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EVALUATION OF PHYTOREMEDIATION FOR HMX AT THE WAINWRIGHT MILITARY BASE: GREENHOUSE TRIALS

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LIST OF ABBREVIATIONS

BRI	Biotechnology Research Institute
B_v	Soil-to-plant transfer factor (concentration in plant/concentration in soil)
°C	degrees Celsius
CAEAL	Canadian Association of Environmental Analytical Laboratories
CCIW	Canada Centre for Inland Waters
COC	Contaminant of Concern
cm	centimetres
DND	Department of Defense
HPLC	High Pressure Liquid Chromatography – Ultraviolet
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
K_{ow}	Octonal–water partition coefficient.
L	litres
µL	microlitre
µm	micrometer
m	metres
m^3	cubic metres
mL	millilitres
mg/kg	milligrams per kilogram
mg/L	milligrams per litre
PASC	Philip Analytical Services Corporation
PGPR	Plant Growth-Promoting Rhizobacteria
QA/QC	Quality Assurance/Quality Control
RPM	Revolutions per Minute
WATC	Western Area Training Centre
WTC	Wastewater Technology Centre

Chemical Parameters:

Ag	Silver
Cd	Cadmium
Cl	Chloride
Cr	Chromium
Cu	Copper
Fe	Iron
HMX	High Melting Explosive (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
Ni	Nickel
RDX	Royal Demolition Explosive (hexahydro-1,3,5-trinitro-1,3,5-triazine)
Zn	Zinc

Study Participants

This study was completed on behalf of the Department of National Defense (DND) and Environment Canada's Wastewater Technology Centre (WTC) and Environmental Protection Branch (Quebec Region). The study was conducted by BEAK International Incorporated (BEAK) in association with the National Research Council's Biotechnology Research Institute (BRI). Individuals at BEAK responsible for design and/or implementation of the study and report preparation include Don Lush, Ran Lifshitz, Virginia Hart, and Neil Morris. Staff at BRI responsible for analytical components of the study and contributions to study design and interpretation include Sylvie Beaudet, Carl Groom, Annamaria Halasz, Jalal Hawari, and Louise Paquet. Charles Dubois of DND contributed to study design and interpretation, and participated directly in study activities completed at WATC Wainwright. Lucie Olivier and Frederic Shooner of Environment Canada also contributed to the design and interpretation of the greenhouse study. Bernard Glick of the University of Waterloo provided comment on the study design and provided procedures and materials for seed inoculation conducted as part of germination trials. The study authors also wish to acknowledge the support provided by WTC staff at the Canada Centre for Inland Waters (CCIW), including Pat Falletta, Stephen Lee, Sandra Skog, and Peter Seto.

Project Summary

In order to evaluate phytoremediation as a possible remediation approach at Canadian Forces firing ranges contaminated with explosive materials, a greenhouse study was designed and implemented primarily to examine the fate of HMX in soil and plant tissues. Five plant species, including alfalfa (*Medicago sativa)*, bush bean (*Phaseolus vulgaris*), canola (*Brassica rapa*), wheat (*Triticum aestivum*), and perennial ryegrass (*Lolium perenne*), were grown in HMX-bearing soil collected from an anti-tank firing range at Wainwright, Alberta. The presence of HMX in soil did not appear to have any significant adverse effects on plant growth or physiology. All plants examined in the study exhibited some capacity to translocate HMX from soil to above-ground plant tissues. The grass species (rye and wheat) exhibited the greatest capacity for such translocation, removing up to 10% of the total HMX mass from soil over a period of only 11 weeks. There was no evidence to suggest that the presence of plants resulted in any significant degradation of HMX. A concurrent evaluation of the potential beneficial effects of adding zero-valent iron as part of *in situ* remediation efforts did not reveal any evidence of enhanced HMX degradation as a result of this approach. Overall, the study results suggest that phytoremediation may be a practical and effective remediation approach at Canadian Forces firing ranges, primarily through the process of phytoextraction.

1.0 INTRODUCTION

1.1 Background

The Department of National Defense (DND) operates military training bases which are contaminated with low levels of various explosive substances as a result of routine training activities. The contaminants of concern (COCs), specifically at anti-tank firing ranges, include hexahydro-1,3,5-trinitro-1,3,5-triazine, also known as *Cyclonite, Hexogen* or *Royal Demolition Explosive* (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, also known as *Octogen* or *High Melting Explosive* (HMX). These two compounds are hereafter simply referred to as RDX and HMX, respectively. HMX is the more recalcitrant of these two substances, and presents greater challenges with respect to remediation efforts.

Remediation of these contaminants in soil is complicated by their physical and chemical properties (e.g. relatively low solubility) as well as the existence of unexploded ordnance and other restrictions routinely encountered at locations of military training activities. The application of conventional remediation strategies is likely to be impractical under these conditions and over the relatively large areas where contamination is present.

Phytoremediation is a general term for technologies that harness the characteristics of selected plant species to remove, contain, accumulate or degrade a variety of COCs in various media (surface and sub-surface soils, groundwater, and surface water). Central to the application of phytoremediation is the ability of many plant species, through their natural metabolic processes, to extract and directly uptake various inorganic and organic chemicals from soil and groundwater (*i.e., phytoextraction*). Once within the plant, chemicals can either accumulate (as with many metals) and/or be transformed (as with some organics) through normal plant metabolism (*i.e., phytodegradation*). In the cases where chemicals simply accumulate in plant tissues, plant harvesting and disposal may provide a remediation alternative analogous to traditional excavation and disposal techniques (Salt *et al.*, 1995). In cases where chemicals are directly transformed, the potential exists to use phytoremediation to destroy the chemicals *in situ.* In some cases, plants

also enhance or are responsible for biotic and abiotic rhizosphere reactions which can be manipulated to assist in degrading a variety of organic and inorganic chemicals. The primary benefit associated with the establishment of plants may also simply be the containment of contaminant-bearing soil or water within confined areas (*i.e., phytostabilization*).

To date, researchers have documented the accumulation and/or transformation of a wide variety of organic and inorganic compounds in plants, including;

- metals (Pierzynski *et al.*, 1994; Dushenko *et al.*, 1995; Brown *et al.*, 1995; Huang *et al.*, 1997; Lawrence and David, 1997; Tossell *et al.*, 1997),
- radionuclides (Cornish *et al.*, 1995; Entry *et al.*, 1994 and 1997; Kochian, 1997),
- phenolic compounds and pentachlorophenol (Adler *et al.*, 1994; Dee and Bollag, 1994; Ferro *et al.*, 1994),
- polyaromatic hydrocarbons (PAHs) (Schwab and Banks, 1994; Reilley *et al.*, 1996; Schnoor *et al*., 1995),
- chlorinated solvents (Walton and Anderson, 1990; Anderson and Walton, 1995; Gordon, 1997), and
- pesticides (Lappin *et al.*, 1985; Zablotowicz *et al.*, 1994; Cole *et al.*, 1995).

Recent studies have also revealed a potential for the application of phytoremediation as a viable approach to remediation of soil or shallow groundwater contaminated with materials associated with the production, use or disposal of explosive materials, including TNT and RDX (Scheidemann *et al.*, 1998; Hughes *et al.*, 1997; Thompson *et al.*, 1998 and 1999; Schnoor *et al.*, 1995; and Best *et al.*, 1997). There is little published research with respect to the interactions of plants with HMX in ambient media.

Overall, phytoremediation strategies can provide a remediation approach that is non-invasive, long-term and self-sustaining, requiring little maintenance and capital input during operation. In addition,

the use of plants in site remediation provides ecological and public relations benefits associated with habitat creation and aesthetic value. Given the general attributes of phytoremediation and the specific conditions at Department of National Defense training bases (*i.e.,* "Sites"), there is reason to believe that the application of phytoremediation techniques may be effective in addressing site-specific requirements for an inexpensive, non-invasive, and relatively long-term remediation strategy. Phytoremediation may be a viable alternative at such sites by virtue of several general phenomena. The processes of phytoextraction, phytodegradation, and phytostabilization may all provide some level of effectiveness.

1.2 Current Study Rationale and Objectives

During the winter of 1999/2000, Beak International Incorporated (BEAK) developed and implemented a greenhouse-based study on behalf of the Department of National Defense and Environment Canada's Wastewater Technology Centre (WTC). The study was focussed on the fate and transport of explosive COCs in soil and plant tissues. The overall purpose of the study was to evaluate the potential suitability of phytoremediation as an alternative approach to reducing current levels of contaminants of concern (COCs), particularly RDX and/or HMX, under the conditions encountered at DND Sites. Specifically, the greenhouse study was completed to address the following issues:

1. the capacity of select commercial plant species to tolerate Site soil conditions, including the presence of elevated concentrations of COCs,

2. the capacity of select plant species to absorb COCs from Site soils,

3. the capacity of select plant species to transform COCs found in Site soils, and

4. the effect of adding zero-valent iron during *in situ* remediation processes.

In order to meet these stated objectives, the greenhouse study encompassed a series of procedures, including a literature review, analytical protocol revision, and additional experimental trials apart from the main experiment.

1.3 Report Structure

The remaining sections of this report provide full documentation of the study content, methodology, and the study results, including data summaries and interpretation. Raw data and other supporting material are also appended to this report.

The content of this report is structured as follows:

- **Section 2.0**: detailed approach and methods for all study components;
- **Section 3.0**: presentation of study results;
- **Section 4.0**: a discussion of the implications of the study findings;
- **Section 5.0**: conclusions and recommendations; and
- **Section 6.0**: full citation of references appearing in the main body of the report.

2.0 APPROACH AND METHODS

Prior to initiation of the greenhouse study, a Study Design (BEAK, 1999) was prepared to identify specific tasks and associated methods that would be required in order to meet the defined objectives. The following sections describe in detail the specific tasks which were identified in the Study Design and undertaken as part of the greenhouse study.

The study was conducted between 09 November 1999 and 24 January 2000 in a climate-controlled greenhouse located at the Canada Centre for Inland Waters (CCIW), in Burlington, Ontario.

2.1 Literature Review

Several preliminary tasks were completed as part of the development of the Study Design to ensure that the best available information was considered in that design. These tasks were generally as follow:

- review of background information regarding candidate military sites in question (e.g. Thiboutot *et al*., 1998);
- discussions with staff from the Department of Defense (DND) and Environment Canada (EC); and
- review of scientific literature relevant to the fate and transport of explosive contaminants in soils and plants, and specific treatises of phytoremediation of these contaminants.

The information obtained via the tasks above was used to optimize the Study Design, serving as part of the basis for selection of plant species, experimental treatment options, and general experimental procedures.

2.2 Experimental Treatments

Experimental treatments were established to encompass specific combinations of various levels of the following four (4) key variables;

1. Contaminant concentration in soil (2 levels – presence/absence (control)),
2. Plant species (5 levels),
3. Iron amendment application and incorporation (4 levels), and
4. Organic amendment application (livestock manure) (2 levels – presence/absence).

Four control series were also established for soil quality evaluation and plant performance evaluation in absence of COCs. The specific levels of the key variables and the proposed experimental combinations of these variables (*i.e.,* experimental treatments) and the rationale for their selection are discussed in detail in the following sections.

2.2.1 Concentration of HMX/RDX

Measured concentrations of explosive contaminants in soil at candidate Canadian Range Sites are heterogeneous. For example, HMX at CFB Valcartier firing ranges has been measured at concentrations ranging from approximately 16 to 3,900 mg/kg, all within several meters of target locations, and HMX at WATC Wainwright has ranged from non-detectable to 3,700 mg/kg (Thiboutot *et al.*, 1998). It is recognized that in localized areas of very high HMX/RDX concentrations, specialized remediation efforts will likely be required and phytoremediation is not currently being considered as a remediation alternative under these conditions. The expected application of phytoremediation will target more typical and widespread conditions, where concentrations of HMX/RDX are in the order of 50 to 100 mg/kg.

In order to obtain soil stocks of the desired concentration of HMX/RDX, surface soils were collected from Range 13 at the Western Area Training Center (WATC) Wainwright, within areas of known levels of contamination (*i.e.*, 50 to 100 mg/kg). A stock of control soil (nominally 0 mg/kg RDX and HMX) was also prepared by collecting soils in the vicinity the Site, from a non-

target area adjacent to Range 13, outside of the known zone of contamination. The collection and preparation of soil stocks is described in Section 2.4 of this report.

Treatments based on soil quality included controls (soil with no contaminants) which were established primarily for the evaluation of plant performance (*i.e.,* plants were planted in both clean and contaminated soils). A series of treatments incorporating contaminated soil in combination with other variables (*i.e.,* iron and manure amendments) were also established. Soil controls (*i.e*., soil with no contaminants) were also established to determine if substances which are known products of degradation of the COCs are also coincidentally produced as a result of manure and/or iron amendment in absence of COCs. For example, the addition of manure to any treatment may result in the elevation of soil concentrations of nitrogen, nitric oxide, or ammonia to levels well above normal background concentrations. These compounds are also a degradation products of RDX/HMX. In the measurement of these compounds in contaminated soil as possible degradation products, the data from the control series (*i.e.,* the concentration of these same compounds in clean soil) serves to adjust for the presence of any secondary sources (*i.e.,* not as a result of degradation of COCs).

2.2.2 Plant Species

Based on the preliminary literature review, a number of plant taxa were identified which could be considered for evaluation in this phytoremediation study. Several studies have focussed on a variety of plants, including both herbaceous plants and tree species, which show some promise with respect to removal, degradation, or containment of contaminants related to the production or use of military explosives. The list of candidate species considered for the current greenhouse studies was confined to herbaceous species (*i.e.,* no trees or shrubs) to take into account operational restrictions at the DND Sites. The selection of plant species also gave consideration to their suitability to meteorological conditions at the candidate Sites.

The candidate list of plant species encompassed several major plant families and included grasses (monocots) and non-grasses (dicots), as well as annuals and perennials. Monocots and dicots are two main sub-classes of plants which are physiologically distinct in many respects. It was possible

that inherent physiological differences between these two plant groups would confer a general distinction with respect to their response to HMX and/or RDX. For this reason, both of these major groups were represented among the plant species selected for the experiment.

Only commercial varieties which were available in reliable supply were selected for the greenhouse study. The use of commercially available varieties minimizes the potential complications of introduction of weedy species and facilitates scaled-up evaluation or application of phytoremediation if the results of the greenhouse study warrant larger scale efforts (refer to Section 5 for a discussion of possible additional trials).

A select number of these major taxa were then selected based on their overall tendency to exhibit characteristics which are desirable for the purpose at hand (e.g., rapid growth, good root penetration, good cover potential, known capacity for contaminant uptake, *etc*.). Ultimately, five commercially available plant varieties were selected for the purpose of the greenhouse study, including:

1. Alfalfa (*Medicago sativa*)
2. Bush Bean (*Phaseolus vulgaris*)
3. Canola (short season) (*Brassica rapa*)
4. Wheat (*Triticum aestivum*), and
5. Perennial ryegrass (*Lolium perenne*)

This group encompasses a range of major plant groups and provides for a relatively broad evaluation of the potential capacity to achieve phytoremediation of soils containing the COCs.

2.2.3 Zero-Valent Iron

The addition of zero-valent iron to RDX-contaminated soil has also been demonstrated as a potential remediation strategy (Singh *et al.*, 1998) which could be used in conjunction with phytoremediation to achieve remediation goals. In order to gain an understanding of the potential enhancement of degradation processes within the rhizosphere, zero-valent iron was added to some experimental treatments. In two series of treatments, two separate concentrations of iron were added to the soil surface and not mixed into the soil medium. In two additional treatments, the same amounts of iron were added and thoroughly mixed into the entire volume of soil within each pot. These two means of application were considered to address the possibility that the capacity to mix iron into the soil matrix, as part of a full scale application at any Site, may be severely restricted by Site activities. Treatments receiving iron included planted (surface iron application only) and unplanted treatments.

