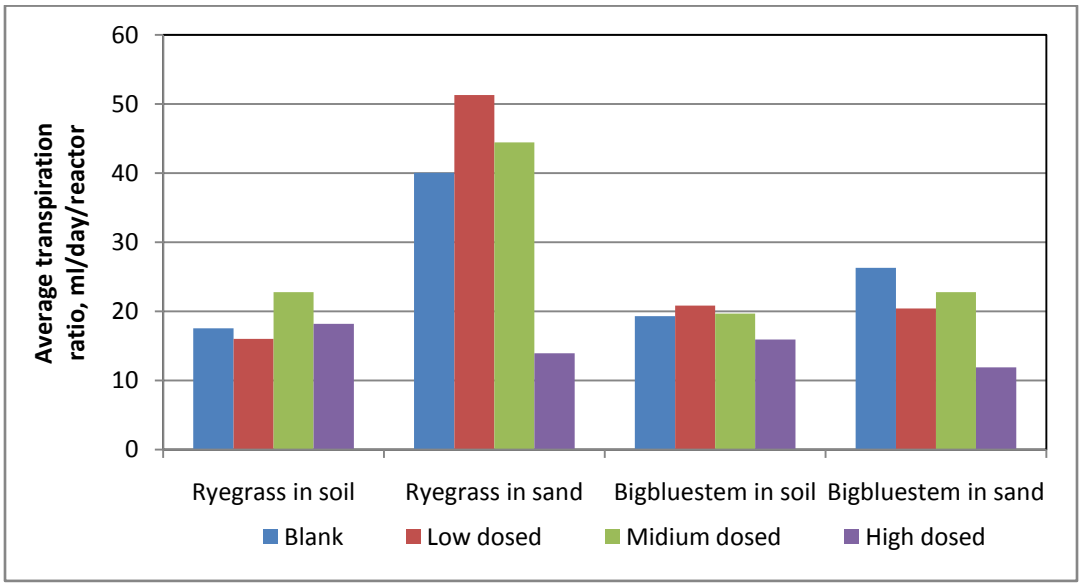

Figure C.2 Transpiration of Grasses

TIME-SERIES GRASS EXPERIMENTS

Same as small reactor planting, the ryegrass had higher transpiration ratio than big bluestem. Although ryegrass was smaller than big bluestem from the point of view of the individual plant, the ryegrass can grow very densely in the whole reactor. The transpiration ratio was impacted by the plant biomass and temperature and humidity in the greenhouse. After every harvest, the removed plants decreased the biomass in the reactors, but the growth of the remaining plants offset the loss. Three sub-samples were taken from dosed reactors for triplication, but just one sub-sample was taken from blank reactors. Thus in the latter exposure period the more plants were remained in black reactors, which resulted in higher transpiration ratio of black reactors compared with the dosed reactors. Due to in this experiment the high dosing concentrations were half of those in small reactors experiment, the high dosing solution were not toxic to ryegrass. And the hermetic effects were exhibited for ryegrass that contaminants stimulated the plant growth. On the contrary big bluestem was more sensitive to the explosives. Plant growth was inhibited by the dosing solution. Higher dose, worse grow.

Figure C.3 showed the transpiration of ryegrass, and Figure C.4 showed the transpiration of big bluestem.

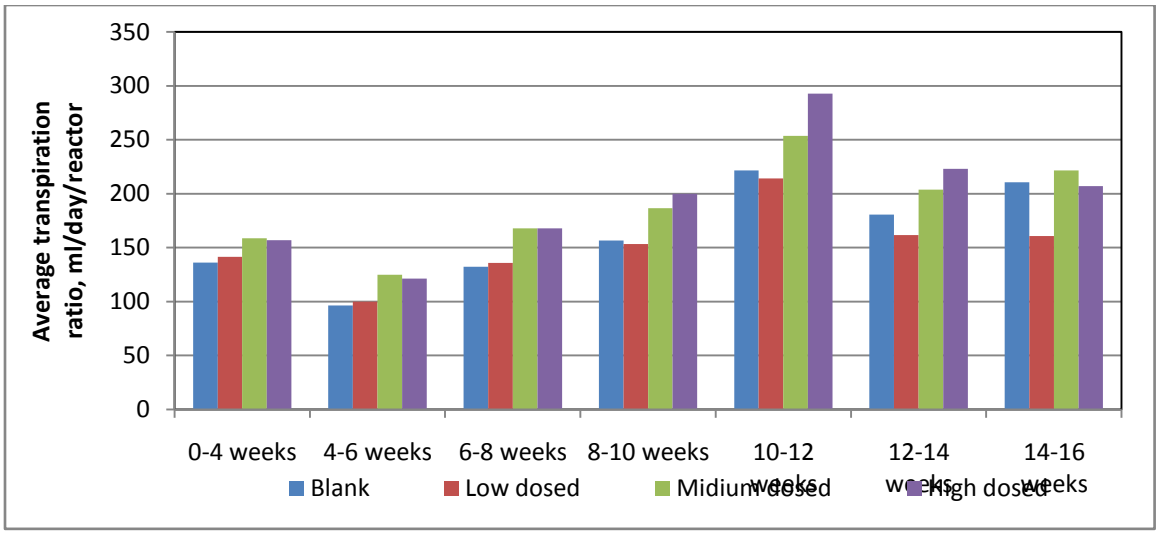

Figure C.3 Transpiration of Ryegrass

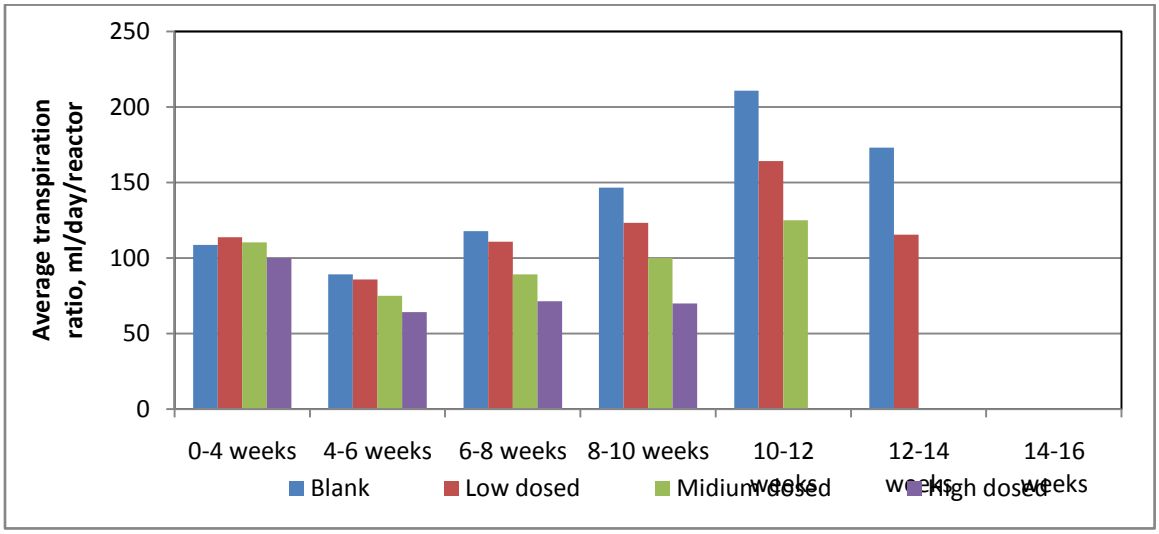

Figure C.4 Transpiration of Big Bluestem

LARGE SCALE TREE AND LONG TERM EXPERIMENT

The volumes of dosing solution added were treated as transpiration flow at once. In the terminal the remaining dosing solutions in the dosing tanks were measured and deducted from the calculation of the transpiration flow. The high values of the first week were due to the water storage capabilities in the system including dosing tanks and reactors which were calculated as transpiration flow. The lower values of the last week in trees dosed by high concentration were due to the deduction of the remaining solution volume in dosing tank.

Several special events needed to be noted. Firstly, reactors of 1#, 2#, and 3# were dosed by low concentration solution at the beginning term of 72 days because the high dosing solution inhibited the growth of willows in the small scale experiment. Then the data from the first harvest showed the strong sorption ability of the commercial soil used in the big scale experiment, which decreased the availability of explosives to the plants. Thus the dosing solution was changed to high concentration. Secondly, the normal bottom dosing pathway was found to be blocked on the approximately 165th day for the 2# reactor. The surface dosing was adopted until the problem was fixed on the 205th day. Since 205th day to the end of the experiment, the concentration of dosing solution was deduced to the half of the normal concentration due to the extremely high *in-planta* concentration in this reactor. The decreasing dosing concentration resulted to higher transpiration ratio. Finally, the tree in 6# reactor grew into the high intensity lamp under the top of green house and was dried by the heat of the lamp, which in fact resulted in the termination of this experiment. Thus the transpiration value of the reactor was abnormally low in the last week.

Although the species, soil, and other environmental conditions were same, the transpiration ratio in the different reactors showed diversity. Thus the individual difference will be a more important impact factor on the results for the big scale plants than small scale plants. Among the medium dosing treatments, the transpiration ratio decreased in the order of 4#>6#>5# during the first half of experiment. But in the last two month 5# tree entered the active growth term along with the rise of the temperature in green house, showing highest transpiration ratio. Among the high dosing treatments the differences among three trees were smaller than the medium dosing treatments. During the first half of experiment the transpiration ratios of trees in high treatments were close to those in medium treatments besides the most actively growing 4# tree. But when temperature rose, the trees in medium treatments increased their transpiration ratio greatly, the trees in high treatments just increased slightly, showing the inhibition effect of the high concentration RDX on willow. Figure C.5 showed the transpiration of all reactors during the whole experiment terms.