In the case of unplanted treatments (both surface application and full incorporation of iron) two rates of iron addition were examined. The two rates of iron amendment were 10 grams per litre (g/L) of soil, and 20 g/L. These rates were based in part on a previous study which found effective degradation of RDX in solution following addition of 10 g/L of zero-valent iron (Singh *et al.*, 1998). In treatments where plants were grown in the presence of iron, a set rate of 10 g/L of iron amendment was used, and all iron in these treatments was surface applied. The iron used for this experiment was of type Connelly UW #185, -8/+50.

A control series comprised of zero-valent iron added to clean (uncontaminated) soil was also established to account for any confounding formation of known HMX/RDX degradation products simply as a result of the presence of iron in clean soil rather than as a result of actual COC degradation.

2.2.4 Manure Application

The application of manure as an experimental treatment (both with and without plants) was included in order to consider the possibility of accelerating the production of a reducing

environment in the presence of zero-valent iron amendments. The biological oxygen demand (BOD) associated with the manure could potentially create a reducing environment at the surface of the root zone, as well as serving as a physical barrier to permeation of atmospheric oxygen into the soil. Reducing conditions would favour COC degradation which may occur as a result of the presence of the iron.

A control consisting of clean soil with manure applied at surface (without iron) was also established to correct for the confounding generation of known COC degradation products simply due to the presence of manure in clean soil rather than actual degradation of COCs.

2.2.5 Treatment Combinations

The experimental treatments in the greenhouse study consisted of the following eight (8) combinations of the various levels of the designated variables:

1. contaminated soil with no plants or amendments (control for soil quality)
2. clean soil with plants (control for plant performance):
    - -2a – alfalfa
    - -2b – bush bean
    - -2c – canola
    - -2d –wheat
    - -2e – ryegrass
3. contaminated soil with plants:
    - -3a – alfalfa
    - -3b – bush bean
    - -3c – canola
    - -3d –wheat
    - -3e – ryegrass
4. contaminated soil with manure applied to surface (no iron, no plants)

5. contaminated soil with iron and manure applied:

    -5a – 10 g/L iron applied at surface

    -5b – 10 g/L iron incorporated throughout soil medium

    -5c – 20 g/L iron applied at surface

    -5d - 20 g/L iron incorporated throughout soil medium

6. Contaminated soil with iron (10 g/L, surface application), manure (surface) and plants:

    -6a – alfalfa

    -6b – bush bean

    -6c – canola

    -6d –wheat

    -6e – ryegrass

7. Clean soil with manure applied at surface (control)

8. Clean soil with iron and manure applied (control):

    -8a – 10 g/L iron applied at surface

    -8b – 10 g/L iron incorporated throughout soil medium

    -8c – 20 g/L iron applied at surface

    -8d - 20 g/L iron incorporated throughout soil medium.

Each of the 26 treatments identified above was replicated four (4) times to allow for statistical evaluation of final measurements (three replicates of each treatment) and to accommodate a single interim analytical sampling event mid-way through the course of the study. In total, 104 pots were established in the greenhouse study.

2.3 Plant Propagation

On October 21, approximately three weeks prior to the onset of the greenhouse study, seeds of each of the five plant species were sown in soil-less potting medium placed in seeding trays at BEAK's Laboratory in Brampton, Ontario. Under this approach, established seedlings would be

available for transplanting at the onset of the greenhouse trials, allowing for greater control and consistency with respect to the number and size of plants in each treatment.

At the time of seeding, all cells in each tray were provided with 10 mL of high phosphate soluble fertilizer (Plant-Prod™ Flowering Plant Fertilizer, 15-30-15) to encourage root development of newly germinated seedlings. Seeds were planted three (3) per cell with the exception of bush beans which were planted at two (2) per cell owing to their relatively large seed size. All cells were monitored daily and watered with de-ionized water on an as-needed basis. The majority of seeds of all five species had germinated by Oct 24.

The seedlings were grown under an artificially maintained photoperiod of 16 hours, consistent with the photoperiod to be experienced during the implementation of the greenhouse study. The seedlings were covered under translucent plastic during early growth stages.

2.4 Soil Collection and Preparation

Soil to be used in the greenhouse study was collected from Range 13 at WATC Wainwright (Photo 1, Appendix B) on 28 October 1999. In total, 125 litres of clean soil and 250 litres of contaminated soil were collected to provide sufficient volume for the greenhouse trials. All soil was sieved (0.5 cm) and homogenized on site (Photos 2 and 3, Appendix B) to prepare a relatively uniform stock of the nominal concentration (*i.e.,* 100 mg/kg). Following transport to the greenhouse location, the soil stock was further homogenized using a clean cement mixer.

The control and contaminated soils were qualitatively examined at the time of collection to ensure that they were generally similar with respect to texture and organic matter content. Samples of both soils were also submitted for laboratory analysis prior to the onset of the study. These analyses were completed to identify the presence of potential contaminants of concern (COCs) other than HMX or RDX, as well to assess the similarity of the two soil stocks with respect to texture and other characteristics (pH, organic matter content, nutrient content). The results of these analyses are presented in Tables 2.1 and 2.2, and the analytical report is provided in Appendix A. The two stocks were reasonably similar in texture, but differed notably with respect to a number of

Table 2.1: Greenhouse Study Soil Stocks: Chemistry

Parameter	MDL	Control Samples		Contaminated Samples		
		DND-1	DND-2	DND-3	DND-4	DND-5
Nutrients:						
Phosphate (as P)	0.5	<2.5	<2.5	<2.5	<2.5	<2.5
Nitrate (as N)	1.00	<1.3	<1.3	2.7	1.3	1.6
Nitrite (as N)	1.00	<1.3	<1.3	<1.3	<1.3	<1.3
Boron (soluble)	0.05	0.13	0.12	0.23	0.49	0.4
Metals:						
Antimony	0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Arsenic	2.0	2.8	2.6	2.5	2.5	2.3
Barium	0.50	97	92	100	120	120
Beryllium	0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Cadmium	0.20	0.22	0.2	5	5.4	5.6
Chromium	0.50	8.1	6.7	12	13	12
Cobalt	1.0	5.1	4.9	7	6.8	6.5
Copper	0.50	11	11	1000	860	790
Lead	0.20	5	4.8	85	90	96
Molybdenum	1.0	1.3	<1.0	1.9	3	2.7
Nickel	1.5	15	9.3	21	20	23
Selenium	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Silver	0.20	<0.20	<0.20	2.3	2.2	2.8
Thallium	0.20	<0.20	<0.20	<0.20	0.22	<0.20
Vanadium	2.0	12	11	14	13	13
Zinc	1.5	47	45	100	120	110
Others:						
pH		6.76	6.72	7.41	7.44	7.52
Cation Exchange Capacity (meq/100g)	0.1	18	18	15	17	18
TOC (%)	0.1	3.4	3.3	2	2.1	2.1

All values provided in units of mg/kg unless otherwise specified
MDL = Method Detection limit

Table 2.2: Greenhouse Study Soil Stocks: Physical Attributes

Component	Control Samples		Exposed Samples		
	DND-1	DND-2	DND-3	DND-4	DND-5
coarse gravel (>4.8 mm)	<0.1	<0.1	<0.1	<0.1	<0.1
fine gravel (2.0 - 4.8 mm)	0.5	8.2	1.1	0.9	1.1
very coarse sand (1.0 - 2.0 mm)	1.4	8.6	1.1	0.7	1.2
coarse sand (0.50 - 1.0 mm)	8.4	8.5	12	6.7	9.1
medium coarse sand (0.25 - 0.50 mm)	26	17	13	15	13
fine coarse sand (0.10 - 0.25 mm)	25	21	20	26	21
very fine coarse sand (0.050 - 0.10 mm)	8.8	11	9.2	14	13
silt (0.002 - 0.050 mm)	22	22	28	25	25
clay (<0.002 mm)	8	4	16	12	16

All values presented as percentages

chemical constituents, including numerous metals (Ba, Cd, Cr, Cu, Pb, Ni, Ag, and Zn) and nutrients (B and NO_2), and also pH. In general, all parameters were relatively high in the contaminated soil stock, reflecting the general influence of training activities at the location of soil collection. The implications of the relative quality of soil are discussed in Sections 3.1 and 5.2 of this report.

Samples of the resulting soil stock were also collected and analyzed by BRI to determine the starting concentration of RDX and HMX. The results of this analyses are summarized in Table 2.3. The absence of HMX and RDX from the control soil stock was confirmed. The initial analyses also revealed that there were no measurable quantities of RDX in the contaminated soil stock. The range of HMX in three samples collected from the contaminated soil stock was reported as 28.8 to 50.7 mg/kg (Table 2.3). Extractions for analyses were repeated in triplicate and the replication revealed a high degree of variability. Following the analysis of mid-point samples, the extraction method was refined and the in-sample variation was reduced through the partial dissolution and redistribution of HMX in soil with the addition of acetone. Following this method modification, re-analysis of one of the initial stock samples returned a concentration of 31.8 mg/kg HMX.

At the time of soil collection at Wainwright, a general inventory of major plant groups at the Site was also completed to identify general trends in the presence or absence of plant groups or species relative to areas of elevated concentrations of COCs in the vicinity of targets. This information provides an indication of general Site tolerance by higher plants (with respect to both climatic conditions and contamination) and assists in the interpretation of the greenhouse study and in the design of any future on-Site studies, if such studies are warranted.

Samples of two common non-woody species of naturally occurring vegetation (one monocot and one dicot) were also collected from the same area from which the contaminated soil stock was collected. These plant tissue samples were shipped to BRI and analyzed for HMX/RDX. The analytical results for these naturally occurring plants are discussed in Section 3.2.2.

Table 2.3: HMX in Preliminary Soil Samples

Soil Sample	HMX[a] (mg/kg)	RSD (%)
DND-1	ND	NA
DND-2	ND	NA
DND-3	28.8	29.1
DND-4	50.7	31.4
DND-5	32.3	24.2

[a] Mean of triplicate extraction.
ND - Not detected.
NA - Not applicable.

2.5 Experimental Set-up

All stages of experimental set-up, excluding the addition of manure, were completed on 09 and 10 November 1999.

Soil collected from Wainwright was placed in a sufficient number of pots to complete all designated treatments, including controls. A volume of approximately 3 litres of soil was added to each pot. The density of the contaminated soil was approximately 875 g/L, resulting in mass of approximately 2,600 g of soil per pot.

Pots measured approximately 15 cm in diameter by 20 cm in depth, for a total volume of approximately 3.5 liters. This allowed for a sufficient depth of soil to avoid significant impairment of root development within the anticipated duration of the study. The pots were sealed (*i.e.,* no drainage holes) to avoid the potential for any loss of COCs in solution during the experiment.

Following the addition of soil, pots representing the appropriate control and contaminated treatments were planted with the designated plant type. A set number of plant seedlings, grown in advance of the set-up of the greenhouse study (Photo 4, Appendix B), were transplanted into the standard mass of soil. For beans, three (3) plants were established per pot, while the other four species were planted at a density of nine (9) plants per pot (Photos 5 to 9, Appendix B).

Where required (Treatments 5, 6 and 8), a fixed mass of iron was added to each pot. The iron was mixed into the soil or gently spread over the soil surface (after transplanting), in accordance with the specific treatment. Transplanting was completed prior to the addition of iron in those planted treatments where iron was applied at the soil surface (*i.e.*, Treatment 6). This approach avoided any unintentional incorporation of the iron into the soil medium as a result of transplanting.

In treatments receiving manure, all required transplanting was completed prior to the addition of any manure. Manure was not added until 16 November 1999 (*i.e*., 1 week following transplanting) to avoid physical damage of frail transplants (primarily alfalfa). The application of manure was the final step in establishing the experimental treatments. A fixed volume of composted

cattle manure (*i.e.,* 400 mL) was added to the soil surface and gently spread over the surface to form a layer approximately one (1) cm thick.

Once all pots were completely set up (*i.e.,* planted and treated with iron and/or manure), they were arranged by treatment within the greenhouse following a complete randomized block (CRB) design, as depicted in Figure 2.1 and illustrated in Photo 10 (Appendix B), to reduce environmental bias. The initial application of water and fertilizer was then completed for all pots.

2.6 Maintenance and Monitoring

2.6.1 Greenhouse Maintenance

The greenhouse was thermally regulated so that the maximum daytime temperature did not generally exceed 25 ^{o}C and the nighttime minimum temperature remained equal to or greater than 15 ^{o}C. Temperature and relative humidity were monitored in the greenhouse approximately 3 times per week for the duration of the study. On average, the maximum daily temperature was 25.0 ºC and the average daily minimum was 17.8 ºC. Relative humidity ranged between 48 and 71%, averaging 59.4%. A photoperiod of 16 hrs (alternating with a dark period of 8 hrs) was artificially maintained with flourescent lighting.

All pots received a standardized volume (250 mL) of de-ionized water at the onset of the study. Subsequent watering was completed as required to maintain a suitable level of soil moisture (less than saturation and greater than the wilting point). Plant condition and soil moisture levels were assessed at a maximum interval of three days to determine if watering was required. Soil moisture adequacy was indicated by observations of plant vigour and also through the use of standard soil moisture probes randomly placed in several pots at the onset of the study. When required, water was applied by hand in increments allowing each application of water to permeate the soil surface until soil moisture levels were satisfactory. The watering schedule is provided in Table 2.4. The source of water in the greenhouse study was locally supplied de-ionized water.

Figure 2.1: Complete Randomized Block Layout

Treatment Assignments

5C-2	3E-1	6D-4	1A-4	3D-1
3B-2	7A-3	6D-3	3B-3	2C-2
3A-1	6A-4	3C-4	8D-1	3B-4
3C-1	4A-4	2C-4	2E-2	3C-3
6C-1	5A-3	2E-4	2C-1	6E-2
2D-3	3E-2	6D-2	2D-2	6C-2
2E-3	5D-3	7A-1	6D-1	3B-1
6B-3	6B-1	4A-2	5A-4	8D-3
5B-4	8B-2	3D-2	5D-2	6C-4
6A-1	2D-1	3A-4	2A-3	8A-2
5D-1	5B-1	Monitoring Instruments	6E-1	2B-3
6A-2	2A-1	1A-1	7A-2	2B-4
3C-2	8C-4	2C-3	6A-3	6B-2
5B-3	3A-3	8B-3	3D-3	4A-1
5B-2	8C-1	1A-2	8B-4	8B-1
2A-4	8A-1	2D-4	5D-4	8D-2
5C-3	3E-3	8D-4	8C-2	8C-3
5A-2	8A-3	3E-4	8A-4	3A-2
5C-1	5A-1	6E-4	6C-3	1A-3
2B-2	2A-2	6E-3	5C-4	6B-4
7A-4	4A-3	2E-1	3D-4	2B-1

Nutrient application (in solution) was initially completed following the transplanting. The rate of nutrient application was based on manufacturer recommended rates and was uniform for all treatments, including those without plants. Stocks of fertilizer solution (Plant-Prod^{TM} fertilizer - 15-30-15) were prepared according to manufacturer instructions using de-ionized water. Fertilizer solution was applied to each pot at the manufacturer recommended rate and frequency. The schedule for fertilizer application is also provided in Table 2.4

2.6.2 Monitoring of Plant Health

On a weekly basis, the general condition of all plants was monitored and recorded on standard data sheets. Observations of plant condition (e.g. wilt, chlorosis, leaf drop), and the incidence of disease and insect pests were included in the monitoring. The presence of diseases and pests could potentially have biased the evaluation of plant biomass production and thus required some degree of quantification. In general, the presence of plant diseases and pests was low and these phenomena did not constitute a significant influence on plant performance.