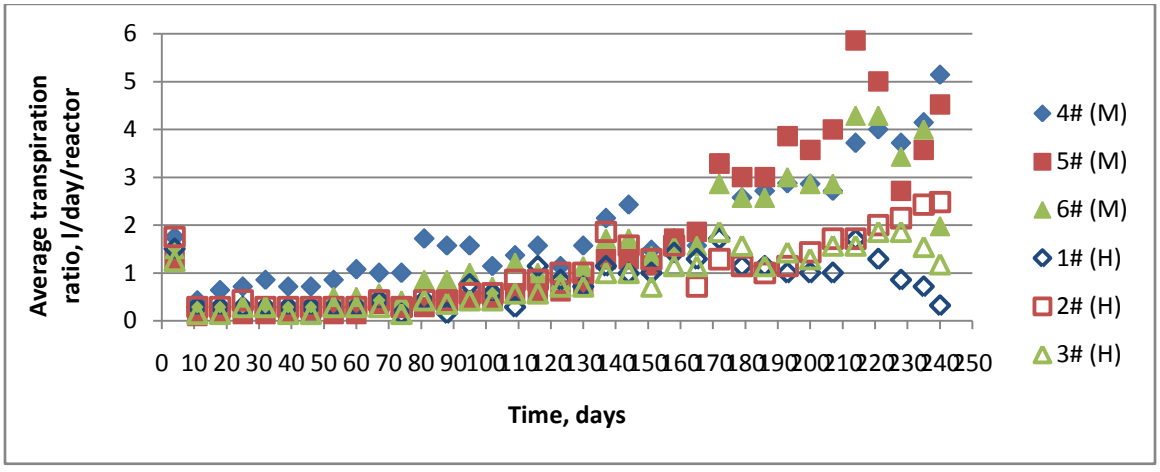

Figure C.5 Transpiration of Large trees

APPENDIX D

DOSING AND PORE WATER CONCENTRATION IN SMALL SCALETREE AND GRASS EXPERIMENTS

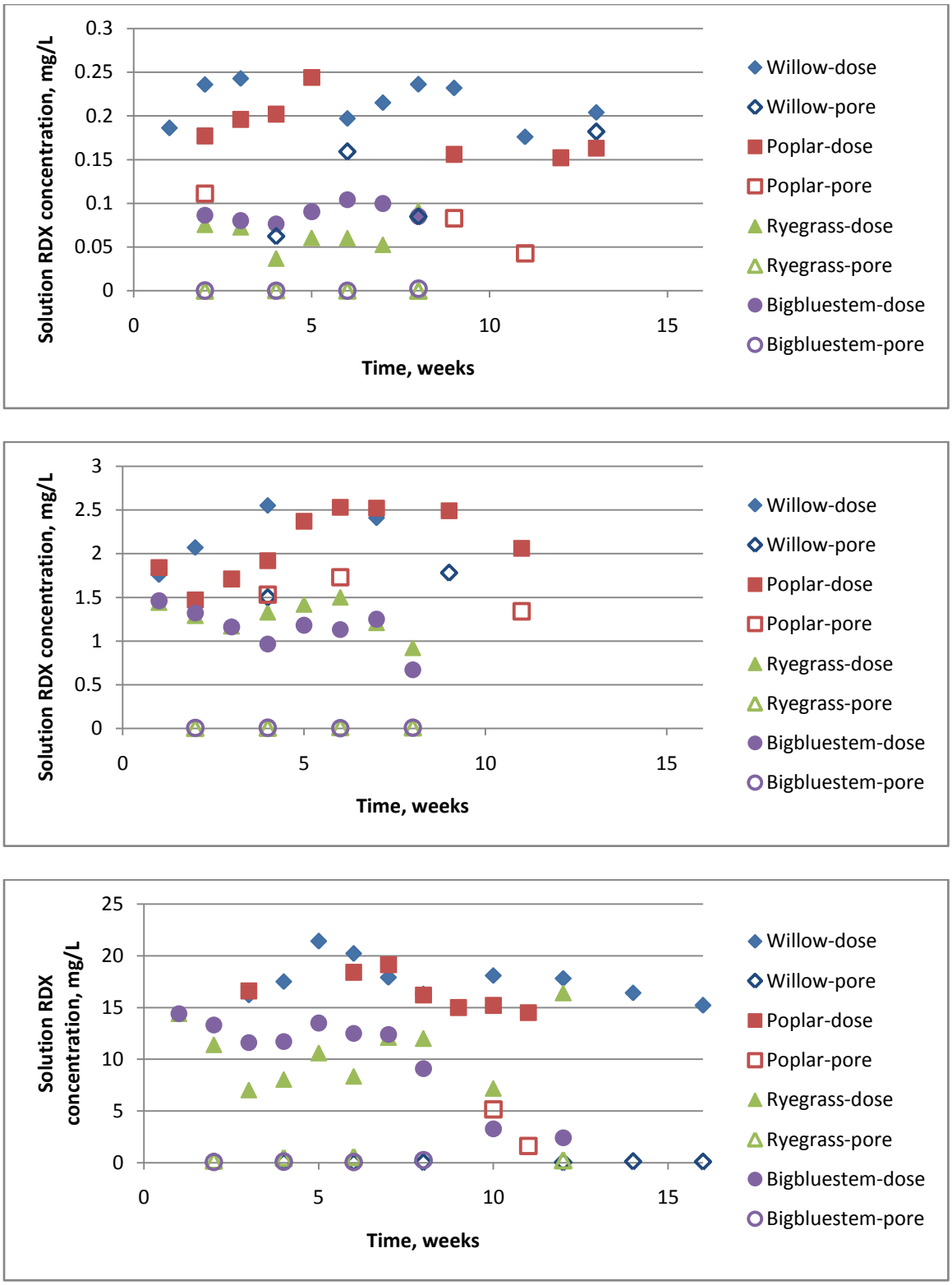

Figure D.1 Dosing and Pore Water RDX Concentration in Soil Reactors
(3 dosing levels)

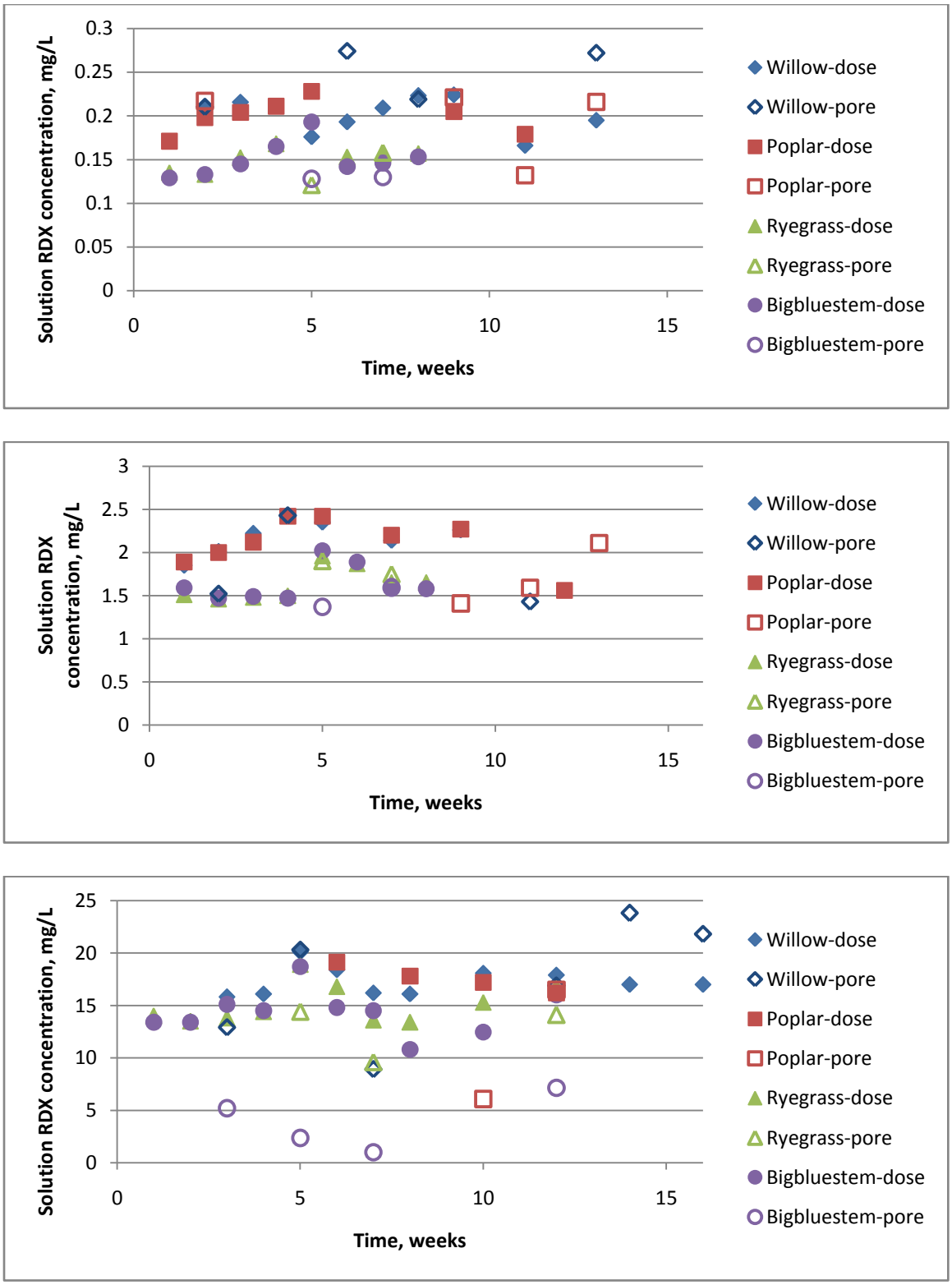

Figure D.2 Dosing and Pore Water RDX Concentration in Sand Reactors
(3 dosing levels)

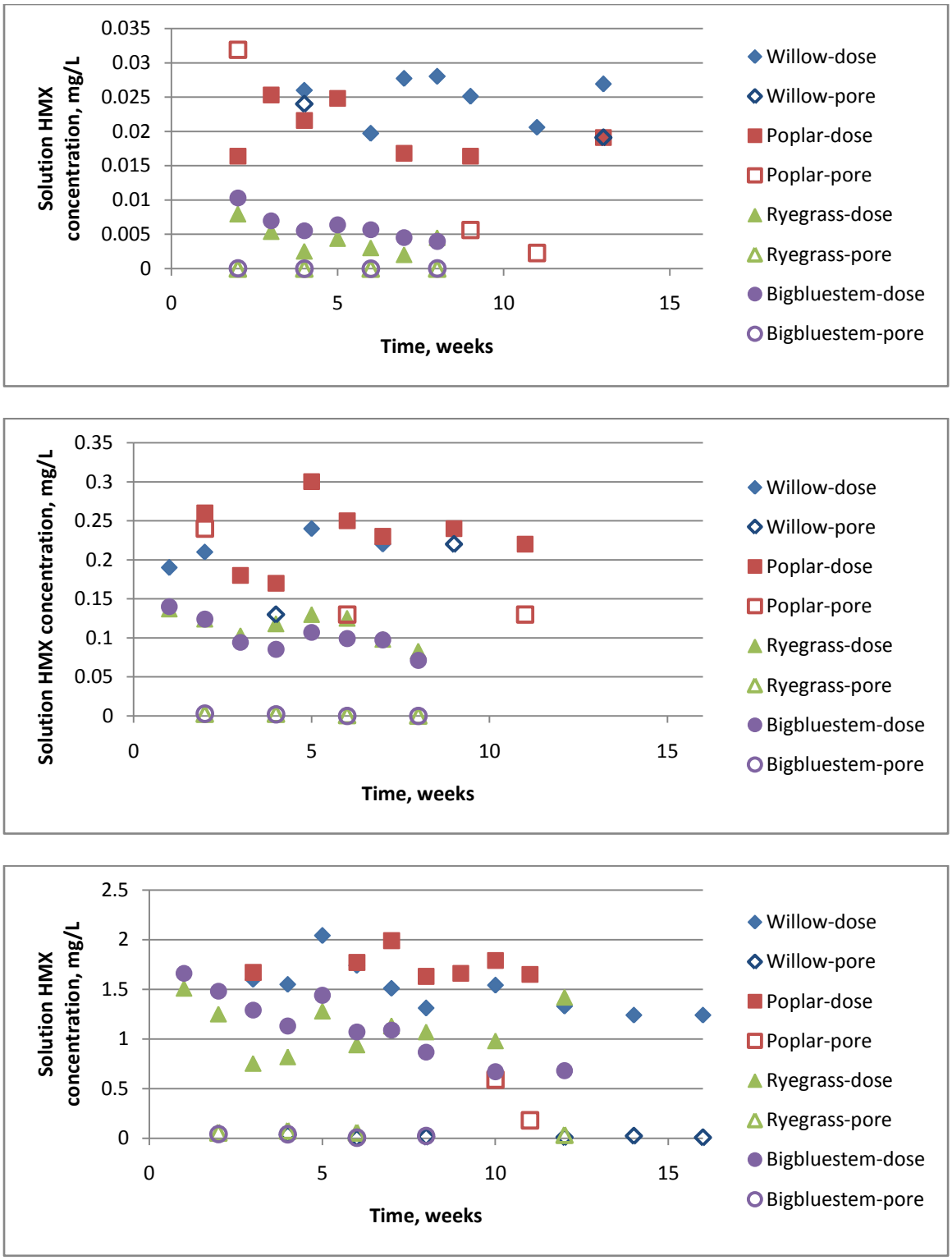

Figure D.3 Dosing and Pore Water HMX Concentration in Soil Reactors
(3 dosing levels)