A photographic record of plant growth and health throughout the study was compiled by regularly taking photographs from several fixed points. A selection of these photographs (Photos 11 to 13) is provided in Appendix B.

2.7 Sample Collection

Sampling of soil and/or plant tissues for the purpose of chemical analyses was completed at the onset, the midway point, and at the termination of the greenhouse study. Preliminary soil sampling was completed at the time of study start-up on 09 November 1999. Mid-term sampling of soil and plant tissues was completed on 20 December 1999, and the final sampling event (soil and plant tissues) took place on 24 January 2000.

Samples of the homogenized soil stocks were collected prior to the establishment of experimental treatments. These samples were analyzed for general parameters (TOC, grain size, pH, nutrients, and metals) and also for RDX and HMX. Three (3) samples of each stock were collected as

Table 2.4: Schedule of Water and Fertilizer Applications

Date	Treatments 1,4, 5, 7 and 8 (no plants)	Treatments 2, 3 and 6 (Planted)					
		Alfalfa	Bush Bean	Canola	Wheat	Rye	
9-Nov	-	250	250	250	250	250	*
12-Nov	250	250	250	250	250	250	
15-Nov	-	250	250	250	250	250	
18-Nov	125	125	250	125	125	125	*
20-Nov	-	125	125	125	125	125	
22-Nov	125	125	125	125	125	125	
23-Nov	-	125	250	125	250	250	
24-Nov	125	125	125	125	125	125	
25-Nov	125	125	125	125	125	125	
26-Nov	-	-	125	-	-	-	
27-Nov	-	250	250	250	250	250	
29-Nov	125	125	250	125	125	250	*
1-Dec	125	250	250	250	250	250	
3-Dec	-	125	250	250	250	250	
6-Dec	-	-	250	250	250	250	
8-Dec	-	125	375	250	250	250	
10-Dec	-	-	375	375	250	250	
13-Dec	-	250	375	375	375	375	
15-Dec	250	125	250	250	250	250	
17-Dec	-	125	375	375	250	250	
20-Dec	125	250	250	250	250	250	*
22-Dec	-	125	375	375	250	250	
24-Dec	-	250	500	500	500	500	
27-Dec	-	125	250	375	375	375	
29-Dec	-	125	375	375	375	375	
31-Dec	-	250	375	375	375	375	
3-Jan	250	250	250	375	375	375	
5-Jan	-	125	250	250	250	250	
7-Jan	-	250	250	375	375	375	
10-Jan	-	375	375	375	375	375	
12-Jan	-	250	250	375	375	375	
14-Jan	250	250	250	375	375	375	
17-Jan	-	375	375	375	375	375	
19-Jan	-	375	125	375	375	375	
21-Jan	250	500	125	500	500	500	
Total	**2125**	**6750**	**9250**	**9875**	**9625**	**9750**	

All values in units of ml

* - application includes 125 ml of maintenance fertilizer solution

random grabs (consisting of approximately 400 grams of soil) taken following final homogenization of the stocks at the greenhouse.

Samples of both soil and plant tissues were collected at the midpoint of the study and at the termination of the study. During the interim event, a single replicate of each of the 26 treatments was sacrificed in order to collect representative plant tissue and soil samples. The interim sampling was completed in part to allow for an assessment of temporal trends through comparison with the data from final samples. The interim sampling also allowed for an assessment and refinement of available techniques for HMX/RDX analysis prior to the final sampling event.

At the termination of the study, soil and plant tissue samples for HMX analysis were collected as representative samples for each of the remaining replicates of each treatment (*i.e.,* 3 samples per treatment).

Plant tissue samples were collected to represent both above-ground (shoots and leaves) and below-ground tissues (roots). For all samples, above-ground plant tissues were collected by cutting all plant stems in each pot at the soil surface. No attempt was made to separate specific above-ground tissue types (*i.e*., leaves, stems, flowers, and seeds), with the exception of bean seed pods which were collected as separate samples for each treatment in addition to the composite samples of the remaining above-ground bean plant tissues. Below-ground plant material was collected by fragmenting the soil into a sieve and extracting all visible root tissues. Upon collection, all plant tissue samples were rinsed with de-ionized water, air dried, weighed (using an electronic balance) and subsequently placed in labeled containers (plastic freezer bags) and kept frozen until submitted for chemical analysis. Sub-samples of plant tissues were also collected for the determination of dry-weight fractions. These samples were weighed fresh, and then oven dried at 65 degrees C for 72 hours for dry weight determination.

Soil samples were collected concurrent to plant tissue samples. As plant samples were collected from each replicate of planted treatments (Treatments 2, 3 and 6), the soil was fragmented and placed in a larger container and homogenized. In treatments which received manure (Treatments 4, 5 and 6) the manure was carefully collected from the soil surface prior to collecting the soil itself.

The entire volume of manure from each treatment replicate was collected in 500-mL plastic jars as the representative sample. Manure samples typically weighed approximately 40 to 50 grams. Plant root material was removed as the soil was collected from each pot, even if the root material was not required for sampling purposes. After the soil from each pot was homogenized, individual soil samples for analyses of RDX/HMX were collected to represent each pot. Each sample consisted of approximately 400 grams of soil. All soil samples were placed in pre-labeled 500-mL plastic jars and kept cool until analyzed. All instruments and containers used for sampling and homogenization were rinsed with de-ionized water between treatments. Control samples (*i.e.*, clean soil) were collected first to further reduce the potential for cross-contamination.

2.8 Analytical Procedures

Analyses completed for the purpose of characterizing soil stocks (both control and contaminated) at the onset of the study included pH, TOC, grain size analysis, macro-nutrients (N, P, K), and micro-nutrients (Cu, B, Zn, *etc*.). These soil samples were also subjected to complete metal scans, completed using ICP-MS, to determine if metal contaminants were present at concentrations which may have phytotoxic implications. Philip Analytical Services Corporation (PASC), certified by the Canadian Association of Environmental Analytical Laboratories (CAEAL), was responsible for the analytical chemistry of the preliminary soil samples. The analytical report from PASC is provided in Appendix A.

Analytes for all soil and plant samples collected during the greenhouse study initially included both RDX and HMX as well as degradation products of RDX and HMX, including DNX, TNX, methanol, formaldehyde, hydrazine, and 1,1-dimethylhydrazine. Analyses for HMX and RDX and their degradation products in both soil and plant tissue samples were completed by BRI. The analytical results for the soil stock samples collected at study onset revealed that there were no detectable concentrations of RDX in the contaminated soil collected at WATC Wainwright. For this reason, subsequent analyses of soil as well as plant tissue samples was focussed solely on HMX and its degradation products.

The procedure used for both soil and plant tissues was based on the US EPA Method 8330. While the determination of the explosive content in soil is a widely recognized standard protocol (EPA 8330), no recognized standard method currently exists for the analysis of explosives and their degradation products in plant tissues. Most of the protocols in current use for plant tissue analyses are variations of EPA 8330 constructed to minimize additional equipment requirements. The selected protocol for plant tissue (Larson et al, 1998) serves well for the comparison of HMX content with soil samples, but the various steps (homogenization, lyophilization, silica chromatographic cleanup) were performed in an iterative manner to allow for method development and to improve general efficiency and the recovery of volatile cyclic nitramine degradation products.

The complete details of analytical procedures have been documented, along with the detailed analytical results, in the report of Groom et al., 2000. The sample preparation, extraction and analytical methods are described below, including any modifications of the EPA 8330 method. The results of analyses completed at BRI are summarized in the main body of this report, and presented in detail in Appendix A.

2.8.1 Sample Preparation

Preliminary and mid-point soil samples were lyophilized (in place of air drying to constant weight) for the initial removal of moisture. The samples were then passed through a 32 mesh sieve before extraction. In the case of final samples, the entire sample (400 g) was spread in a Pyrex dish, mixed with acetone to a paste, and then air dried for 24 to 48 hours before sieving to reduce the spatial heterogeneity of crystalline HMX in the soil.

The primary step in the preparation of plant tissue samples was the measurement of total sample weight. After weighing, approximately 5 g of material was selected from each sample with care taken to provide equal amounts for each tissue present (*i.e.*, stems, leaves, fine roots, coarse roots, *etc*). The sample was then finely cut into 2 mm pieces and the cut weight was recorded as the sample fresh weight using an analytical balance. Samples were stored on ice for immediate homogenization. The finely cut samples (approximately 4 g) were then suspended in 10-20 mL of

ice cold deionized water (18 M? cm resistivity) and homogenized using a Kinematica (Kriens Switzerland) Homogenizer fitted with a Brinkman Polytron PTA 20 S saw tooth generator (Brinkman Instruments, Mississauga ON) suitable for fibrous plant or animal tissues. Homogenization was initially performed at 5,000 RPM for 2 minute intervals with immersion of the beaker into ice. The samples were then homogenized in the same manner at full setting (20,000 RPM) until a frothy granular paste was obtained.

Immediately after homogenization, the samples were transferred to 120-mL Labconco lyophilization flasks and the sealed flask assemblies were immersed in a dry ice acetone bath for 20 minutes. The flasks were then connected to either a Flexi Dry FDX-1-84ACD (Flexi Dry Inc, Stone Ridge NY) or a Virtis Freezmobile 24 (Virtis, Gardner NY) lyophilizer. The samples were lyophilized until no further change in flask weight was observed (average time 20 h). Lyophilized samples were transferred to polypropylene vials and the lyophilized weights were recorded. The freeze dried samples were stored under aluminum foil at 4 ^{0}C.

2.8.2 Extraction

Preliminary soil samples were extracted with the addition of 2 g of sieved soil to 10 mL acetonitrile in an ultrasonic bath for 16 hours. For midpoint soil samples, 4 g of soil were added to 10 mL of acetonitrile to account for soil heterogeneity. Final samples were extracted using 16 g of soil added to 40 mL of acetonitrile in 60-mL bottles. The use of such large volumes reduced the sample capacity of the sonicator and a time study revealed that 6 hours sonication was sufficient to extract all available HMX from the samples of Wainwright soil (Groom *et al*., 2000). Final soil samples were accordingly sonicated either for 16 hours overnight, or for 6 hours during the day to increase sample through-put. Preliminary samples were extracted in triplicate. Mid-point samples were extracted singularly except in the case of Treatments 1 and 4 which were extracted in triplicate. Final samples were normally extracted in duplicate. When the HMX concentration deviation exceeded 15 % of the mean for a given set of duplicates, a third extraction was completed.

For plant tissue samples, approximately 0.2 g of freeze dried material was transferred to a 15-mL Kimax screw cap culture tube with the subsequent addition of 10 mL of acetonitrile. The capped

tubes were then vortex mixed and placed in a Blackstone Ultrasonics Neptune Ultrasonic Generator (Blackstone Ultrasonics, Jamestown NY) cooled to 10 ^{0}C using a Lauda RM6 refrigerated circulating bath (Brinkman instruments, Mississauga ON). The sonication duration was 18 hours. After sonication, the Kimax tubes were centrifuged at 5,000 RPM for 15 minutes using a Fisher Centrific benchtop centrifuge (Fisher Scientific, Montreal QC). The supernatant was then decanted and allowed to settle for 20 minutes. A 2-mL aliquote of the supernatant was then mixed with an equal volume of deionized water and filtered using Millex HV 0.45 µm filter cartridges. The samples were then immediately analyzed (HPLC-UV).

2.8.3 Analyses

HPLC-UV analysis was completed using a Waters chromatographic system composed of a Model 600 pump, a Model 717 Plus injector, a Model 996 Photodiode-Array Detector and a Temperature Control Module. The column was a Supelcosil LC-CN (25 cm, 4.6 mm, 5 µm) with the column temperature held at 35 ^{0}C. The solvent system consisted of a methanol/water gradient at a flow rate of 1.5 mL/min. The initial solvent composition was 30% methanol and 70% water, which was held for 8 minutes. A linear gradient was then run from 30 % to 65 % methanol over 12 minutes. The solvent ratio was then returned to initial conditions over 5 min and then maintained for 5 min for a total run time of 30 minutes. The detector was set to scan from 200 to 325 nm with extraction of chromatograms at 254 nm. The injection volume was 50 µl. In general, this method has proven to be superior to that of EPA 8330 (C18 column with an isocratic 50 % water / methanol mobile phase) for the reduction of interference. The limits of detection and quantification for HMX with this method were respectively 0.05 mg/kg and 0.1 mg/kg.

For LC-MS analysis, a Micromass Plattform benchtop single quadrupole mass detector fronted by a Hewlett Packard 1100 Series HPLC system equipped with a Photodiode-Array detector was used. Samples (50 µl) from the extract were injected into a Supelcosil LC-CN column (25 cm, 4.6 mm, 5 µm) thermostated at 35 ^{0}C. Two different methanol/water gradients were used at a flow rate of 1 mL/min. For the first HPLC method applied, initially, a linear gradient was run from 10 % to 20 % over 15 min, followed by a second linear gradient from 20% to 60 % over 5 min

which was then held for 3 min. This solvent ratio was returned to the initial conditions over 2 min and held for an extra 10 min. For the second method, the initial solvent composition was 40 % methanol and 60 % water held for 8 min, then a linear gradient was run from 40 % to 65 % methanol over 12 min. This solvent ratio was changed to the initial conditions over 5 min and held for an extra 10 min. Analyte ionization was done in a negative electrospray ionization mode (ES -) producing [M-H] mass ions. The electrospray probe tip potential was set at 3.5 kV with a cone voltage of 30 V at an ion source temperature of 150 ^{0}C. The mass range was scanned from 25 to 400 Da with a cycle time of 1.6 s and the resolution was set to 1 Da (width at half height). The limit of detection for this method was less than 4 ppb.

All analytical data were verified through instrumental calibration curves, blank runs, reproducibility and accuracy checks. Recoveries were verified by spiking non-contaminated soil samples with HMX and extracting them under the same conditions as for the contaminated soils.

2.9 Germination Trials

Additional trials to examine any limitations on seed germination as associated with HMX-bearing site soil were initiated on November 20. These trials were completed in order to evaluate the effect of soil-borne contaminants on the germination and early growth of seedlings of the same plant species included in the main study (*i.e.,* alfalfa, bush bean, canola, wheat and rye). The basic approach of these germination trials was to plant both contaminated and uncontaminated soil with seeds of the five plant types of concern, and to record the rate and extent of germination and the extent of early growth. In addition, separate treatments were established to evaluate the effects of bacterial inoculation of seeds. For this purpose, seeding of contaminated and clean soils was repeated using inoculated seeds. The seed inoculant was provided by Bernard R. Glick of the University of Waterloo. The application of the bacterial inoculant was completed at the University of Waterloo, and the germination trials themselves were completed at the CCIW greenhouse facility, where the main experiment was in progress.

The soils used for the germination trials, both clean and contaminated, were those obtained from WATC Wainwright. Consistent volumes of soil (clean or contaminated) were placed in individual cells within seeding trays. In total, four trays containing 40 cells each were set up (Photos 14 and 15, Appendix B) in the following manner:

- Tray 1: clean soil with untreated seed,
- Tray 2: clean soil with treated seed,
- Tray 3: contaminated soil with untreated seed, and
- Tray 4: contaminated soil with treated seed.

All seeds, both the seeds to be inoculated and those serving as controls, were initially surface sterilized by soaking for 10 minutes in 1.5 % sodium hypochlorite solution. Following this procedure, all seeds were successively rinsed 5 times with sterilized distilled water. Following the final rinse, and prior to planting, seeds designated for inoculation were incubated for 1 hour in a suspension of bacteria in 0.85% NaCl solution, prepared at the University of Waterloo. Simultaneously, control seeds were incubated for 1 hour at room temperature in 0.85% NaCl solution alone. Seeds were planted immediately following incubation in either of two soil preparations.

In each tray, each seed type (alfalfa, bush bean, canola, wheat and rye) was sewn in a consistent manner into a total of 8 cells per tray. Beans were planted two seeds per cell, while all other plants, having substantially smaller seeds, were planted at four seeds per cell.