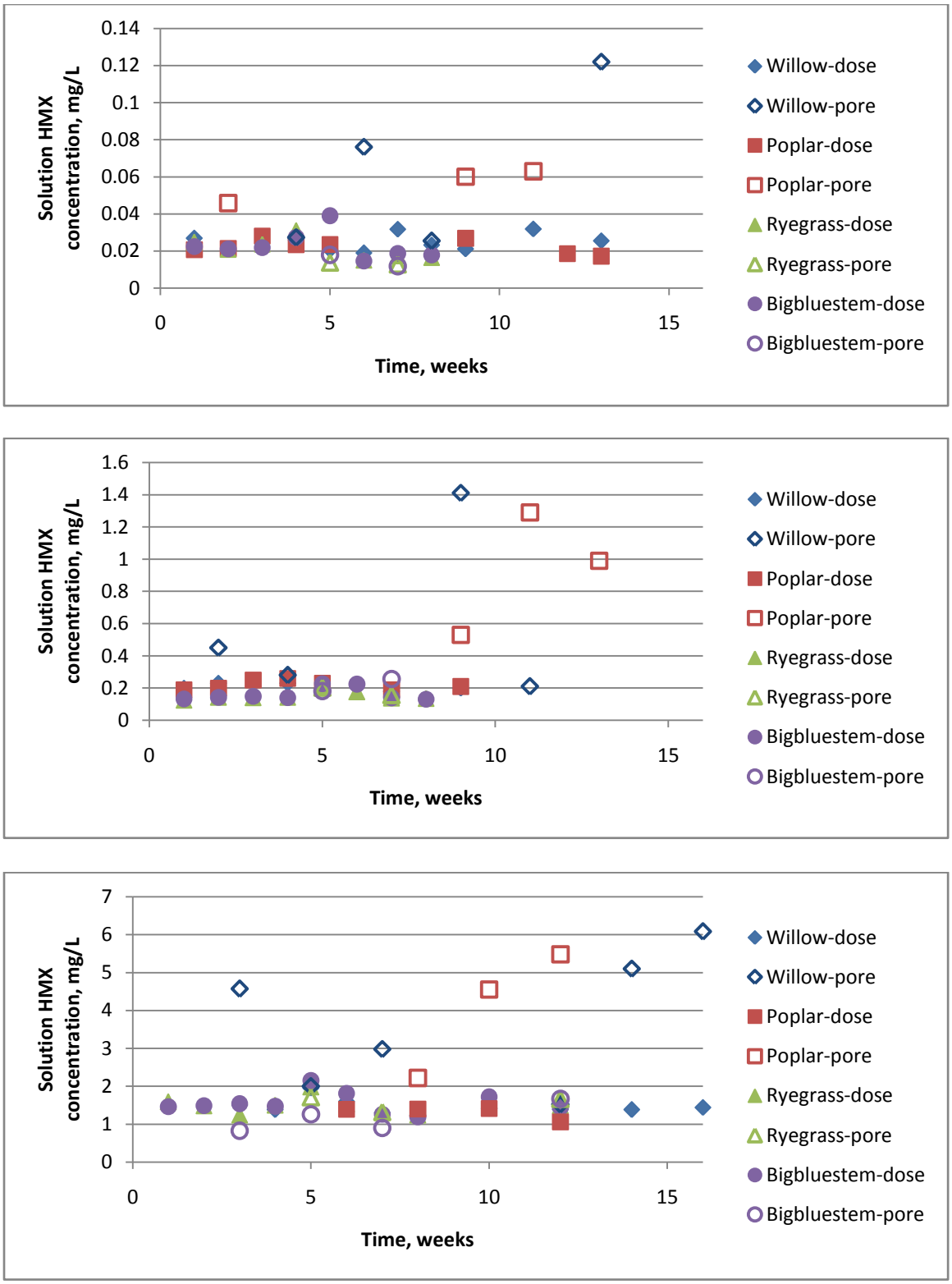

Figure D.4 Dosing and Pore Water HMX Concentration in Sand Reactors
(3 dosing levels)

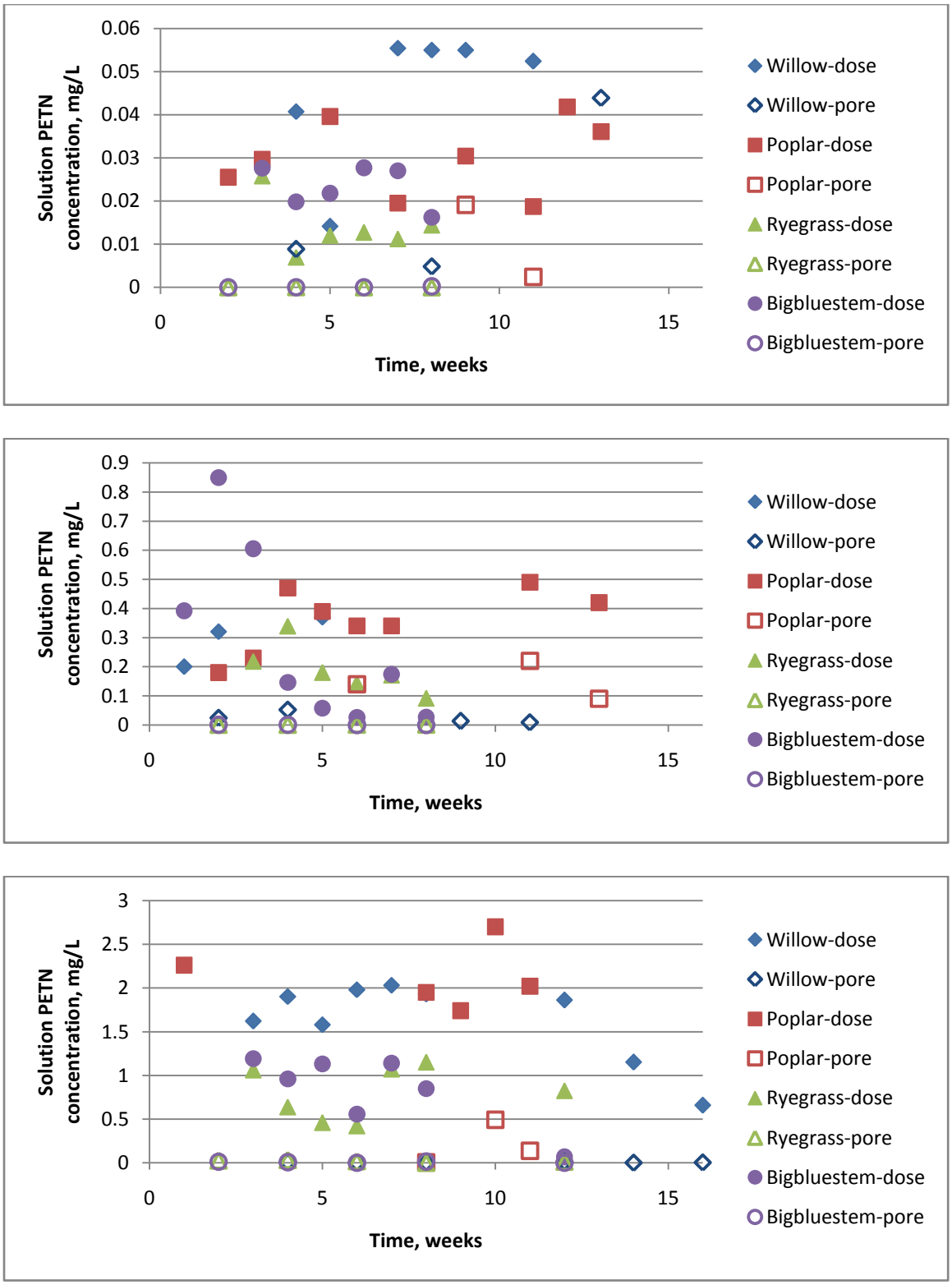

Figure D.5 Dosing and Pore Water PETN Concentration in Soil Reactors (3 dosing levels)

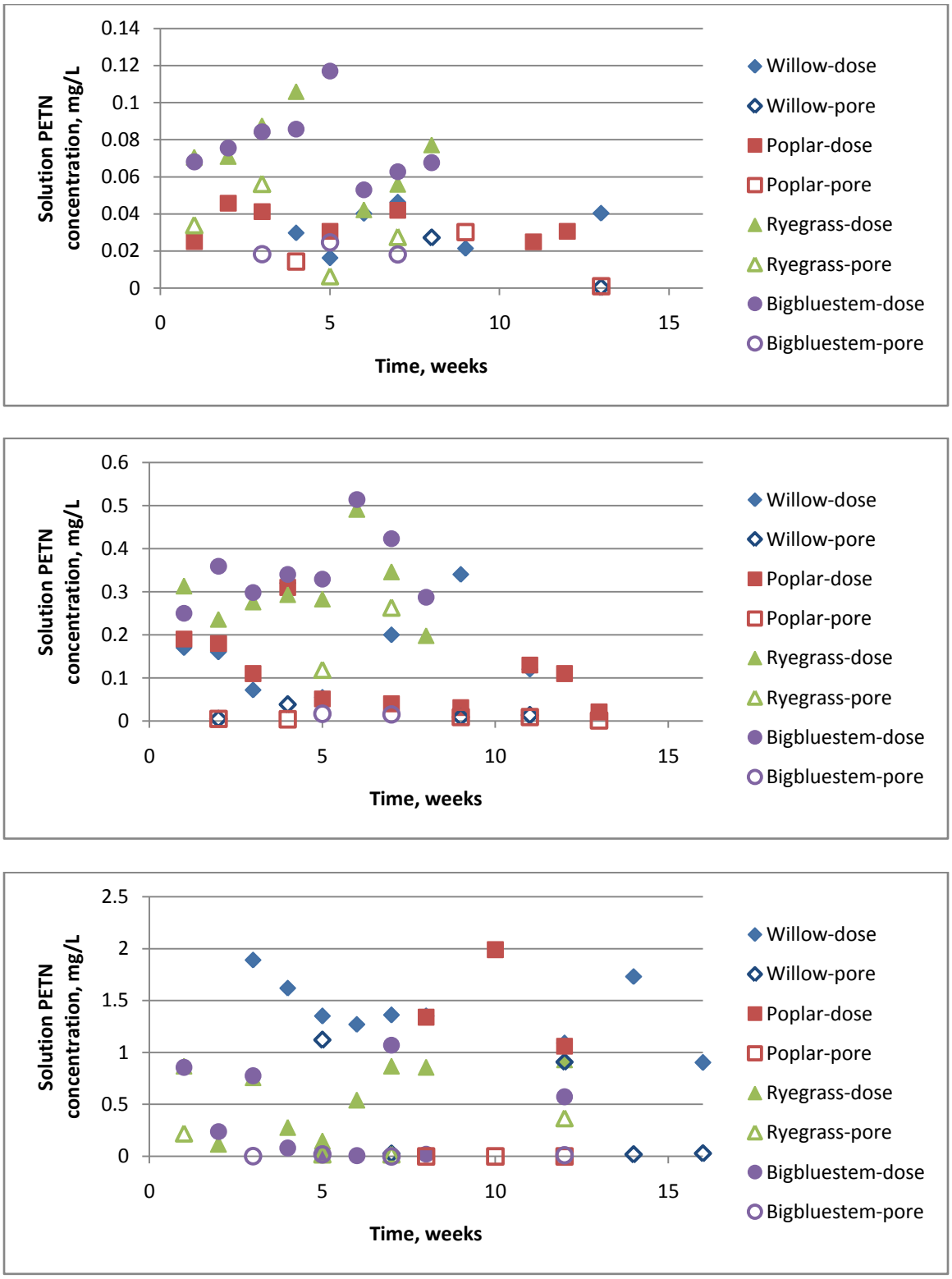

Figure D.6 Dosing and Pore Water PETN Concentration in Sand Reactors (3 dosing levels)

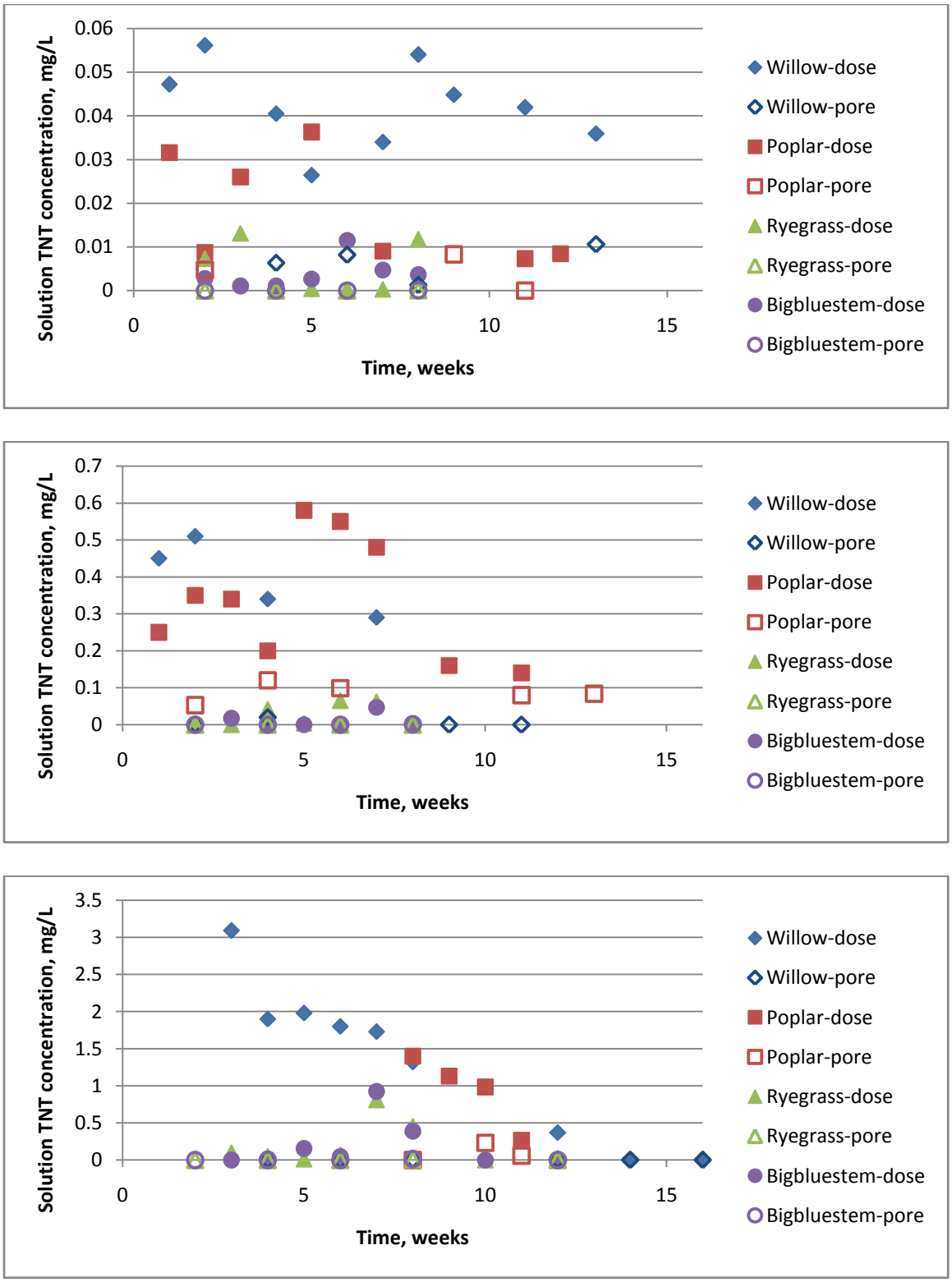

Figure D.7 Dosing and Pore Water TNT Concentration in Soil Reactors (3 dosing levels)

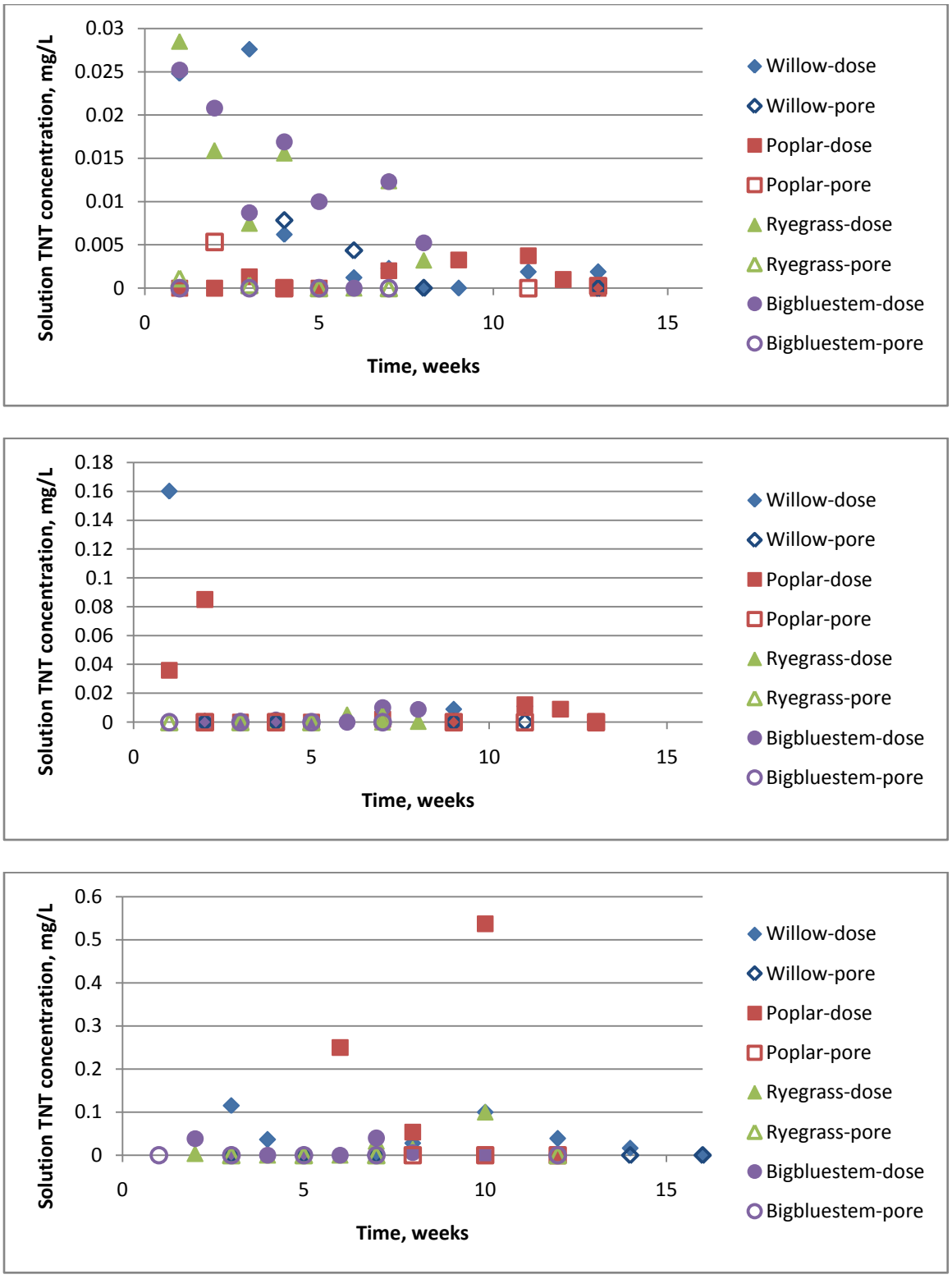

Figure D.8 Dosing and Pore Water TNT Concentration in Sand Reactors (3 dosing levels)

High degradation potential of TNT and PETN results in the variable concentration in dosing bottle and very low pore water concentration even in sand reactors. TNT degraded too quickly in Hoagland solution due to the presence of nutrients so that the concentrations in dosing bottles linking to sand reactors were far away the nominal value.

After harvest of plants, the substrate contaminant distributions were assessed by sampling pore water at other 2 higher levels of reactors. The high layer was 1/3 height below the surface, the height of trees inserted, standing for the dense root zone, or about 3 cm below the surface for grasses. The medium layer was 2/3 height below the surface for trees or 1/2 height below the surface for grasses, standing for the medium of high level and bottom. Low concentration dosed trees were the first batch to be harvested. According to original experiment plan pore water only sampled from bottom and Middle layers. Middle layer stood for the root area. After the differences of concentration among the heights were revealed, the higher layer was added to evaluate the contaminant distribution in the reactors. The bottom pore water was sampled by syringe linked to long needle or sampling tube. Only one sample was taken from per reactors.

For trees in low dosed reactors, the 8 pore water samples were centrifuged out from medium layer. Then every 4 samples were combined into one sample to get enough volume for auto sampler of HPLC-MS/MS. The standard deviations were from 2 combined samples and thus were smaller. For trees in medium and high dosed reactors, the 3 pore water samples were centrifuged out from medium or high layer. Then every sample was diluted and injected into HPLC-MS/MS. The standard deviations were from 3 single samples and thus were larger.

For grasses, the 4 pore water samples were centrifuged out from medium or top layer. Then all 4 samples were combined into one sample to get enough volume for auto sampler of HPLC-MS/MS. Thus there were no standard deviations for grass data. But all data can stand for the concentration of specific height as they were the average of several samples from this height.

The concentration of pore water in the whole reactor was not consistent spatially. Due the bottom dosing strategy was adopted in these planting experiments, the contaminant concentration would be expected to increase with regard to the height. The highest concentration was in bottom. Of course the flush of the water level in the reactors will result in more variable concentration distribution. For trees, the sand reactors better showed the predicted distribution pattern. While the soil reactors revealed lower concentration in bottom and medium layers, which maybe were contributed by the active rhizosphere organism activities.

For grasses, the most sand reactors showed the pore water concentration decreased in the order: top layer > medium layer > bottom. The grasses were planted by surface sowing, thus the dense root areas were in the half upper reactor although roots extended to the bottom of the reactors. The top layer was sampled just below the surface thus the evaporation and selective uptake resulted in the highest concentration. While higher concentrations in middle layer than bottom maybe were contributed by the plants' selective uptake. In sand reactor the biodegradation of HMX and RDX were limited.