All cells were initially provided with a fixed volume of fertilizer solution (in de-ionized water). Cells received uniform volumes of de-ionized water as required throughout the duration of the trials. Cells were routinely monitored to record the number of seeds which had germinated at any given time.

All cells remained covered under a translucent plastic enclosure for the first 12 days of the study. The plastic enclosure was removed on day 13 to avoid any physical interference of plant growth.

Seedlings were maintained for a period of approximately 3 weeks following the initial planting, at which point (*i.e.,* 09 December 1999) the plants from each cell were harvested for the purpose of measuring shoot length and plant tissue weights (dry weight). Plants were oven dried at 65 ^{0}C for 72 hours for dry weight determination.

2.10 Data Management and Statistical Analysis

All greenhouse data were transcribed from field sheets into an electronic spreadsheet (*Microsoft® Excel® 7.0*). After the data were subjected to QA/QC procedures and screened for outliers, basic descriptive statistics were performed including means, standard deviations and sample sizes. Subsequent statistical procedures included univariate analysis of variance (ANOVA) and Scheffes multiple means tests. Statistical analyses were performed using SPSS for Windows (Version 8).

3.0 STUDY RESULTS

3.1 Plant Growth

One of the keys to the success of effective remediation of contaminated media by plants is the survival and growth of those plants in those media. Previous studies of plant survival and performance as related to explosive contaminants have revealed contrasting results to the current study. For example, severe effects of TNT on plant performance (especially root growth) were documented by Palazzo and Leggett (1986). Similarly, TNT contamination of soil was found to significantly reduce the growth of grass species (Krishnan *et al*., 2000). This highlights the importance of conducting evaluations on a site-specific basis to account for the potential presence of other inhibiting COCs. The potential application of phytoremediation to military sites may be limited if certain contaminants are present. In this specific case, as discussed in the sections below, the presence of HMX is not indicated as being limiting through any phytotoxic effects. This permits the establishment of plants at the site in question – a necessary first step in effective remediation.

3.1.1 Germination Trials

Germination trials showed no significant inhibition of germination of seeds of all 5 test species in contaminated site soil (compared to clean site soil), with germination rates exceeding 85% for all species (except beans, which exhibited relatively low germination success ranging from 38 to 69%) and showing no clear trends relative to treatment (Table 3.1).

The germination trials do suggest possible impairment of early seedling growth in contaminated site soil. Both shoot length and shoot weight tended to be lower for those seedlings grown in the contaminated soil in comparison to their counterparts in clean soil (Tables 3.2 and 3.3). The reasons for this apparent inhibition are not clear, and it is not necessarily related to the presence of HMX. Toxicological benchmark values for HMX have not been adequately defined for plant life at this point in time (Maxwell and Opresko, 1996), precluding a comparative evaluation of soil HMX concentrations in context of potential growth-inhibiting phytotoxic effects. Initial characterization of site soils (refer to Table 2.1) revealed that the concentrations of several metals

Table 3.1: Germination Trial - Percent Germination

Treatment	Alfalfa	Beans	Canola	Wheat	Rye
1: Clean Soil, Un-treated Seed	94%	63%	97%	97%	100%
2: Clean Soil, Treated Seed	100%	38%	100%	97%	100%
3: Contaminated Soil, Un-treated Seed	97%	50%	97%	88%	88%
4: Contaminated Soil, Treated Seed	94%	69%	88%	100%	97%

All values are treatment averages

Table 3.2: Germination Trial Harvest Measures - Shoot Length

Treatment	Alfalfa	Beans	Canola	Wheat	Rye
1: Clean Soil, Un-treated Seed	7.0	21.8	9.1	23.2	26.6
2: Clean Soil, Treated Seed	6.9	20.2	8.2	23.2	24.9
3: Contaminated Soil, Un-treated Seed	5.3	17.4	5.5	20.4	20.8
4: Contaminated Soil, Treated Seed	4.7	17.7	6.1	20.0	19.6

All values are treatment averages, presented in units of cm

Table 3.3: Germination Trial Harvest Measures - Shoot Weight

Treatment	Alfalfa	Beans	Canola	Wheat	Rye
1: Clean Soil, Un-treated Seed	0.005	0.233	0.012	0.020	0.019
2: Clean Soil, Treated Seed	0.005	0.212	0.011	0.018	0.016
3: Contaminated Soil, Un-treated Seed	0.005	0.212	0.007	0.017	0.012
4: Contaminated Soil, Treated Seed	0.003	0.198	0.006	0.016	0.012

All values are treatment averages, presented in units of g (dry weight)

were also elevated in the contaminated soil. Several metals (Cd, Cu, Pb and Zn) were present at concentrations which exceed lowest reported phytotoxic thresholds (CCME, 1997). It is possible that these elements contributed to the observed inhibition of seedling growth. It is also possible that soil texture and water holding capacity of the two soil types may have had some influence, as qualitative observations during the germination trials indicate some degree of difference with respect to these soil characteristics. The analysis of soil texture (refer to Table 2.2) did reveal a slightly higher clay and silt content of contaminated soil stocks which could effect soil binding and water holding capacity.

Recent studies have indicated that plant growth-promoting rhizobacteria (PGPR) can improve the performance of seedlings under poor conditions, including the presence of elevated levels of potentially toxic contaminants in the soil matrix (Burd *et al.*, 1998; Glick *et al.*, 1998). The results of the current study do not provide evidence to suggest that such bacteria have beneficial effects regarding the germination and early growth of the plant species in question when grown in contaminated soil obtained from WATC Wainwright.

3.1.2 Main Trials

Plants were transplanted as young seedlings for the purpose of the main trials, so issues of germination and early seedling growth and survival cannot be directly considered. All transplanted seedlings did survive in both clean and contaminated soil.

The primary measure of growth in the main trials was above-ground plant tissue biomass (dry weight) recorded during the interim sampling event and during the final sampling event. Table 3.4 presents the treatment averages of above-ground biomass of all five plant species.

There were clear differences in plant growth (*i.e.,* biomass production) among the five species, with alfalfa producing the lowest above-ground biomass, and canola producing the greatest biomass among all Treatments (2, 3 and 6). It is important to note that this relative indication of growth is relevant only to the time-period encompassed in the study (*i.e.,* 11 weeks). Over this period, beans and canola reached maturity while alfalfa and the grasses were still in early or middle stages

Table 3.4: Greenhouse Trials - Plant Biomass[1] Production

Plant Species	**Treatment 2**: Clean Soil	**Treatment 3**: Contaminated Soil	**Treatment 6**: Contaminated Soil with Iron and Manure
Alfalfa	9.95	6.62	7.97
Bean:			
- leaves and stems	6.21	13.84	12.62
- seed pods[2]	48.40	38.10	47.40
Canola	24.69	17.85	25.59
Wheat	14.98	15.66	14.07
Rye	16.51	14.85	15.42

All values are treatment averages

1 - presented in units of g (dry weight), unless specified otherwise

2 - presented in units of g (fresh weight)

of growth. Over a protracted period, these three species would produce substantially greater biomass than reported for this study.

Bean and canola plants all flowered and produced seed pods at approximately equivalent rates for each plant type, regardless of the treatment. This observation supports the conclusion that there was no significant inhibition of the growth or reproductive physiology of these plants as a result of being grown in contaminated Site soil.

The addition of iron and manure to contaminated soil (Treatment 6) did appear to slightly improve the yield of canola, with an average biomass production of 25.6 g (dry weight) in Treatment 6 compared to 17.9 g in Treatment 3. This observation is possibly a result of additional nutrients associated with the manure or iron itself. Overall, however, there were no clear and consistent trends in growth (biomass production) when comparing the three planted treatments, suggesting that the contaminated soil had no consistent inhibitory effects on plant growth.

3.2 Fate of HMX

3.2.1 Soil and Manure Partitioning

Manure:

In those treatments in which manure was applied to the soil surface (*i.e.,* Treatments 4, 5, and 6), the concentrations of HMX in that manure (2.0 to 16.8 mg/kg) were relatively low compared to soil concentrations (28.3 to 38.7 mg/kg in the final sampling event)(Table 3.5).

Concentrations of HMX in manure were fairly consistent within treatments, but differed between treatments (*i.e.,* ranging from 2.0 to 5.0 mg/kg in Treatment 6, and 13.6 to 16.8 mg/kg in Treatment 5). This difference may reflect the fact that Treatment 6 (planted) received regular watering while Treatment 5 (not planted) was watered less frequently (refer to watering schedule in Table 2.4). The application of water likely leached some portion of the HMX down from the soil/manure interface, and thus more frequent watering would reduce the level of HMX in manure originating at this interface.

Table 3.5: HMX Concentration in Soil and Manure Samples

Treatment ID	Treatment Description	Soil				Manure	
		Interim Sampling		Final Sampling			
		Mean	Std. Dev	Mean	Std. Dev.	Mean	Std. Dev.
1	Contaminated soil - no amendments or plants	22.4	NA	35.0	4.3	NA	NA
2A	Clean soil with alfalfa	ND	NA	trace	NA	NA	NA
2B	Clean soil with beans	ND	NA	trace	NA	NA	NA
2C	Clean soil with Canola	ND	NA	0.5	NA	NA	NA
2D	Clean Soil with wheat	ND	NA	trace	NA	NA	NA
2E	Clean soil with rye	ND	NA	trace	NA	NA	NA
3A	Contaminated soil with alfalfa	30.2	NA	33.8	2.6	NA	NA
3B	Contaminated soil with beans	55.6	NA	32.7	1.8	NA	NA
3C	Contaminated soil with Canola	47.8	NA	34.8	1.4	NA	NA
3D	Contaminated Soil with wheat	51.2	NA	34.2	1.3	NA	NA
3E	Contaminated soil with rye	32.8	NA	34.1	1.1	NA	NA
4	Contaminated soil with manure	39.1	9.1	30.9	2.6	4.3	0.2
5-A	Contaminated soil with manure and iron (10 mg/kg at surface)	23.7	11.6	30.9	1.3	16.8	3.2
5-B	Contaminated soil with manure and iron (10 mg/kg at incorporated)	37.0	12.3	28.3	0.8	15.9	2.2
5-C	Contaminated soil with manure and iron (20 mg/kg at surface)	32.1	5.1	35.3	2.6	13.6	0.9
5-D	Contaminated soil with manure and iron (20 mg/kg at incorporated)	25.4	7.2	30.2	2.4	16.5	6
6A	Contaminated soil with manure, iron and alfalfa	31.0	1.3	33.0	2.6	2.0	0.1
6B	Contaminated soil with manure, iron and beans	19.0	10.9	28.3	1.3	3.2	0.1
6C	Contaminated soil with manure, iron and Canola	27.5	4.7	38.7	0.8	4.7	0.1
6D	Contaminated Soil with manure, iron and wheat	87.4	6.1	32.4	2.6	4.6	0.3
6E	Contaminated soil with manure, iron and rye	31.9	2.3	38.7	2.4	5.0	1.5
7	Clean soil with manure	ND	ND	ND	ND	NA	NA
8-A	Clean soil with manure and iron (10 mg/kg at surface)	ND	ND	ND	ND	NA	NA
8-B	Clean soil with manure and iron (10 mg/kg at incorporated)	ND	ND	ND	ND	NA	NA
8-C	Clean soil with manure and iron (20 mg/kg at surface)	0.3	ND	ND	ND	NA	NA
8-D	Clean soil with manure and iron (20 mg/kg at incorporated)	2.3	ND	ND	ND	NA	NA

All values in units of mg/kg
ND Not dectected.
NA Not applicable

The presence of HMX in manure was likely an artifact of physical mixing at the interface of the manure and soil surface. In the collection of the manure samples, the complete removal of all adherent soil was not possible. It is important to note that the mass of manure in each pot was very small (*i.e.,* approximately 50 grams or less – dry weight) relative to the mass of soil, and thus the total mass of HMX in manure is also very small and insignificant in context of the HMX mass contained in soil.

Soil:

Concentrations of HMX measured in soil samples collected during the interim sampling event were highly variable both within and between treatments (Table 3.5). Reported concentrations ranged from 19 to 87.4 mg/kg with no distinct trends with respect to treatment.

As noted in Section 2.7.2, the initial protocol used for the analysis of soil samples for HMX was not perfectly suitable to account for the inherent heterogeneity of Site soil, and thus tended to produce results with a relatively high level of variability. The method was refined prior to final sampling to reduce this variability. The soil concentrations reported for the samples collected at study termination were within a much narrower range (28.3 to 38.7 mg/kg). Despite the resolution of issues of variability, the results of final soil analysis still do not exhibit any clear trends with respect to treatment effects on soil concentrations of HMX. The concentrations reported for the soils treated with plants, iron, and/or manure (Treatments 3, 4, 5 and 6) did not differ significantly from each other or from the concentrations in Treatment 1 (contaminated soil with no plants or amendments). These results do not suggest any positive effect of the various treatments on soil quality. However, this finding is likely confounded by a natural degree of variability in soil HMX concentrations which masks any marginal effects of treatments. A review of the data describing plant uptake does indicate beneficial effects of plants on soil quality (refer to Section 4.1).

3.2.2 Plant Tissue Uptake

Interim Sampling:

Plant tissue samples collected at the mid-point sampling event (*i.e.,* following only 6 weeks of exposure) were found to contain concentrations of HMX ranging from 60.7 to 292.5 mg/kg (dry weight) (Table 3.6). This represents substantial bioaccumulation of HMX from soil to plant tissues in a relatively short period of time. Soil-to-plant transfer factors (B_v), (calculated as the dry weight concentration in plant tissues divided by the concentration in soil), ranged as high as approximately 10 (assuming a starting concentration of 30 mg/kg in soil).

The analytical data for interim plant samples revealed no clear and consistent trends with respect to plant type or the addition of amendments (iron and manure). However, there were distinct differences in comparison of different plant tissue types (e.g. leaves vs. roots), with the concentration of HMX in roots being significantly lower than the concentrations in above-ground tissues of all plant types in both Treatments 3 and 6.

Final Sampling:

As in the interim sampling event, tissues of all plants sampled at study termination were found to contain significant concentrations of HMX, ranging from 61.5 to 713 mg/kg (dry weight) depending on plant species and the specific condition of tissues at the time of sampling (*i.e.,* fresh vs. senescent) (Table 3.7). Soil-to-plant transfer factors (B_v) calculated from the final analytical data ranged from approximately 2 to 20.

In contrast to the interim sampling event, there were several identifiable trends with respect to HMX in plant tissues. In general, grasses (rye and wheat) tended to have higher tissue concentrations of HMX than dicots (alfalfa, beans, and canola) with the lowest concentrations detected in beans (exclusive seed pods). The analysis of bush bean seed pods, which comprised a significant portion of the total bean plant biomass, revealed a complete absence of HMX in all

Table 3.6: Concentration of HMX in Plant Tissue Samples - Interim Sampling

Treatment ID	Treatment Description	Shoots (dry weight)		Shoots (fresh weight)		Roots	
		Mean	Std. Dev	Mean	Std. Dev	Mean (dry weight)	Mean (fresh weight)
2A-4	Clean soil with alfalfa	ND	NA	ND	NA	ND	ND
2B-4	Clean soil with beans	ND	NA	ND	NA	ND	ND
2C-4	Clean soil with Canola	ND	NA	ND	NA	ND	ND
2D-4	Clean Soil with wheat	ND	NA	ND	NA	ND	ND
2E-4	Clean soil with rye	ND	NA	ND	NA	ND	ND
3A-4	Contaminated soil with alfalfa	121	3	18.6	0.4	NA	NA
3B-4	Contaminated soil with beans	164.5	0.5	23.8	0	NA	NA
3C-4	Contaminated soil with Canola	197	NA	20.7	NA	16.4	3.15
3D-4	Contaminated Soil with wheat	60.7	10.2	16.2	2.7	18.3	4.53
3E-4	Contaminated soil with rye	171	2	50.6	0.6	44.9	14.3
6A-4	Contaminated soil with manure, iron and alfalfa	75.7	1.2	18	0.3	NA	NA
6B-4	Contaminated soil with manure, iron and beans	115.5	1.5	21.8	0.2	15.8	10.6
6C-4	Contaminated soil with manure, iron and Canola	282.5	17.5	51	1	8.19	1.01
6D-4	Contaminated Soil with manure, iron and wheat	292.5	0.5	61.1	0.2	13.4	1.69
6E-4	Contaminated soil with manure, iron and rye	67.4	0.7	17.4	0.2	13.2	3.21

All values are treatment averages, presented in units of mg/kg
ND Not dectected.
NA Not applicable - sample size insufficient

Table 3.7: Concentration of HMX in Plant Tissue Samples - Final Sampling Event.