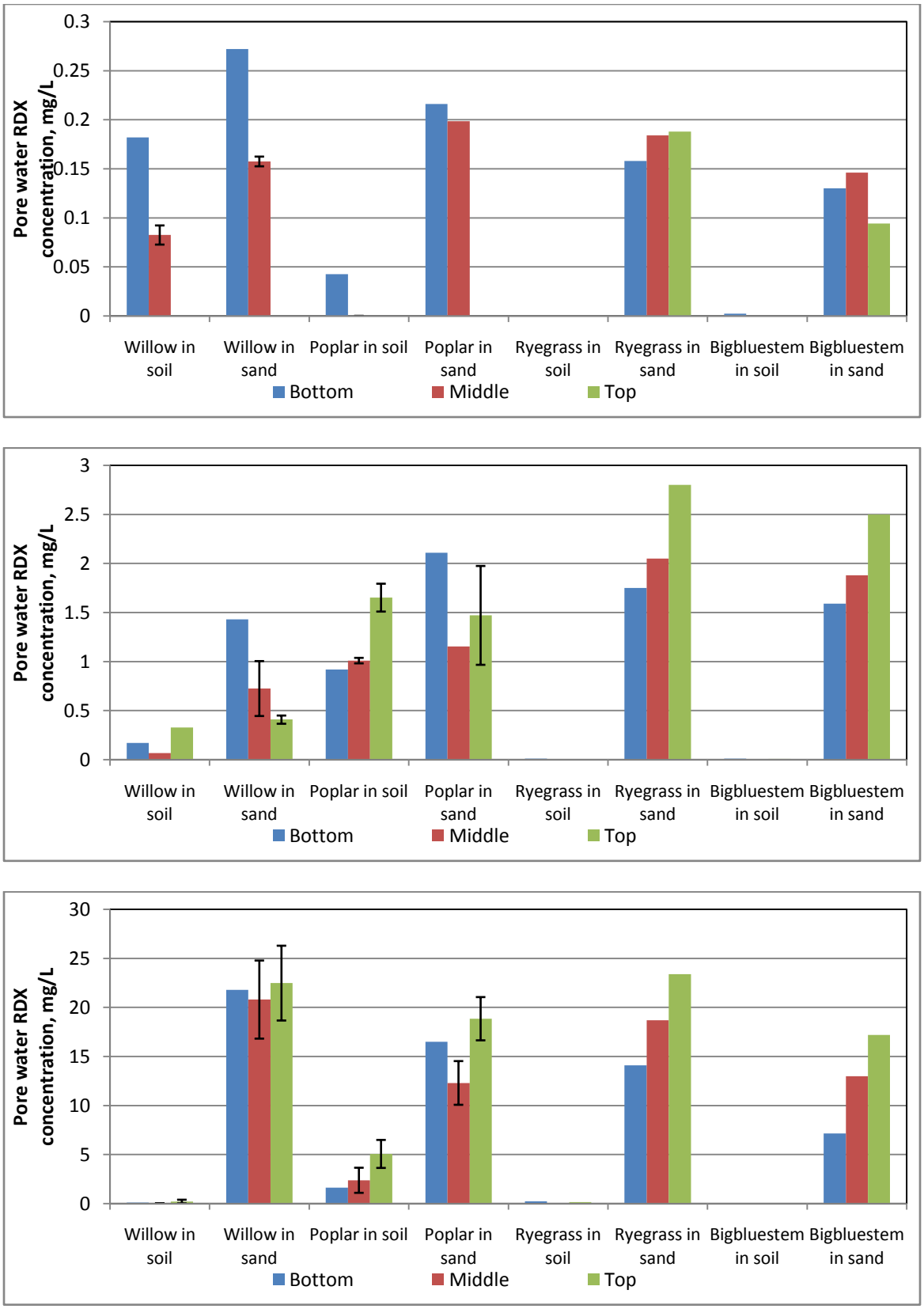

Figure D.9 Pore Water RDX Concentration Spatial Distribution

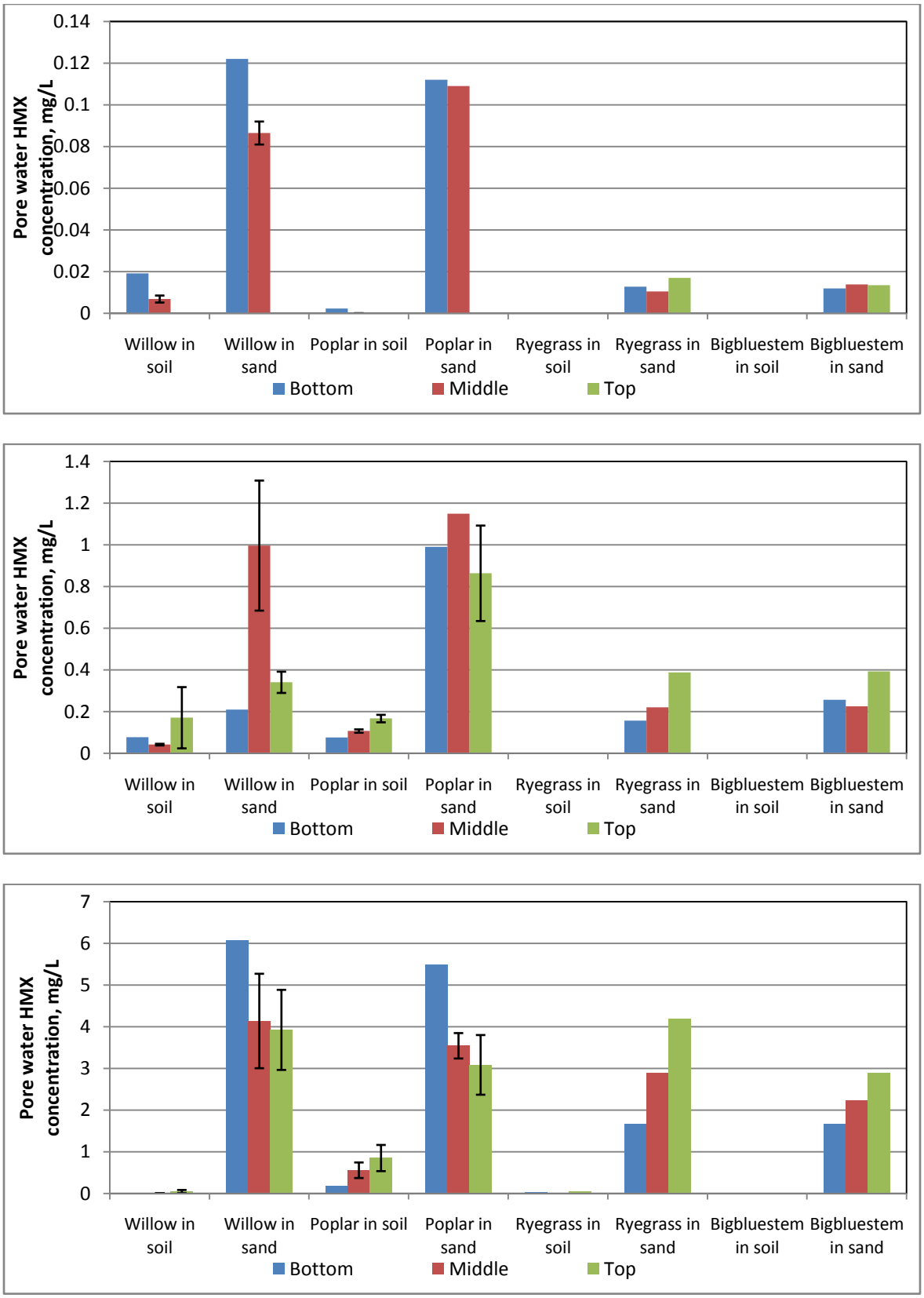

Figure D.10 Pore Water RDX Concentration Spatial Distribution

APPENDIX E

DOSING AND PORE WATER CONCENTRATION IN LARGE SCALE EXPERIMENTS

The pore water concentrations during the whole exposure periods for each test unit in large scale tree and long term experiment were shown in Figure E.1and Figure E.2for RDX and for HMX respectively. The RDX concentrations in dosing tanks were consistent in the whole experiment term. The HMX concentrations in dosing tanks were consistent in the whole experiment term except the last 3 month. The RDX stock solution was used up. The new coming RDX with higher impurity of HMX, which resulted in higher HMX in prepared dosing solution in order to keep RDX constant.

Pore water concentrations in the bottom of reactors were consistently lower than those in dosing tanks, which was in accordance with the results of the small scale experiment. It should be noted that the pore water concentration determined just represented the bottom of reactor. The difference of spatial distribution was much larger in big reactors than small reactors. The soil closer to surface was not determined any explosives even at the termination of the experiment. Also the distances of sampling tubing with inlet of dosing tubing were at random in every reactor, which possibly led to the higher deviation.

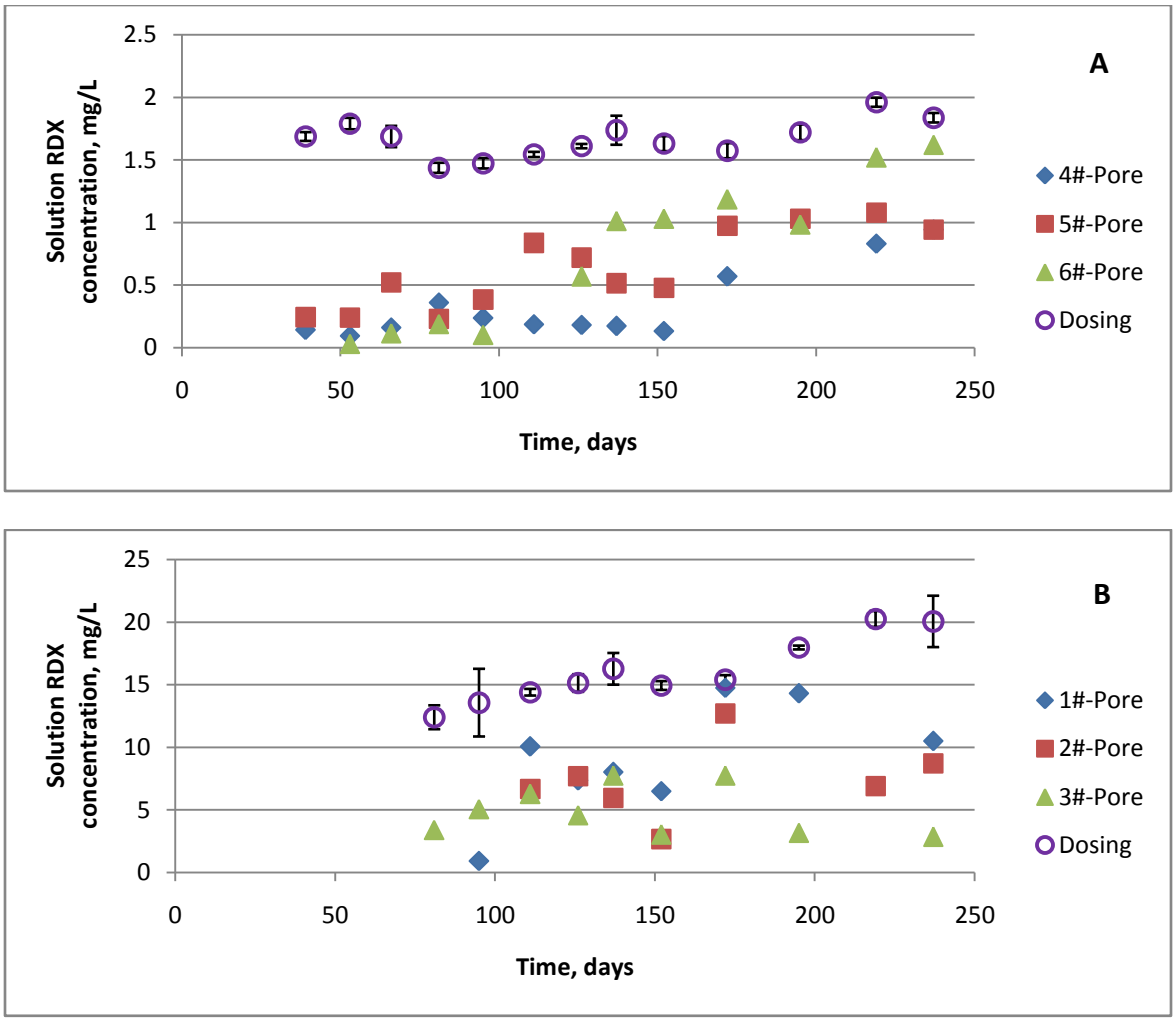

Figure E.1 Dosing and Pore Water RDX Concentration

Data from large scale tree experiment during the Whole Exposure Period. The dosing concentration represented the average value of the same treatment 3 test units. The error bars represented the standard deviation. A showed the test units dosed by medium levels; B showed the test units dosed by high levels.

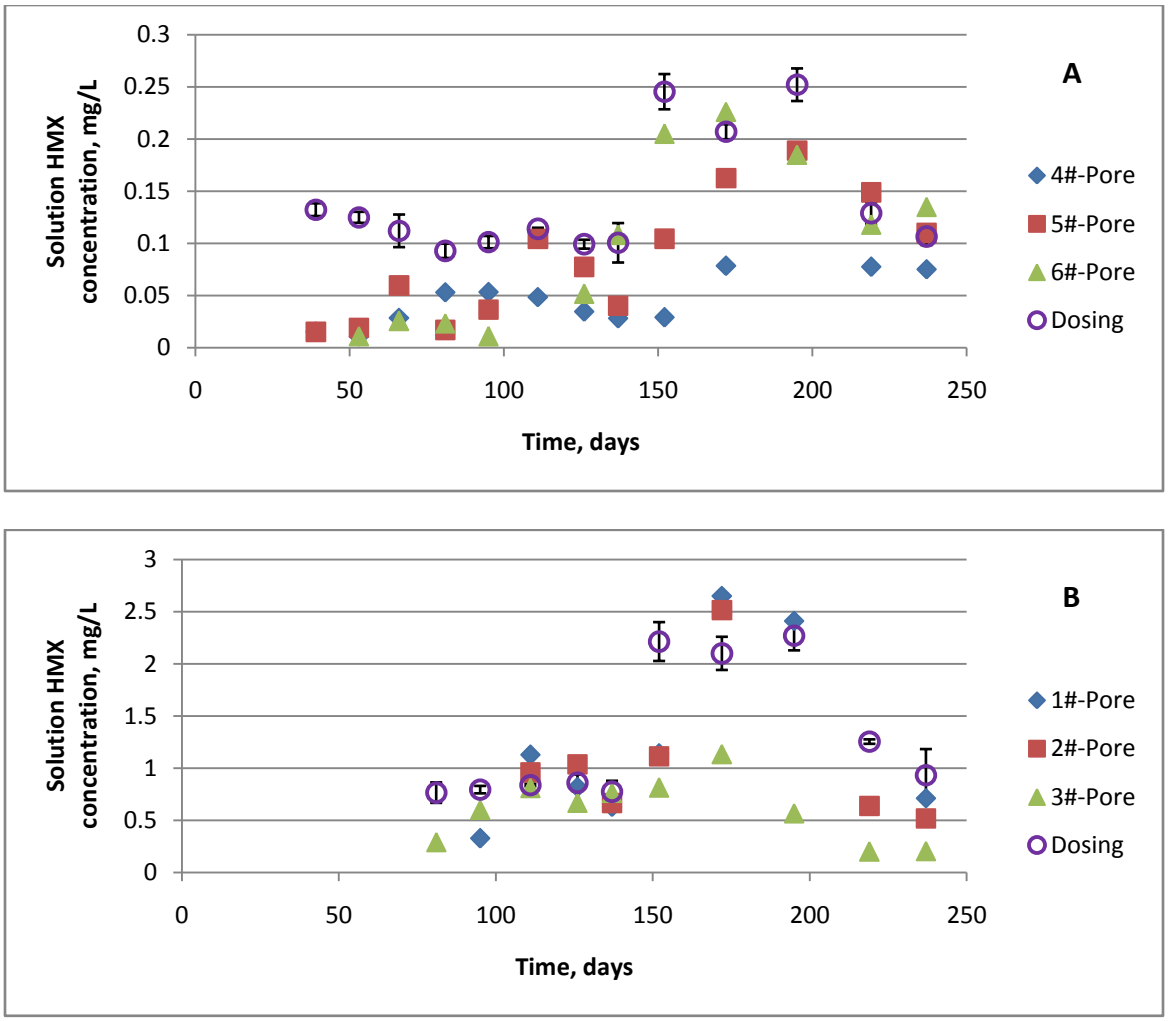

Figure E.2 Dosing and Pore Water HMX Concentration

Data from large scale tree experiment during the Whole Exposure Period. The dosing concentration represented the average value of the same treatment 3 test units. The error bars represented the standard deviation. A showed the test units dosed by medium levels; B showed the test units dosed by high levels.