Treatment ID	Treatment Description	Fresh Shoots		Senescent Shoots		Roots	
		Mean (Dry weight)	Mean (fresh weight)	Mean (Dry weight)	Mean (fresh weight)	Mean (dry weight)	Mean (fresh weight)
2A-4	Clean soil with alfalfa	ND	ND	ND	ND	ND	ND
2B-4	Clean soil with beans	ND	ND	ND	ND	ND	ND
2C-4	Clean soil with Canola	ND	ND	ND	ND	ND	ND
2D-4	Clean Soil with wheat	ND	ND	ND	ND	ND	ND
2E-4	Clean soil with rye	ND	ND	ND	ND	ND	ND
3A-4	Contaminated soil with alfalfa	215	46	NA	NA	NA	NA
3B-4	Contaminated soil with beans	61.5	14.4	204.8	111.1	18.4	6.0
3C-4	Contaminated soil with Canola	100	22	677	233	35	7
3D-4	Contaminated Soil with wheat	668	220	378	317	14	7
3E-4	Contaminated soil with rye	485	123	624	418	16	5
6A-4	Contaminated soil with manure, iron and alfalfa	298	82	n.a	NA	NA	NA
6B-4	Contaminated soil with manure, iron and beans	42	9	180	52	19	4
6C-4	Contaminated soil with manure, iron and Canola	168	48	713	536	19	12
6D-4	Contaminated Soil with manure, iron and wheat	418	118	428	299	36	9
6E-4	Contaminated soil with manure, iron and rye	261	71	259	174	16	5

All values are treatment averages, presented in units of mg/kg
ND - Not dectected.
NA - Not applicable - sample not available in sufficient quantitiy for reliable analysis

treatments, whereas other tissues (roots, stems and leaves) did contain HMX. The underlying causes of this finding and its implications with respect to phytoremediation are unclear at this time.

The observed differences between plant species with respect to HMX uptake were largely consistent across the two treatments in which plants were exposed to contaminated soil (*i.e.,* Treatment 3 and Treatment 6). However, this is in contrast to the findings regarding HMX in samples of tissues of naturally occurring plants at Wainwright. The analyses of the plant samples collected during the initial Site visit reveal higher concentrations of HMX in the above-ground tissues of dicot plants (43.1 to 51.0 mg/kg dry weight) compared to grasses (7.7 to 10.0 mg/kg dry weight).

The results of plant tissue analysis (both interim and final) indicate that HMX taken up by plants is largely translocated to above-ground tissues. The concentration of HMX in the roots of any given plant in any of the contaminated treatments was typically a factor of 10 or more lower than the corresponding concentration of HMX in tissue samples comprised of stems and leaves. This is consistent with the known fate of structurally similar RDX in plant tissues. For example, hybrid poplars grown hydroponically exhibited significant bioaccumulation of RDX, with the majority of the contaminant translocated to leaf tissues (Thompson *et al.*, 1999). The levels of RDX accumulation reported in that study (ranging from 354 to 723 mg/kg (dry) in leaves) were similar to those observed for HMX in this study (ranging as high as 713 mg/kg). RDX has also been reported to accumulate preferentially in leaf tissues of bush beans (Harvey *et al.*, 1991) as was the case for HMX in bush bean tissues in this study. The translocation of HMX to above-ground tissues differs markedly from the fate of TNT, which has been shown to remain primarily in the root tissues of plants grown in contaminated soil or water (Palazzo and Leggett, 1986; Thompson *et al.*, 1998).

The capacity of plants to uptake COCs is partly dependent on the octonal – water partitioning coefficient (K_{ow}) of the COC. Moderately hydrophobic COCs (log K_{ow} = 0.5 to 3) are most easily taken up, while compounds with log K_{ow} of less than 0.5 are reported to undergo poor sorbtion to roots and poor active transport across cell membranes (Schnoor *et al.*, 1995). The

K_{ow} of HMX is relatively low (log K_{ow} = 0.06 to 0.26, Maxwell and Opresko, 1996; Spanggord *et al*., 1983) which has been interpreted to be indicative of a low potential for bioaccumulation. However, in contradiction to this expectation, the results of the greenhouse study clearly indicate that HMX is readily accumulated in plant tissues.

The concentrations of HMX in plant tissues recorded during this study (*i.e*., 60.7 to 713 mg/kg on a dry weight basis) were consistently higher than the concentrations detected in the plant tissue samples collected on-site at WTC Wainwright (i.e., 7.7 to 51.0 mg/kg dry weight). Since the uptake of HMX is in part dependent on the degree to which it enters solution, it is likely that the very dry conditions at Wainwright are an underlying factor in the low rates of *in situ* HMX uptake in comparison to the regularly watered potted plants in the greenhouse study.

3.2.3 Degradation

The degradation of HMX may result in the formation of any of several known degradation products. The analysis of both soil and plant tissues completed as part of this project included screening for the presence of the key HMX degradation products.

In the case of TNT, the covalent linking of aminonitrotoluene metabolites to complex glycols (starches) in root tissue is known to occur and the hydrolysis of these samples in hot acid is recommended to free bound analyte. This treatment can be applied to HMX or RDX, but the formation of stable amino RDX or HMX derivatives has not been reported in the literature. No amino derivatives were observed in the LC-MS analyses of soil and plant samples. Small quantities of 1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazacylooctane (mononitroso-HMX) were observed in all of the HMX contaminated soil samples, as detected by HPLC and periodically verified by LC-MS. In addition, minute quantities (< 1 mg/kg) of TNT and tetryl were infrequently observed in the contaminated soil samples. This observation is consistent with the explosive formulations employed on the anti-tank range.

In general, HMX biodegradation is reported to proceed most rapidly under aerobic conditions (Maxwell and Opresko, 1996), but overall HMX undergoes relatively slow biodegradation

(Spanggord *et al.*, 1982). In this study, the addition of zero-valent iron and the presence of plants were two factors which may have resulted in accelerated degradation of HMX.

Effects of Plants:

As mentioned in Section 1.1, one of the potentially beneficial effects of plants is either the enhanced degradation of contaminants in the rhizosphere or the direct degradation of contaminants within plant tissues (*i.e.,* phytodegradation). This general phenomenon has been examined with respect to other explosive contaminants. For example, TNT is reported to readily bio-transform in plant tissues (Thompson *et al.*, 1998) and several species of both aquatic and terrestrial plants have been found to have an intrinsic ability to transform TNT in ambient media (Hughes *et al.*, 1997; Palazzo and Leggett, 1986). However, the general environmental behaviour of TNT has been found to differ from that of HMX in a number of ways, and similar effects with respect to phytodegradation are not necessarily expected. On the other hand, HMX bears a greater structural similarity to RDX, and an expectation of similar trends in environmental fate is not unreasonable. With respect to RDX, the available data with respect to plant-mediated degradation are mixed. Best *et al.* (1997) found that RDX was bio-transformed by wetland plant species under hydroponic conditions, as was the case for RDX in tissues of hydroponically grown bush bean (Harvey *et al.*, 1991). In contrast to this, no significant transformation of RDX occurred in hybrid poplar tissues following hydroponic exposure to RDX (Thompson *et al*., 1999). In the current greenhouse study, soil or plant tissue samples were frequently found to contain small quantities of mononitroso-HMX, regardless of treatment. However, there were no significant quantities of HMX degradation products detected exclusively in soil or in plant tissues from planted treatments. Based on this finding, the greenhouse study indicates that HMX does not undergo any appreciable degradation in the root zone of the soil or in plant tissues.

Effects of Iron:

The addition of zero-valent iron to contaminated media (soil or water) has been shown to have some promise with respect to achieving reductive degradation of a number of contaminants, including RDX (Singh *et al.*, 1998), TNT and other nitroaromatic compounds (Hofstetter *et al.*,

1999). However, the results of the greenhouse study do not indicate that the presence of zero valent iron had any significant effect on soil-borne HMX as a result of reductive degradation. No significant supporting evidence from soil samples (i.e. the detection of degradation products or the reduction in HMX concentrations) was obtained for treatments using zero valent iron, although the high degree of in-sample variability in soil concentrations of HMX for these samples made the assessment of marginal remediation difficult. The effectiveness of the iron amendment may also have been limited as a result of relatively poor contact between iron particles and the widely dispersed and relatively insoluble HMX, especially in treatments where the iron was applied to the soil surface and not incorporated into the soil profile.

4.0 IMPLICATIONS to PHYTOREMEDIATION

4.1 Mass Transfer

As discussed in Section 3.2.1, the available analytical data for soil samples do not reveal any reduction of HMX concentrations in soil within any of the treatments. On this basis alone, the effectiveness of plants in remediating soil appears to be poor. However, an evaluation of the relative distribution of the HMX mass among soil and plant tissues enables a quantitative evaluation of the effectiveness of plants which is not directly confounded by any variability in the soil HMX data.

Tables 4.1 and 4.2 present estimates of the mass transfer of HMX from soil to plant tissues in context of the entire mass originating from the soil. At the time of interim sampling (*i.e.,* six weeks following study initiation), the calculated mass of HMX in plant tissues (above-ground only) accounted for 0.54% to as much as 3.85% of the total HMX mass in the soil. This translates to a decrease in soil concentration of HMX by as much as 1.15 mg/kg. At the time of final sampling, the relative partitioning of HMX from soil to plant tissues was notably higher, ranging from 1.33% to a maximum of 11.26%. The partitioning of HMX at study termination is depicted in Figure 4.1 (Treatment 3) and Figure 4.2 (Treatment 6). This level of transfer of HMX to plant tissues translates to an upper limit of almost 4 mg/kg in terms of potential decreases in the HMX content of soil.

A previous study of the effects of plants on concentrations of explosive contaminants in soil revealed that nitroaromatics in rhizosphere soil (the root zone) were depleted as a result of uptake of these compounds into plant tissues, despite a low degree of bioaccumulation (Scheidemann *et al.*, 1998). The results of the current study reveal relatively high rates of bioaccumulation (up to 20-fold), which subsequently indicates that it may be possible to achieve measurable reduction of soil HMX through the establishment of plants, especially grasses which exhibited the highest mass transfer of HMX from soil to plant tissues.

Table 4.1: HMX Partitioning among Soil and Plants - Interim Sampling

Treatment	HMX Mass in Fresh Shoots (mg)	Calculated Decrease in Soil HMX [1]	
		mg/kg	%[2]
3A	0.42	0.16	0.54
3B	1.06	0.41	1.36
3C	1.28	0.49	1.64
3D	0.53	0.20	0.68
3E	1.58	0.61	2.03
6A	0.23	0.09	0.29
6B	1.14	0.44	1.46
6C	3.00	1.15	3.85
6D	2.08	0.80	2.67
6E	0.61	0.23	0.78

1 - calculated based on a mass of 2.6 kg of soil per pot
2 - assumes an initial HMX concentration of 30 mg/kg in all pots
NA - Not applicable - sample not available or mass not sufficient for analysis
All values represent treatment averages

Table 4.2: HMX Partitioning among Soil and Plants - Final Sampling

Treatment	HMX Mass in Fresh Shoots	HMX Mass in Senescent	HMX Mass in Roots (mg)	Total HMX Mass in Plant	Soil HMX Concentration	Calculated Decrease in Soil HMX	
						mg/kg[1]	%
3A	1.43	NA	NA	1.43	33.8	0.55	1.62
3B	0.58	0.63	0.10	1.30	32.7	0.50	1.53
3C	1.70	0.56	0.15	2.41	34.8	0.93	2.66
3D	9.05	0.72	0.24	10.01	34.2	3.85	11.26
3E	5.45	1.43	0.46	7.34	34.1	2.82	8.27
6A	2.37	NA	NA	2.37	33.0	0.91	2.76
6B	0.37	0.40	0.21	0.98	28.3	0.38	1.33
6C	3.81	1.47	0.06	5.34	38.7	2.06	5.31
6D	5.06	0.51	0.71	6.28	32.4	2.42	7.45
6E	3.30	0.43	0.40	4.14	38.7	1.59	4.11

1 - calculated based on a mass of 2.6 kg of soil per pot
NA - Not applicable - sample not available or mass not sufficient for analysis
All values represent treatment averages