APPENDIX F

TISSUE CONCENTRATION IN SHORT-TERM HYFROPONIC EXPERIMENTS

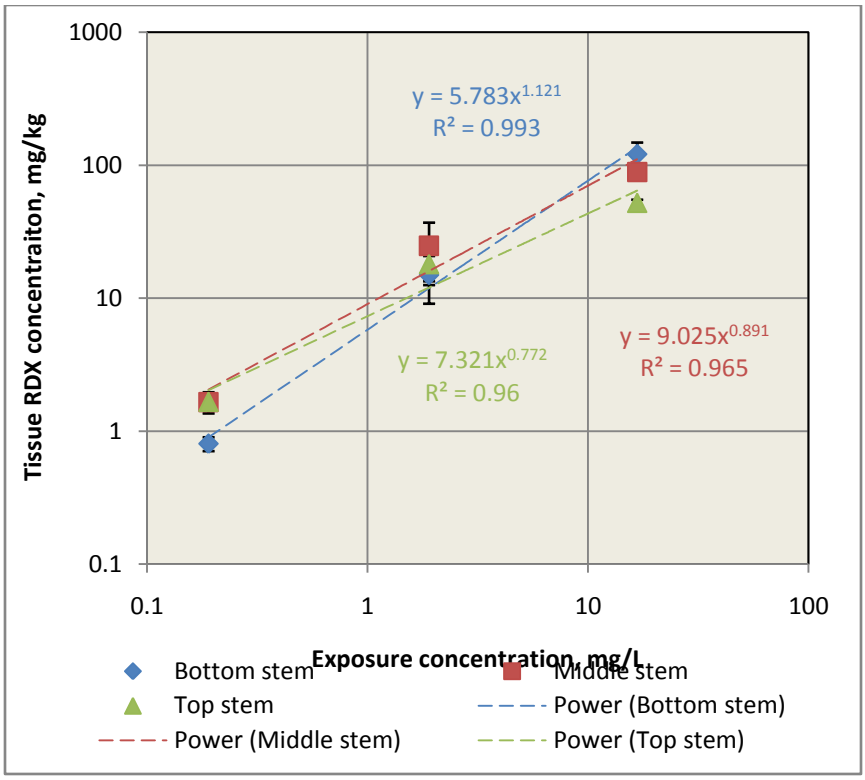

Figure F.1 Stem RDX Concentration in Hydroponic Test

RDX concentration in bottom section appeared highest for high dosing. This discordance was resulted from the short-term exposure as the contaminant cannot distribute throughout the whole trees. The concentration difference in height was smaller than HMX, which can be found from the regression equation coefficients. This result further proved that the plant was resistant to uptake HMX and the RDX was less sorption to wood. When dosing concentration was 16.7mg/L, the bottom, middle and top stem concentration was 121.05±27.14mg/kg, 88.72±5.45mg/kg and 52.19±2.87mg/kg respectively (mean ± SDV). When dosing concentration was 0.19mg/L, the bottom and top stem concentration was 0.80±0.10mg/kg, 1.66±0.30mg/kg and 1.67±0.24mg/kg respectively (mean ± SDV).

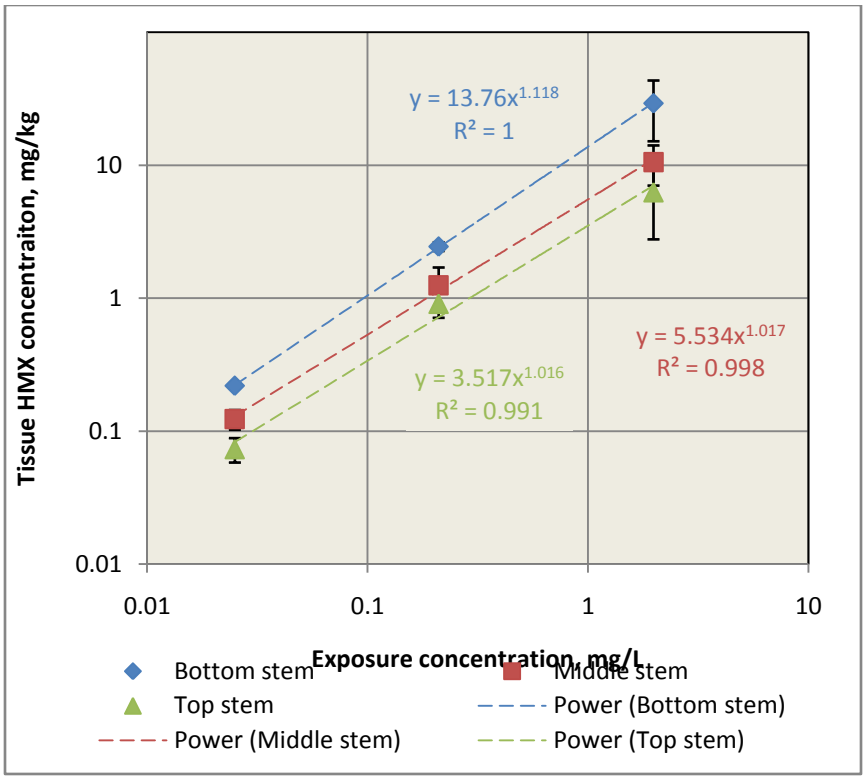

Figure F.2 Stem HMX Concentration in Hydroponic Test

The HMX concentration decreased from bottom to top consistently in all three dosing concentration. The concentration difference in height was clear and was greater in high dosing concentration than in low dosing concentration. When dosing concentration was 1.98mg/L, the bottom, middle and top stem concentration was 29.28±14.13mg/kg, 10.56±3.53mg/kg and 6.28±3.51mg/kg respectively (mean ± SDV). When dosing concentration was 0.025mg/L, the bottom, middle and top stem concentration was 0.22±0.01mg/kg, 0.12±0.02mg/kg and 0.073±0.015mg/kg respectively (mean ± SDV). The results from small scale experiments also showed some difference among different height of the stems, but the differences were considerably lower.

APPENDIX G

TISSUE CONCENTRATION IN SMALL SCALE TREE EXPERIMENTS

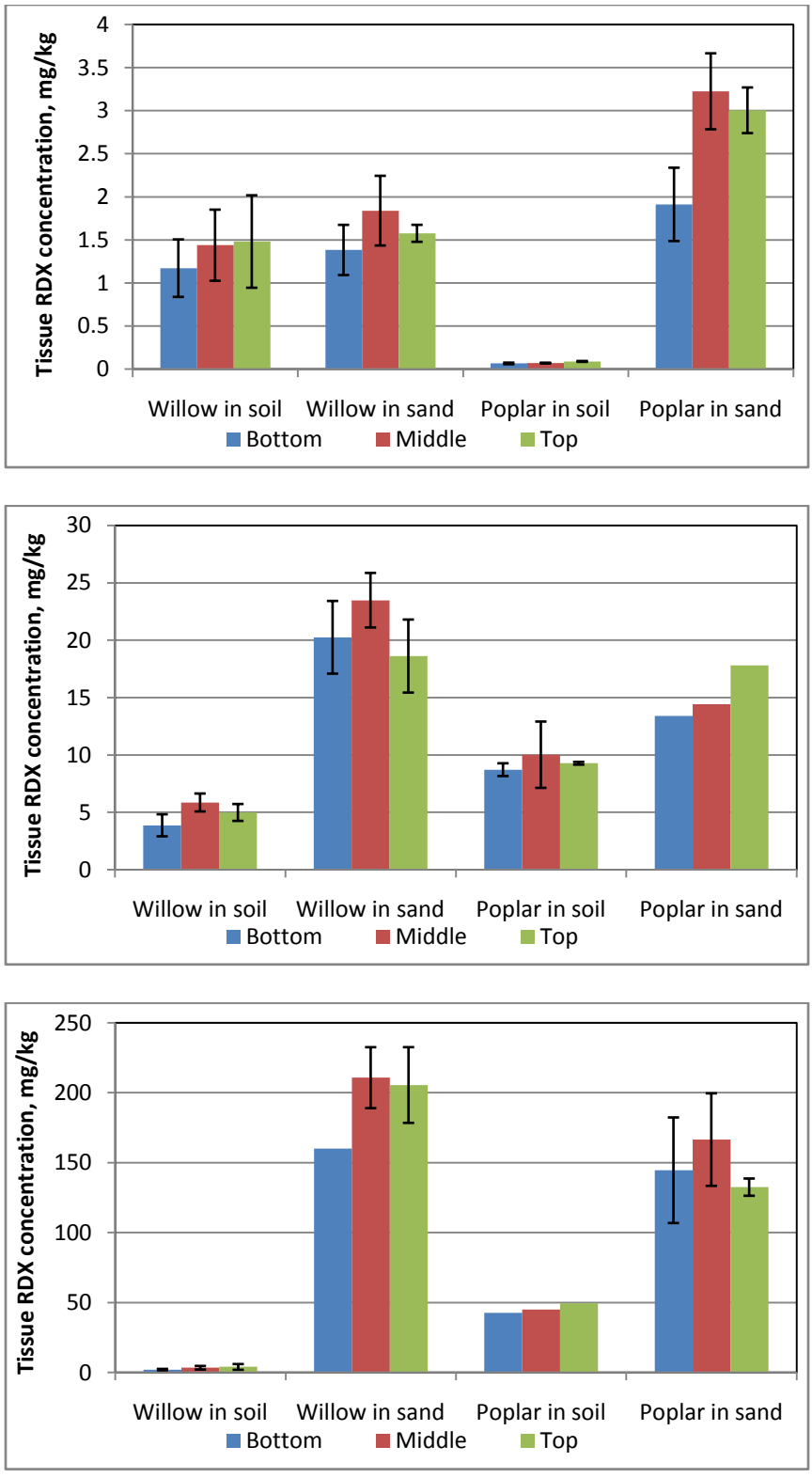

Figure G.1 Stem RDX Concentration in Small Scale Experiment (3 dosing levels)

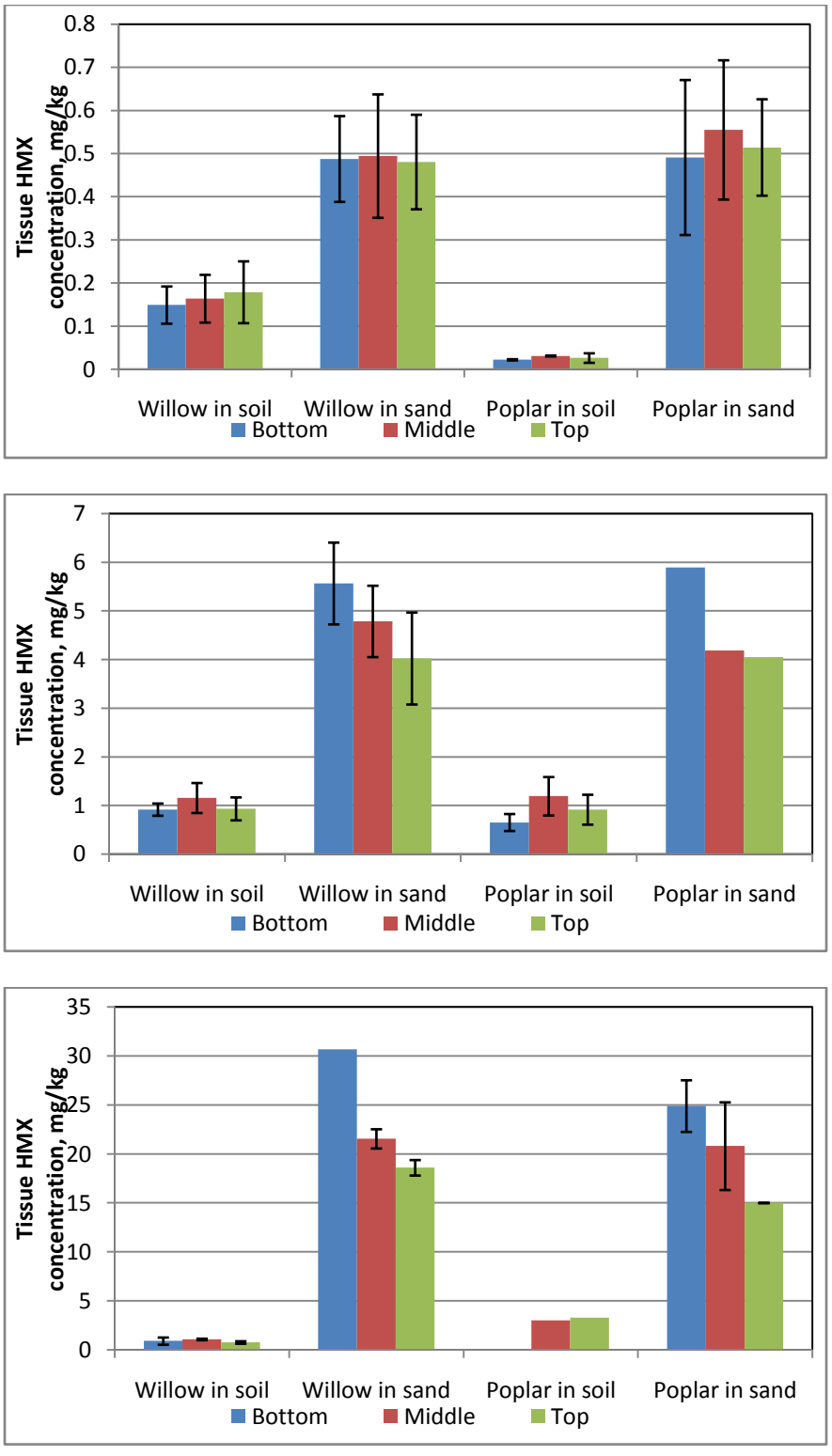

Figure G.2 Stem HMX Concentration in Small Scale Experiment (3 dosing levels)

The concentration differences in the stems were assessed. The tree stems were cut into three sections as described in the method section. Some data were listed for willows rooting in sand in order to compare to the data from hydroponic tests.

RDX:

For low concentration exposure, the middle and top stems concentrations were 1.84±0.40mg/kg and 1.58±0.10mg/kg (mean±SDV). For medium concentration exposure, the middle and top stems concentrations were 23.48±2.38mg/kg and 18.61±3.18mg/kg (mean±SDV). For higher concentration exposure, the bottom, middle and top stem were160.10, 210.81±21.78mg/kg and 205.55±27.06mg/kg (mean±SDV).

HMX:

For high concentration dosing, the bottom, middle and top stem concentration were 30.68mg/kg, 21.55±0.98mg/kg and 18.60±0.79mg/kg respectively (mean ± SDV). For low concentration dosing, the bottom, middle and top stem concentration were 0.49±0.10mg/kg, 0.49±0.14mg/kg and 0.48±0.11mg/kg respectively (mean ± SDV).

APPENDIX H

EXPLOSIVE DEGRADATION TESTS IN SOLUTION

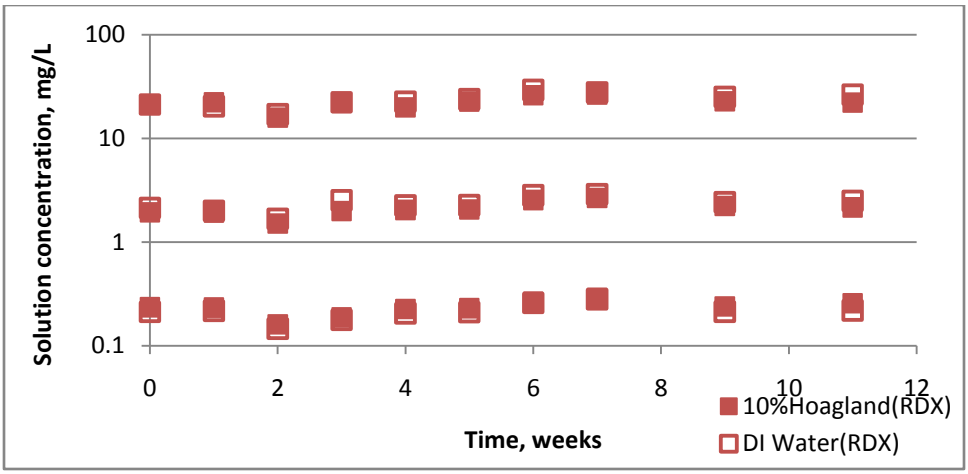

Figure H.1 Solution Degradation Tests (RDX)

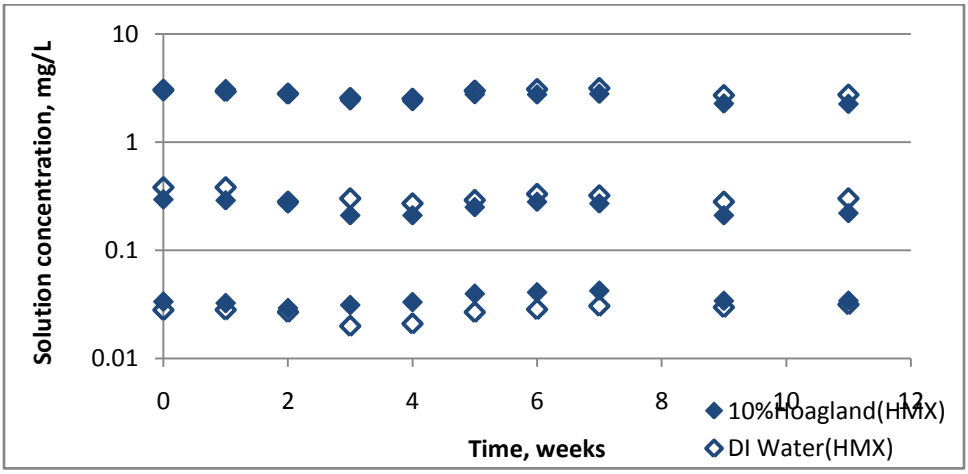

Figure H.2 Solution Degradation Tests (HMX)

RDX and HMX keep steady in both solutions in all three test concentrations.

PETN keep steady in both solutions in the low and medium concentrations and DI water in the high concentration, but showed clearly degradation in 10% Hoagland solution for the high concentration.

TNT degraded rapidly in both solutions in all three test concentrations and TNT degraded much quicker in 10% Hoagland than in DI water as expected. In low spiked solution TNT concentration is below detection limits only after one week. Even in high spiked solution, TNT totally disappeared after three weeks.

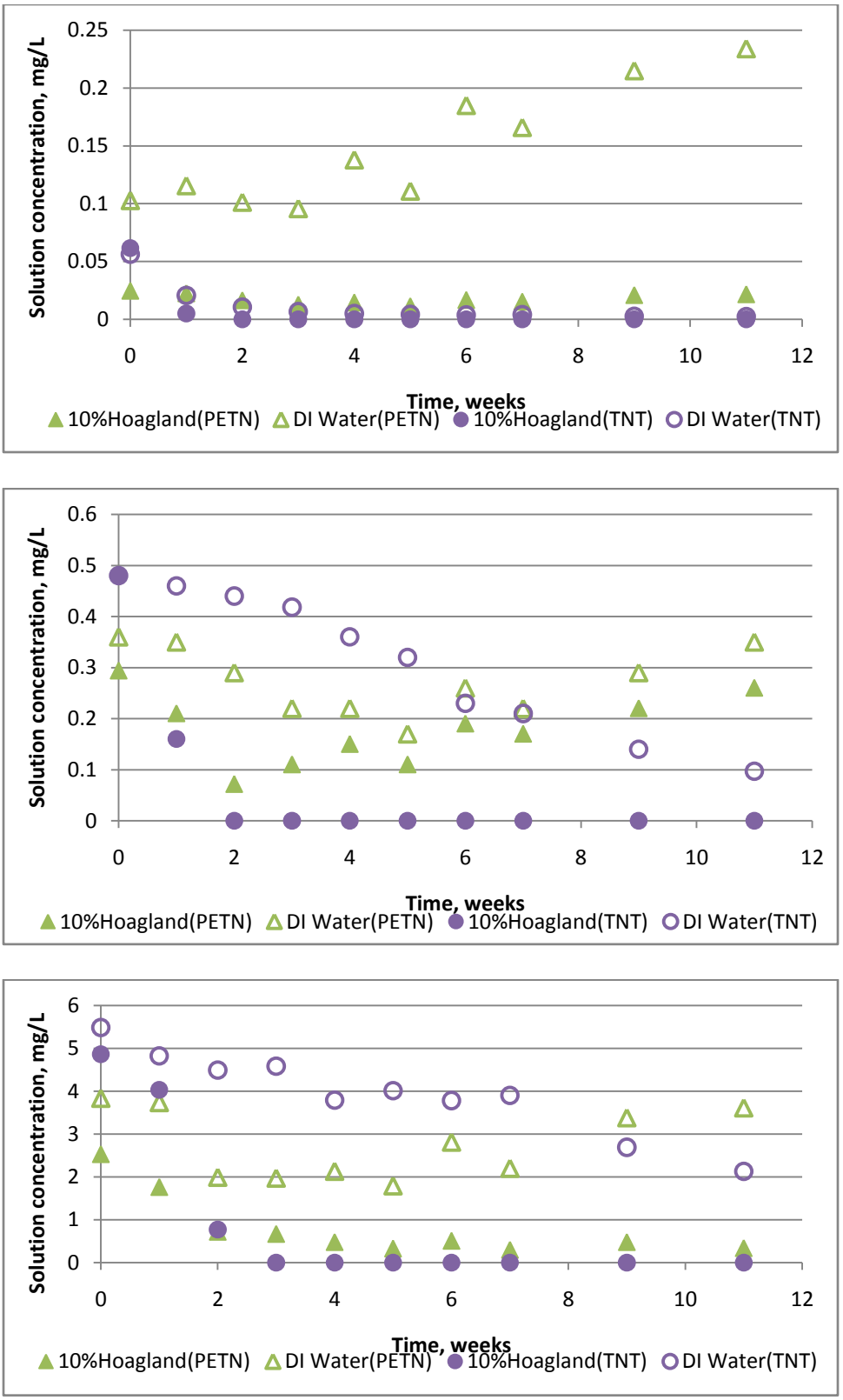

Figure H.3 Solution Degradation Tests (TNT and PETN)

APPENDIX I

TOXIC TEST (SHORT-TERM HYDROPONIC EXPERIMENT)

Test 1

Dosing concentration:

Group name	HMX/ppm	RDX/ppm	TNT/ppm	PETN/ppm	MeCN/ppm
0 dosed without MeCN	0	0	0	0	0
0 dosed with MeCN	0	0	0	0	2430
50% of high dosed	1.15	10.2	2.64	1.5	1215
75% of high dosed	1.65	13.64	4.01	2.25	1823
High dosed	2.07	16.89	5.3	3	2430
High- RDX&HMX	2.04	20.09	0	0	1215

Results

Group name	Tree Number	Growth description
0 dosed without MeCN	2	Normal
0 dosed with MeCN	3	Dead earliest, dying after 9 days; all dead after 12 days
50% of high dosed	3	Grow very slowly. One tree dead after 18 days
75% of high dosed	3	Grow very slowly. All trees dead after 15 days
High dosed	3	One tree dead after 12 days; the other two dead after 15 days
High- RDX&HMX	3	At the beginning grow normally. One tree dead after 12 days; the other two dead after 21 days

Test 2

Dosing concentration:

Group name	HMX/ppm	RDX/ppm	TNT/ppm	PETN/ppm	MeCN/ppm
0 dosed without MeCN	0	0	0	0	0
0 dosed with MeCN	0	0	0	0	1094
0 dosed with50% MeCN	0	0	0	0	547
High dosed	1.16*	16.3	4.88	1.26*	1094
50% of high dosed	1.19	8.89	2.34	1.57	547
High-after sparge	1.98	16.7	4.6	1.9	

*The measured values were may underestimated.