Figure 4.1: HMX Partitioning -Treatment 3

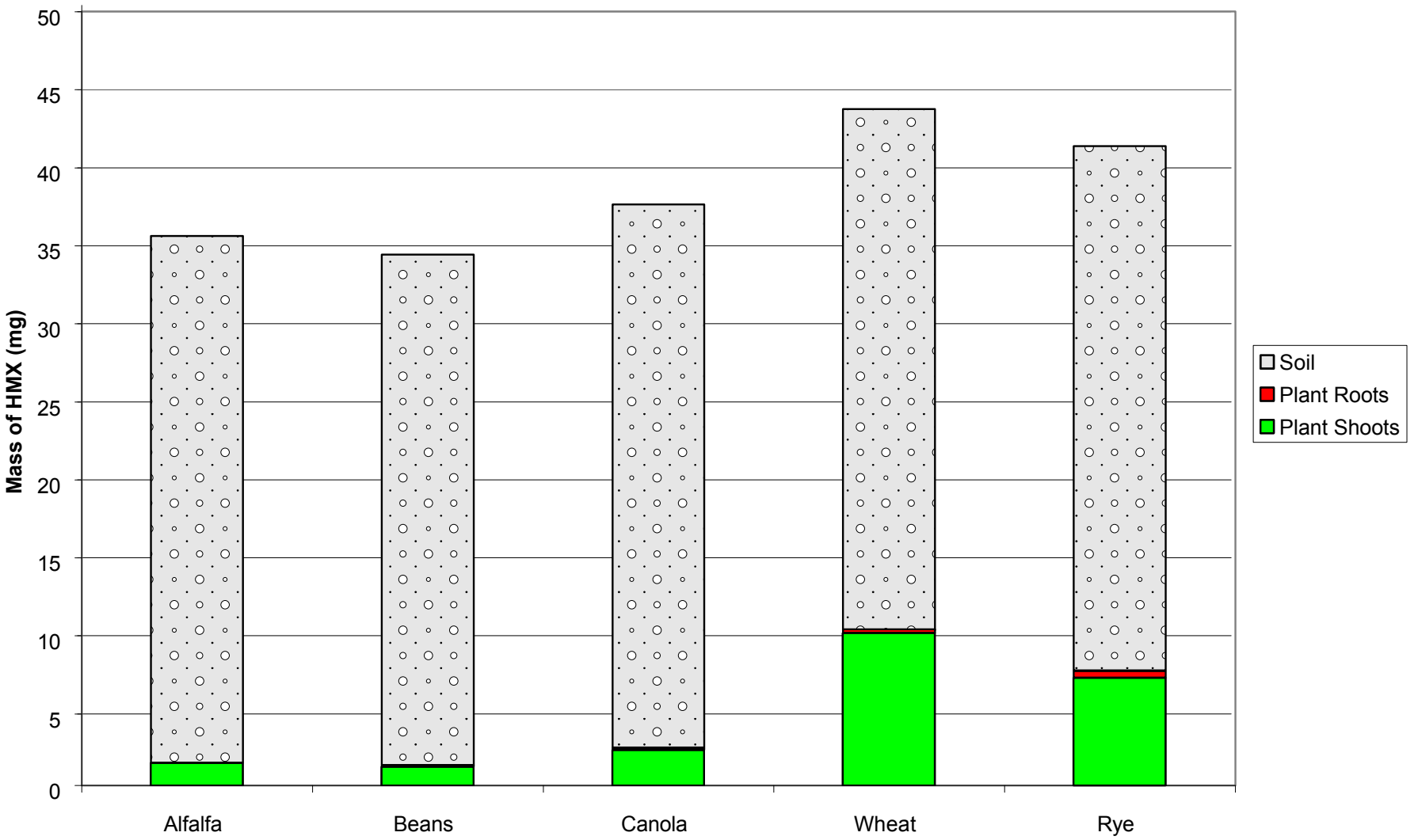
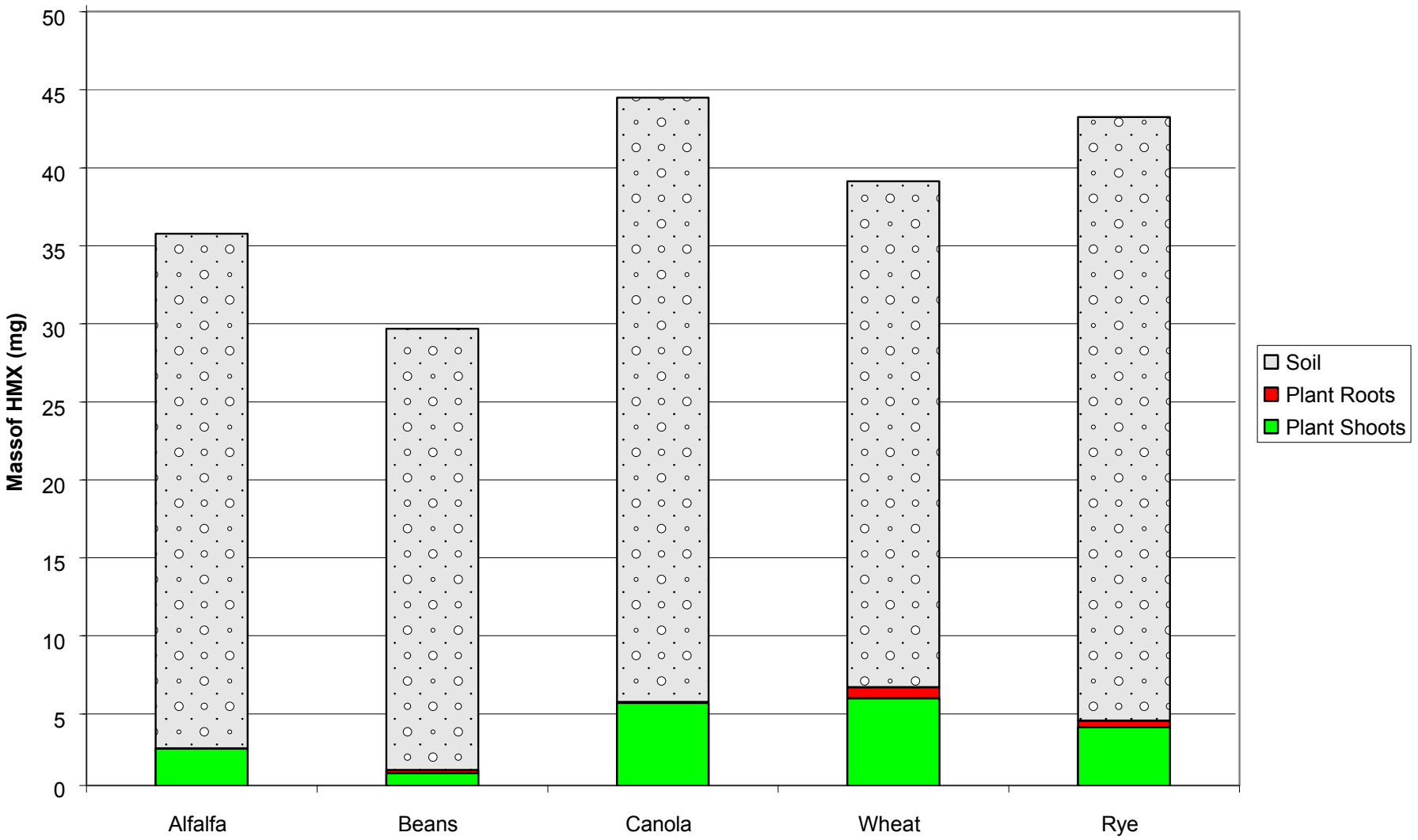


Figure 4.2: HMX Partitioning - Treatment 6



4.2 Key Considerations

There are four key findings relating to the fate of HMX in soil and plant tissues which should be considered in the evaluation of the potential to apply a phytoremediation approach for HMX-contaminated soils at WATC Wainwright, and possibly at other Canadian Firing Ranges. These key findings are as follow:

1. HMX-bearing Site soil did not have significant adverse effects on growth of any of the plant species in question.

2. Neither the presence of plants nor the addition of zero-valent iron appeared to result in any substantial degradation of HMX.

3. All plants examined showed an intrinsic capacity to accumulate HMX from soil to above-ground tissues, with grasses exhibiting the highest capacity for HMX accumulation.

4. It has been estimated (refer to Section 4.1) that plants could translocate up to 10% or more of the total mass of HMX in soil to above-ground plant tissues .

These four findings in summation suggest that plants (especially grasses) could be established and survive in HMX contaminated areas at WATC Wainwright, and potentially remove measurable proportions of HMX from the rhizosphere within relatively short periods of time. Based on greenhouse study results, no degradation of HMX is expected as a result of the establishment of plants. The primary effectiveness of plants would be through the process of phytoextraction rather than phytodegradation.

4.3 Limitations

This assessment has been completed to address some general questions regarding the applicability of phytoremediation as an approach to dealing with HMX contamination. The study results are primarily reflective of site-specific conditions at WATC Wainwright. The performance of plants in this study is related in part to the nature of soil at this Site, including the presence/absence of a

range of chemical constituents that could be found at military ranges (e.g. TNT, RDX, *etc*.). The capacity to establish plants at other sites and achieve significant rates of COC uptake may be limited by site-specific soil quality.

Depth of root penetration may also be a general limiting factor, with the roots of the species which have been considered in this study typically confined to the upper meter of soil. The exception is alfalfa, the roots of which may penetrate as deep as several meters. Under field conditions, HMX or other COCs (e.g. RDX or metals) may be distributed in soil so that much of the total mass is out of reach of shallow-rooted plants.

In the current study, plants were regularly watered whereas plants under field conditions may be exposed to periods of prolonged drought if irrigation is not practical, especially at WATC Wainwright which experiences the dry climate of north-eastern Alberta. The solubility of HMX is very low (*i.e*., 6.6 mg/l at 20 ^{o}C) and the amount available for plant uptake may be limited simply by the available volume of water in the pore space of the rhizosphere soil.

Ecological risk benchmarks for HMX in food stuffs for herbivorous animals have been reported as 5.6 and 22 mg/kg (fresh weight) depending on the animals species (Maxwell and Opresko, 1996). Plant tissue concentrations measured in the greenhouse study were often in excess of both of these benchmarks, exceeding 100 mg/kg fresh weight in some cases. This does not necessarily confer adverse ecological effects as a result of establishing plants on HMX bearing soil, but it does represent a potentially limiting factor that should be considered prior to any potential Site applications. In the event that plant material were harvested or incinerated on site at regular intervals, the potential for ecological effects through food-chain exposure would be significantly reduced.

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 General Conclusions

The results of the present study provide significant positive support for the premise that phytoremediation may be a viable approach to remediation of HMX contaminated soils at Canadian firing regions (e.g. Test Range 13 at WATC Wainwright). The effectiveness is likely to be achieved in the form of phytoextraction as opposed to phytodegradation. Further examination of specific aspects of the plant/soil interactions with respect to HMX, and other contaminants, will likely build on this initial conclusion. The careful design and implementation of field trials will resolve some of the current uncertainties and enable further progress in the overall science. Field trials would also provide the opportunity to demonstrate feasibility directly under Site conditions prior to any potential progression to full scale applications.

5.2 Further Research Considerations

5.2.1 Application Scale

The study results suggest that an effectiveness of 10% removal is possible in a relatively short period of time (refer to Section 4.1), with grasses showing the greatest capacity for HMX removal from soil. In a full growing season, higher rates of translocation of HMX from soil to plant tissues are certainly possible as a result of greater overall plant biomass production and a greater duration of exposure of root systems to HMX-bearing soil.

A theoretical examination of the potential effectiveness of larger and longer scale applications can be completed through extrapolation from the current study results. For example, in a standard growing season, alfalfa may produce in the order of 2 kg (fresh weight) of harvestable plant biomass per square meter (Jones, 1998). The results of this study reveal that HMX can accumulate in alfalfa tissues to concentrations in the order of 50 mg/kg (fresh weight) or more in a relatively short period of time. Assuming similar rates of uptake on a larger scale, this leads to a theoretical removal rate of 100 mg HMX per square meter of contaminated soil.

5.2.2 Climatic Conditions

As noted in Section 4, the relatively low solubility of HMX indicates that the potential effectiveness of phytoextraction may be limited in part by available water, although the uptake of HMX observed in the greenhouse study suggests that the uptake of HMX may not have been entirely water-dependent. Given that field scale applications would potentially encounter highly variable degrees of available water (in the absence of controlled irrigation), some further investigations of the relationship between water availability and HMX uptake would be beneficial.

5.2.3 Post-harvest Procedures

This study has not addressed the issue of the fate of HMX observed in plant tissues following uptake from contaminated soil. There is therefore a question as to the ultimate fate of plant-absorbed HMX, which is major factor determining the ultimate effectiveness of the phytoremediation approach.

There are several options which could be followed in the handling of HMX-bearing plant material, including (but not limited to) controlled land-filling, composting, or incineration. An evaluation of these options, at least in the form of literature review, is warranted.

5.2.4 Other Contaminants

Current study results address only HMX in terms of the potential for phytoremediation. Soils and shallow groundwater at most military sites may also contain other explosive (e.g. RDX and TNT) and/or non-explosive (e.g., metals) COCs. For this reason, expansion of research objectives should include the evaluation of the potential effectiveness of phytoremediation, as potentially applied for HMX, with respect to these other COCs.

5.3 Field Trials

The greenhouse study, which used Site soils from Wainwright, suggests that significant accumulation of HMX from Site soils to plant tissues is likely to occur if field applications are implemented. Sampling and analysis of plant tissues prior to the onset of the greenhouse study has revealed that HMX does naturally accumulate in plant tissues on Range 13 (HMX concentrations ranging from approximately 10 to 50 mg/kg), paralleling plant-mediated processes which were observed during the greenhouse trials. In consideration of this, transition from greenhouse to experimental field trials is likely to provide positive results to some degree. The completion of field trials would allow for further research with respect to most of the outstanding needs discussed above. Some specific recommendations for field trials are presented below.

Grasses exhibit the greatest total uptake of HMX over this short term study, and therefore the field trials should incorporate grasses as species to be evaluated. However, the field studies should not necessarily exclude those plants which faired less well in the greenhouse study. Alfalfa, although ranking relatively low in terms of the mass of HMX translocated from soil to plant tissues, should be considered for inclusion in any field trials that may be initiated. As noted, the potential for alfalfa to exhibit considerable biomass accumulation, well beyond that demonstrated in the greenhouse study, has positive implications with respect to its field performance in contrast to the greenhouse performance. Alfalfa is also a relatively deep-rooted plant that may perform beyond the greenhouse standard in comparison to more shallow rooted species, depending on the distribution and dynamics of HMX (and other contaminants) in the soil profile.

Field trials should also attempt to address the issue of mobility and dynamics of HMX, and possibly other COCs, in the rhizosphere soils. The greenhouse study was established so that there was no capacity for downward migration of HMX out of the rhizosphere soils. This may not be the case under field conditions.

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APPENDIX A

Biotechnology Research Institute (BRI) Report
Detailed Analytical Results

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Table 1. HMX in plant samples, Mid-point Sampling Event.

Treatment	Sample Prefix	Fresh shoots				Roots	
		HMX[a] (mg/kg)	Deviation (±)	HMX[b] (mg/kg)	Deviation (±)	HMX[a] (mg/kg)	HMX[b] (mg/kg)
2	2A-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2B-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2C-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2D-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2E-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
3	3A-4	121.0	3.0	18.6	0.4		
	3B-4	164.5	0.5	23.8	0		
	3C-4	197.0	n.a.	20.7	n.a.	16.4	3.15
	3D-4	60.7	10.2	16.2	2.7	18.3	4.53
	3E-4	171.0	2.0	50.6	0.6	44.9	14.3
6	6A-4	75.7	1.2	18.0	0.3		
	6B-4	115.5	1.5	21.8	0.2	15.8	10.6
	6C-4	282.5	17.5	51.0	1.0	8.19	1.01
	6D-4	292.5	0.5	61.1	0.2	13.4	1.69
	6E-4	67.4	0.7	17.4	0.2	13.2	3.21

[a] HMX concentration reported as mg/kg plant dry weight, mean of duplicate extractions.
[b] HMX concentration reported as mg/kg plant fresh weight, mean of duplicate extractions.
n.d. Not dectected.
n.a. Not applicable.

Table 2. HMX in plant samples, Final Sampling Event.

Treatment	Sample Prefix	Fresh shoots		Dried leaves		Roots	
		HMX[a] (mg/kg)	HMX[b] (mg/kg)	HMX[a] (mg/kg)	HMX[b] (mg/kg)	HMX[a] (mg/kg)	HMX[b] (mg/kg)
2	2A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2B-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2C-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2D-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2E-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	3A-1	227.8	47.7			traces[c]	traces[c]
	3A-2	157.7	37.7			traces[c]	traces[c]
	3A-3	259.7	52.3			traces[c]	traces[c]
	3B-1	61.5	11.4	193.5	103.2	20.2	8.16
	3B-2	61.8	15.0	216.0	119.0	20.0	7.60
	3B-3	61.1	16.8			14.9	2.16
	3C-1	108.8	20.9	763 .0	261.0	31.3	7.46
	3C-2	85.4	24.4	677.2	204.0	34.1	5.12
	3C-3	107.2	22.0			39.1	9.44
	3D-1	195.6	43.6	267.2	227.0	19.6	11.2
	3D-2	739.6	215.0			11.2	4.20
	3D-3	1068	402.0	489.6	407.0	11.4	5.64
	3E-1	388.8	79.7	451.0	313.5	17.8	5.88
	3E-2	499.2	69.2	797.0	522.1	18.9	6.14
	3E-3	566.0	219.1			9.96	3.68
6	6A-1	301.8	80.0			traces[c]	traces[c]
	6A-2	349.8	97.7			traces[c]	traces[c]
	6A-3	243.2	67.9			traces[c]	traces[c]
	6B-1	69.4	14.8			19.0	3.26
	6B-2	29.6	6.52	162.5	51.1	21.4	5.78
	6B-3	27.4	4.30	197.0	52.2	16.2	1.90
	6C-1	106.8	48.6	768.0	566.2	17.5	10.6
	6C-2	219.6	48.8	658.0	506.6	14.5	12.6
	6C-3	177.2	45.9			24.1	13.6
	6D-1	612.4	184.2	533.8	322.8	6.80	1.16
	6D-2	369.4	96.1	322.8	276.0	53.1	17.6
	6D-3	272.3	73.5			48.8	8.64
	6E-1	101.3	31.4	212.8	135.6	11.7	4.64
	6E-2	476.8	121.7	305.4	212.4	24.9	6.14
	6E-3	205.4	59.1			11.5	3.68

[a] HMX concentration reported as mg/kg plant dry weight.
[b] HMX concentration reported as mg/kg plant fresh weight.
[c] Majeur interference masking HMX, but traces identified by LC-MS.
n.d. Not detected.