Results

Group name	Tree Number	Growth description
0 dosed without MeCN	3	Normal
0 dosed with MeCN	3	All dead after 9 days
0 dosed with50% MeCN	3	One dead after 9 days, another one dead after 12 days, the last one alive
High dosed	3	All trees dead after 12 days
50% of high dosed	3	One dead after 12 days; another one dead after 15 days, the last one dead after 21 days
High-after sparge	3	One dead after 21 days, the other two keep alive

MeCN was proven to be toxic to willow even at the concentration of 0.55g/L. Sparging the dosing solution by N_2 showed effectively decreased MeCN concentration as two trees survived from the test group of three trees.(Test 2) Evidently willows were sensitive to TNT (or PETN). Dosing solution of "50% high" and "High-RDX&HMX" are the same concentration of MeCN, but the plants growth response are totally different. Plants grown in the solution of "50% high" were seriously inhibited, while those grown in the solution of "High-RDX&HMX", grew normally at least at the beginning.(Test 1) Under the same MeCN concentration of 0.55g/L, one tree dosed by solution without explosive was alive, while all three trees dosed by solution with explosive were dead. The phyto- toxicity came from the integrated effects of explosive and MeCN.(Test 2)

APPENDIX J

LARGE SCALE TREE AND LONG TERM EXPERIMENTS SIMULATION

The developed model was conducted to simulate the large scale tree and long term experiments. The input parameters kept consistent with those used in the small tree simulations only if the change was justified. Among the parameters related to the plant, the original biomass, growth rate, branch and stem water content was updated based on the experimental measurements. All parameters related to soil had to be changed due to the great difference between the characteristics of two soils. The commercial soil used in the large scale trees cultivation had awesome organic content 50.3±1.6% in contrast to 8.4±0.3% for the soil used in the small scale experiment and great water holding capacity. The organic matter was determined by ashing a 2 gram sieved dry sample at 550ºC for 1 hour in a muffle furnace. The soil water content, distribution coefficient, fresh soil mass and bare soil area for evaporation calculation were derived from experimental measurement. The biodegradation rate was derived from model calibration. All updated input parameter were determined by the average of all reactors rather than one specific reactor. Table 5.6, Table 5.7 and Table 5.8 listed these parameter values.

Four simulations were done on two dosing levels for both RDX and HMX. A time step of 1day was used and the integration method was fourth-order Runge– Kutta methods. Figure J.1 showed the comparison of simulation and experimental measurement for RDX, and Figure J.2 showed the comparison for HMX. The best conformity appeared on stems. All four simulations predicted the dynamic developments quite well. The worst conformity appeared on leaf concentration. The simulations failed to predict the early high concentrations of leaves. This result suggested that the metabolism rate in plant might not be constant. Plants maybe need time to learn how to transform it and the rates increase along with the time. The other possibility is that the plants translocate the

compounds up more efficiently actually. The stem sap maybe includes stationary tissue liquids other than the mobile sap. The mobile sap had higher chemical concentration. The sap measurement was diluted by the stationary liquids with lower concentration resulting in the underestimation of the mass transported into branches and leaves. Although the plant concentrations kept increasing over the whole 250 days of simulation, the surrounding soil concentrations improved also. The ratio of plant concentration to soil concentration (or pore water concentration) was relative steady especially for stem over most of exposure time besides the beginning 60 days. These results validated the phytoforensic approach on bigger scale trees. The stem concentration correlated to the exposure concentration quite well and was suitable for quantitative prediction. The enriched leaf concentration was higher but more varied and can be used as semi-quantitative screening.

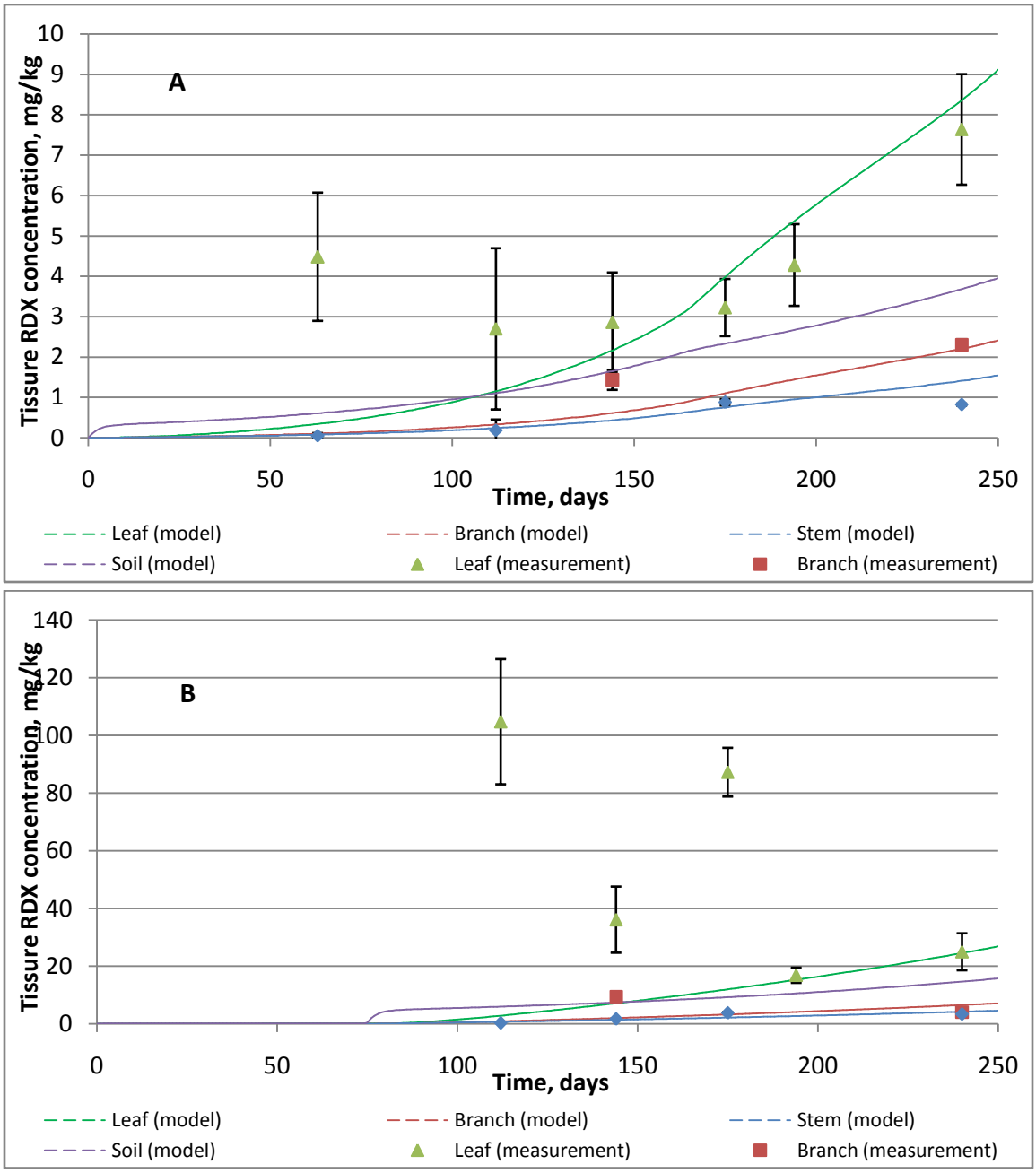

Figure J.1 Model Prediction and Experiment Measurement Comparisons: RDX, Large Scale Tree Experiment

The error bars represented the standard deviation. A showed the test units dosed by medium levels; B showed the test units dosed by high levels.

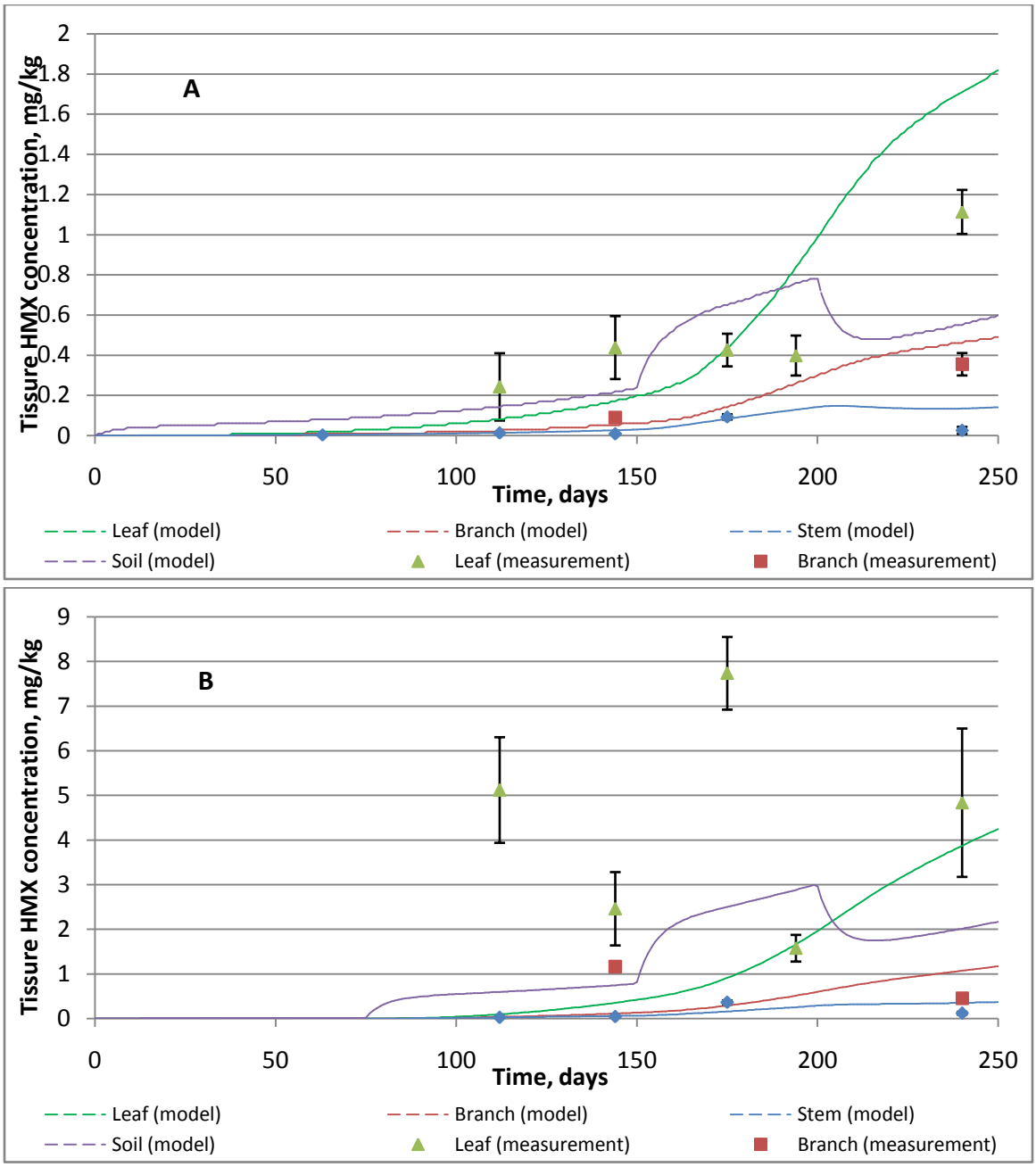

Figure J.2 Model Prediction and Experiment Measurement Comparisons: RDX, Large Scale Tree Experiment

The error bars represented the standard deviation. A showed the test units dosed by medium levels; B showed the test units dosed by high levels.

For high dosing treatment, at the beginning 72 days reactors were treated at low level (100 times lower than high level). From about 150th to 200th day, the supplied solid RDX had higher impurity of HMX resulting in the higher dosing concentration to keep RDX level constant.

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