Table 3**.** HMX in soil samples, Preliminary Sampling Event.

Sample Prefix	Soil Sample	
	HMX[a] (mg/kg)	RSD (%)
DND-1	n.d.	n.a.
DND-2	n.d.	n.a.
DND-3	28.8	29.1
DND-4	50.7	31.4
DND-5	32.3	24.2

[a] Mean of triplicate extraction.
n.d. Not detected.
n.a. Not applicable.

Table 4**.** HMX in soil samples, Mid-point Sampling Event.

Treatment	Sample Prefix	Soil Sample	Manure Sample
		HMX[a] (mg/kg)	HMX[a] (mg/kg)
1	1-4-A	22.4	
2	2A-4	n.d.	
	2B-4	n.d.	
	2C-4	n.d.	
	2D-4	n.d.	
	2E-4	n.d.	
3	3A-4	30.2	
	3B-4	55.6	
	3C-4	47.8	
	3D-4	51.2	
	3E-4	32.8	
4	4-4	39.1	9.1
5	5A-4	23.7	11.6
	5B-4	37.0	12.3
	5C-4	32.1	5.1
	5D-4	25.4	7.2
6	6A-4	31.0	1.3
	6B-4	19.0	10.9
	6C-4	27.5	4.7
	6D-4	87.4	6.1
	6E-4	31.9	2.3
7	7-4	n.d.	n.d.
8	8A-4	n.d.	n.d.
	8B-4	n.d.	n.d.
	8C-4	0.3	n.d.
	8D-4	2.3	n.d.

[a] One replicate was extracted, except for treatments 1 and 4 that were extracted in triplicate with respective RSD of 18.1% and 10.8%.

n.d. Not dectected

Table 5. HMX in soil samples, Final Sampling Event.

Treatment	Sample Prefix	Soil Sample	
		HMX[a] (mg/kg)	Deviation (±)
1	1-1	36.6	5.5
	1-2	30.2	3.4
	1-3	38.1	4.1
2	2A-1	traces [b]	n.a.
	2B-3	traces [b]	n.a.
	2C-1	0.5	n.a.
	2D-1	traces [b]	n.a.
	2E-1	traces [b]	n.a.
3	3A-1	37.9	4.3
	3A-2	34.7	2.9
	3A-3	28.8	0.5
	3B-1	34.7	2.1
	3B-2	41.0	1.5
	3B-3	26.9	0.2
	3C-1	34.5	1.5
	3C-2	28.6	0.1
	3C-2	28.9	0.7
	3D-1	33.6	3.4
	3D-2	30.3	1.9
	3D-3	30.9	2.6
	3E-1	27.6	1.1
	3E-2	39.5	4.2
	3E-3	42.0	1.8

[a] Mean of duplicate extraction, except for the samples from treatment 2.
[b] Below the instrumental quantification limit (0.1 ppm), but identified by LC-MS.
n.a. Not applicable.

Table 5. HMX in soil samples, Final Sampling Event, continued.

Treatment	Sample Prefix	Soil Sample		Manure Sample	
		HMX[a] (mg/kg)	Deviation (±)	HMX[a] (mg/kg)	Deviation (±)
4	4-1	31.5	4.3	15.5	0.2
	4-2	27.9	2.9		
	4-3	33.2	0.5		
5	5A-1	29.3	2.1	16.8	3.2
	5A-2	32.9	1.5		
	5A-3	30.6	0.2		
	5B-1	27.5	1.5	15.9	2.2
	5B-2	29.8	0.1		
	5B-3	27.6	0.7		
	5C-1	35.0	3.4	13.6	0.9
	5C-2	32.1	1.9		
	5C-3	38.9	2.6		
	5D-1	33.6	1.1	16.5	6.0
	5D-2	32.1	4.2		
	5D-3	24.8	1.8		
6	6A-1	32.4	4.3	2.0	0.1
	6A-2	37.2	2.9		
	6A-3	29.4	0.5		
	6B-1	27.3	2.1	3.2	0.1
	6B-2	27.7	1.5		
	6B-3	29.9	0.2		
	6C-1	34.3	1.5	4.7	0.1
	6C-2	37.8	0.1		
	6C-3	44.1	0.7		
	6D-1	28.4	3.4	4.6	0.3
	6D-2	36.2	1.9		
	6D-3	32.7	2.6		
	6E-1	41.8	1.1	5.0	1.5
	6E-2	32.1	4.2		
	6E-3	36.4	1.8		
7	7-1	traces[b]	n.a.	n.d.	n.a.
8	8-1	4.1	n.a.	n.d.	n.a.

[a] Mean of duplicate extraction, except for the samples from treatment 7 and 8.
[b] Below the instrumental quantification limit (0.1 ppm), but identified by LC-MS.
n.a. Not applicable.
n.d. Not detected.

Table 6. Effect of the addition of acetone during the soil preparation on the RSD value.

Description	Sample Prefix	Soil Sample	
		HMX[a] (mg/kg)	RSD (%)
Preliminary Sampling	DND-3	39.6	17.7
Mid-Point Sampling	1-4-A	29.5	13.6
	4-4	26.6	6.3
Final Sampling	1-1	31.3	40.6
	1-2	29.1	23.5
	1-3	43.9	28.8
	4-1	40.5	23.9
	4-2	20.7	10.2
	4-3	30.3	13.4

[a] Mean of triplicate extraction, 4 g of soil extracted with 10 ml acetonitrile.

Table 7. Effect of the amount of soil extracted on the RSD value and the extraction time on HMX recovery.

Amount of soil	Soil Sample DND-3	
	HMX (mg/kg)	RSD (%)
2[a]	28.8	29.1
4[a]	39.6	17.7
8[a]	32.5	12.1
8[b]	31.7	17.5
16[a]	25.3	1.7
16[b]	31.8	3.3
32[a]	33.4	8.5

[a] Mean of triplicate extraction (16 hours extraction).
[b] Mean of triplicate extraction (6 hours extraction).

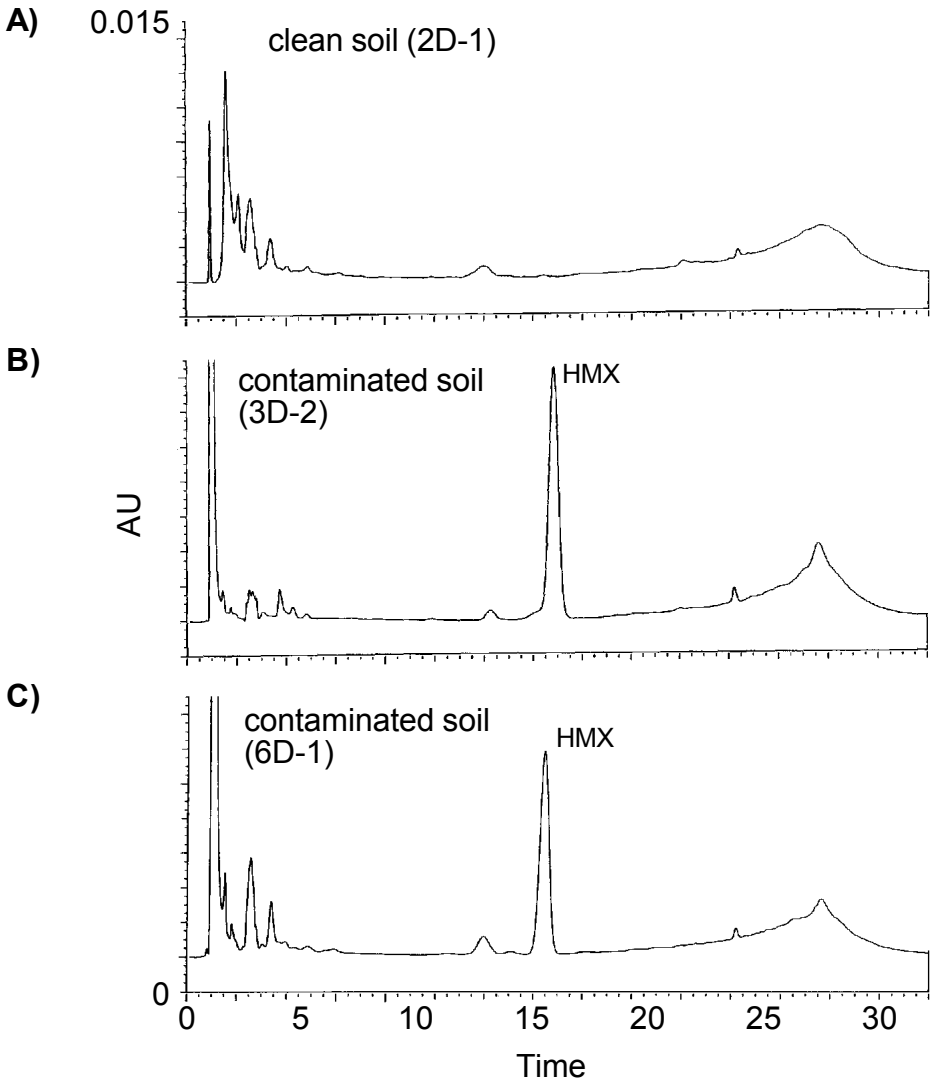

Figure 1: HPLC-UV chromatograms (254 nm) for wheat tissue extracts; (A) shoots grown in clean soil, (B) shoots grown in soil containing HMX (treatment 3), (C) shoots in soil containing HMX, iron and manure (treatment 6).

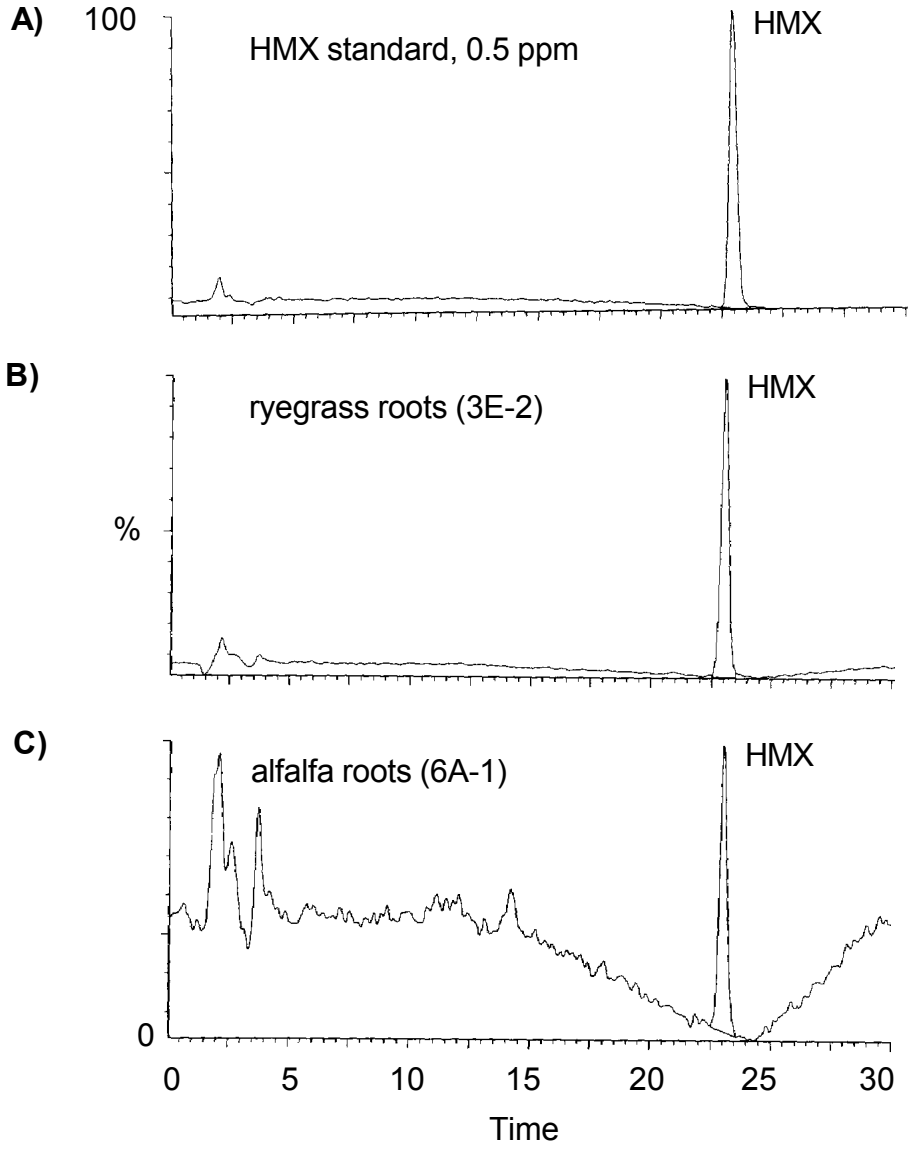

Figure 2: LC-MS characteristic mass ion extractions for HMX in root tissues; (A) 0.5 ppm HMX standard solution in acetonitrile, (B) ryegrass root extract (treatment 3), (C) alfalfa root extract (treatment 6).

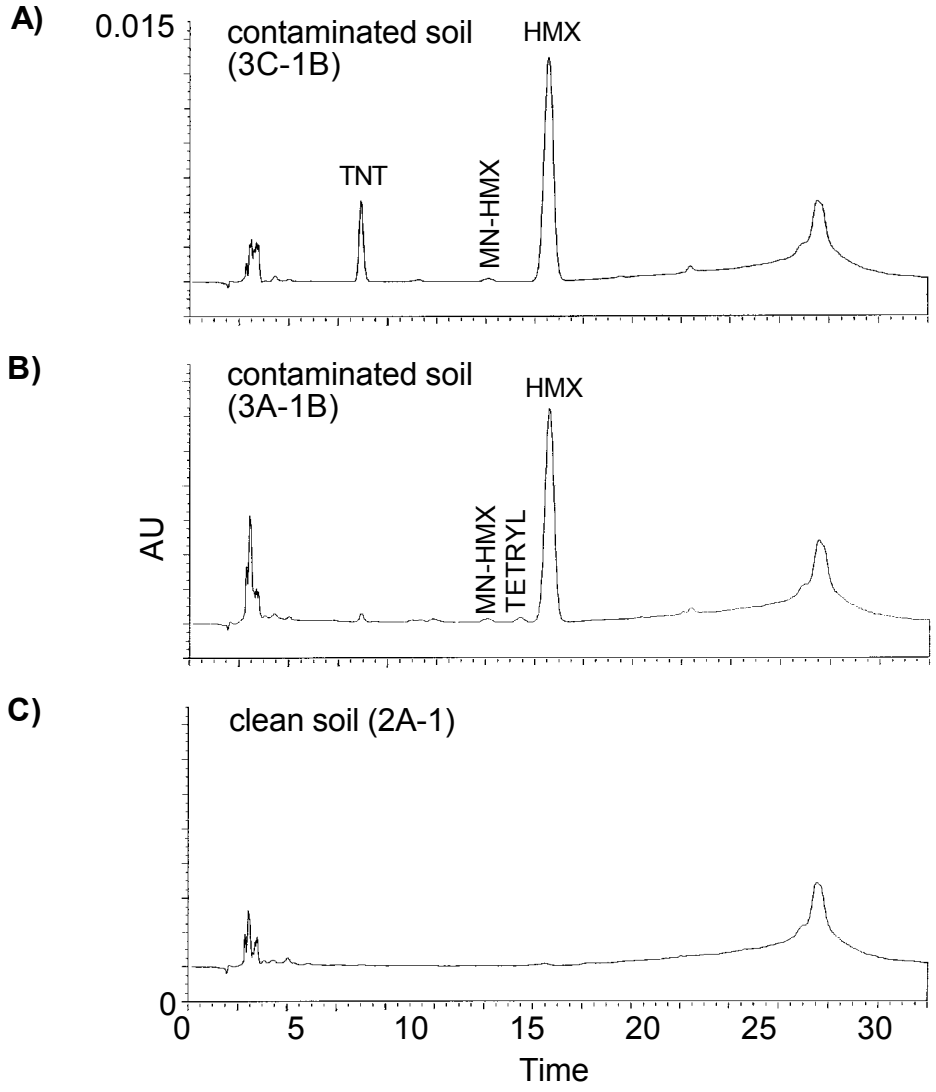

Figure 3: HPLC-UV chromatograms (254 nm) for soil extracts; (A) HMX contaminated soil treated with canola (treatment 3), (B) HMX contaminated soil treated with alfalfa (treatment 3), (C) clean soil (treatment 2). Note small quantities of TNT, Tetryl and MN-HMX in HMX contaminated soil.

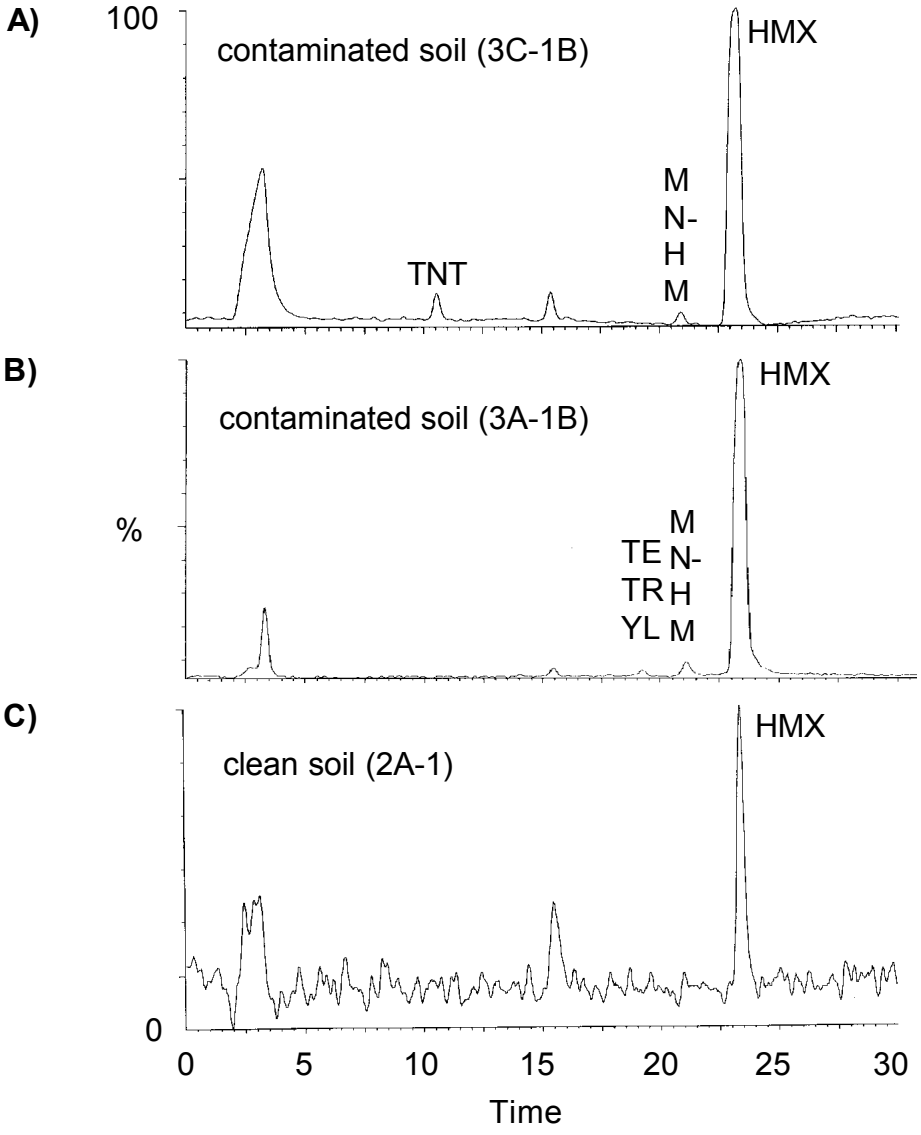

Figure 4: LC-MS characteristic mass ion extractions for TNT, Tetryl, 1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazacylooctane (MN-HMX) and HMX in soil extracts; (A) HMX contaminated soil treated with canola (treatment 3), (B) HMX contaminated soil treated with alfalfa (treatment 3), (C) clean soil (treatment 2).

Philip Analytical Services Corporation (PASC)
Analytical Report

PHILIP SERVICES

Certificate of Analysis

CLIENT INFORMATION

Attention:	Neil Morris
Client Name:	Beak International
Project:	21590.3
Project Desc:	DND Phytoremediation
Address:	14 Abacus Rd. Brampton, Ontario L6T 5B7
Fax Number:	905-794-2338
Phone Number:	905-794-2325

LABORATORY INFORMATION

Contact:	Dave Howell
Project:	AK991491
Date Received:	99/11/24
Date Reported:	99/12/13
Submission No.:	9K0940
Sample No.:	070598-070602

NOTES: ***"-' = not analysed '<' = less than Method Detection Limit (MDL) 'NA' = no data available***
LOQ can be determined for all analytes by multiplying the appropriate MDL X 3.33
All organic data is blank corrected except for PCDD/F, Hi-Res MS and CLP volatile analyses
Solids data is based on dry weight except for biota analyses.
Organic analyses are not corrected for extraction recovery standards except for isotope dilution methods, (i.e. CARB 429 PAH, all PCDD/F and DBD/DBF analyses)

Methods used by PASC are based upon those found in 'Standard Methods for the Examination of Water and Wastewater', Nineteenth Edition. Other methods are based on the principles of MISA or EPA methodologies. New York State: ELAP Identification Number 10756.

All work recorded herein has been done in accordance with normal professional standards using accepted testing methodologies, quality assurance and quality control procedures except where otherwise agreed to by the client and testing company in writing. Any and all use of these test results shall be limited to the actual cost of the pertinent analysis done. There is no other warranty expressed or implied. Your samples will be retained at PASC for a period of three weeks from receipt of data or as per contract.

COMMENTS: (1) Matrix interference suspected

Certified by: _____

PHILIP ANALYTICAL SERVICES CORPORATION
5555 North Service Road, Burlington, Ontario, Canada L7L 5H7 Tel: (905) 332-8788 Fax: (905) 332-9169

PASC - Certificate of Analysis

	Client ID:		DND-1	DND-2	DND-3	DND-4	DND-5
	Lab No.:		070598 99	070599 99	070600 99	070601 99	070602 99
	Date Sampled:		99/11/12	99/11/12	99/11/12	99/11/12	99/11/12
Component	**MDL**	**Units**					
pH (20 DEG C)			6.76	6.72	7.41	7.44	7.52
Clay (<0.002mm)	0.1	(%)	8.0	4.0	16	12	16
Coarse Gravel (>4.8mm)	0.1	"	<	<	<	<	<
Coarse Sand (0.50-1.0mm)	0.1	"	8.4	8.5	12	6.7	9.1
Fine Coarse Sand (0.10-0.25mm)	0.1	"	25	21	20	26	21
Fine Gravel (2.0-4.8mm)	0.1	"	0.5	8.2	1.1	0.9	1.1
Med. Coarse Sand (0.25-0.50mm)	0.1	"	26	17	13	15	13
Silt (0.002-0.050mm)	0.1	"	22	22	28	25	25
V. Coarse Sand (1.0-2.0mm)	0.1	"	1.4	8.6	1.1	0.7	1.2
V. Fine Coarse Sand (0.050-0.10mm)	0.1	"	8.8	11	9.2	14	13
Phosphate (as P)	0.5	mg/kg	<2.5	<2.5	<2.5	<2.5	<2.5
Nitrate (as N)	1.00	mg/kg	<1.3	<1.3	2.7	1.3	1.6
Nitrite (as N)	1.00	"	<1.3	<1.3	<1.3	<1.3	<1.3
Cation Exchange Capacity	0.1	meq/100g	18	18	15	17	18
Boron (soluble)	0.05	mg/kg	0.13 **(1)**	0.12	0.23	0.49	0.40
Antimony	0.50	mg/kg	<	<	<	<	<
Arsenic	2.0	"	2.8	2.6	2.5	2.5	2.3
Barium	0.50	"	97	92	100	120	120
Beryllium	0.50	"	<	<	<	<	<
Cadmium	0.20	"	0.22	0.20	5.0	5.4	5.6
Chromium	0.50	"	8.1	6.7	12	13	12
Cobalt	1.0	"	5.1	4.9	7.0	6.8	6.5
Copper	0.50	"	11	11	1000	860	790
Lead	0.20	"	5.0	4.8	85	90	96
Molybdenum	1.0	"	1.3	<	1.9	3.0	2.7
Nickel	1.5	"	15	9.3	21	20	23
Selenium	1.0	"	<	<	<	<	<
Silver	0.20	"	<	<	2.3	2.2	2.8
Thallium	0.20	"	<	<	<	0.22	<
Vanadium	2.0	"	12	11	14	13	13
Zinc	1.5	"	47	45	100	120	110
TOC(Solid)	0.1	(%)	3.4	3.3	2.0	2.1	2.1

APPENDIX B

Selections from the Photographic Record of the Greenhouse Study

Photo 1: Range 13 - WATC Wainwright

Photo 2: Collection of Control (uncontaminated) Soil

Photo 3: Collection of Contaminated Soil

Photo 4: Seedlings prior to Transplanting at Study Start-up

Photo 5: Transplanted Alfalfa

Photo 6: Transplanted Bush Bean

Photo 7: Transplanted Canola

Photo 8: Transplanted Wheat

Photo 9: Transplanted Rye

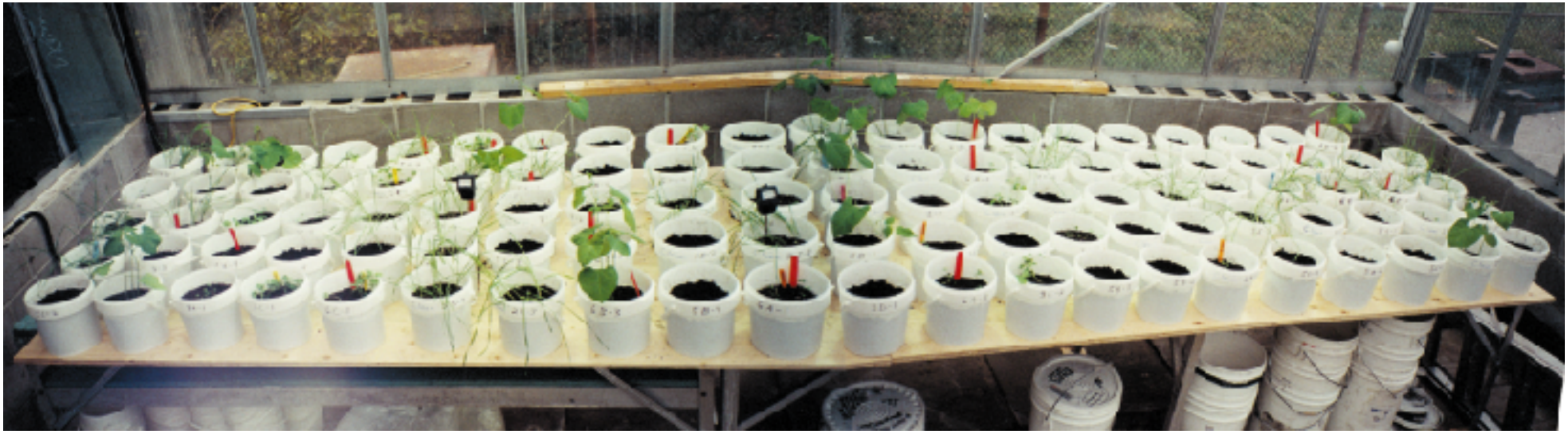

Photo 10: Greenhouse Study Experiment Layout

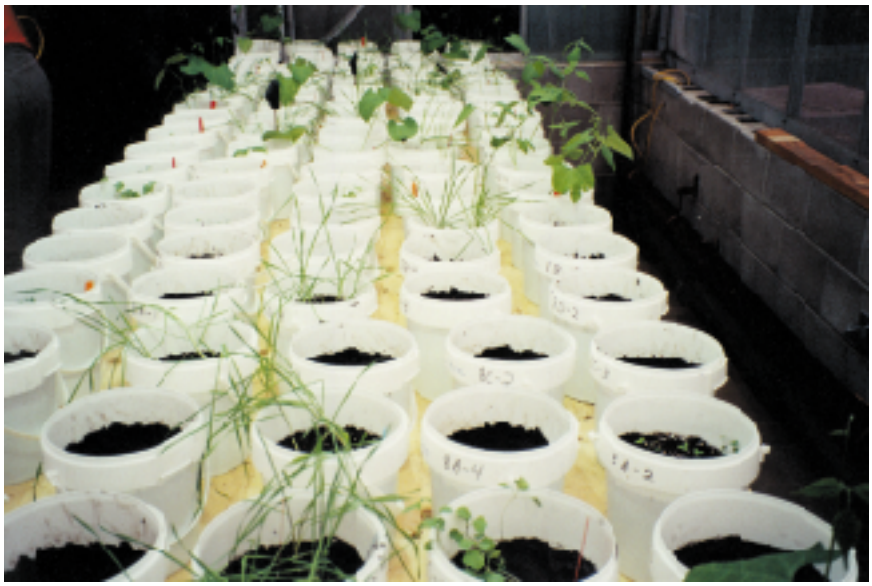

Photo 11: Plant Growth at Week 2

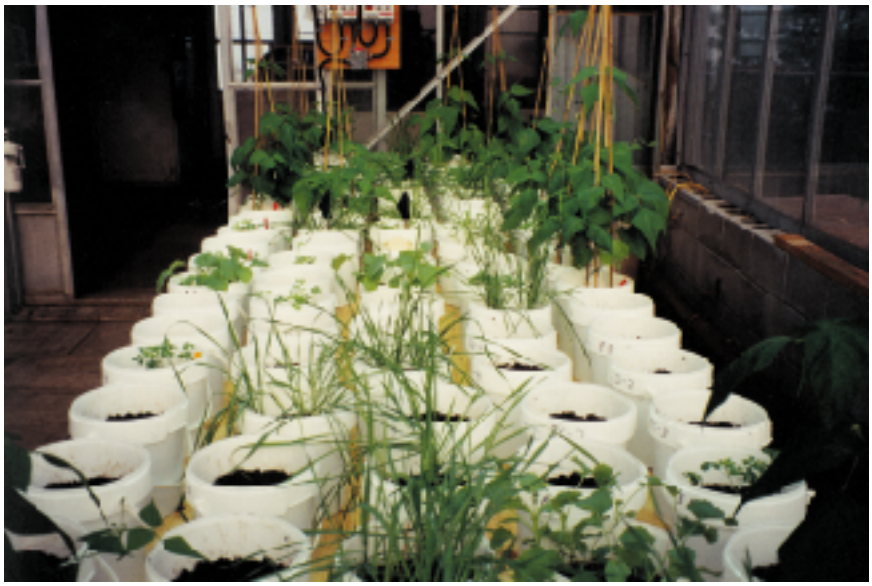

Photo 12: Plant Growth at Week 4

Photo 13: Plant Growth at Week 6

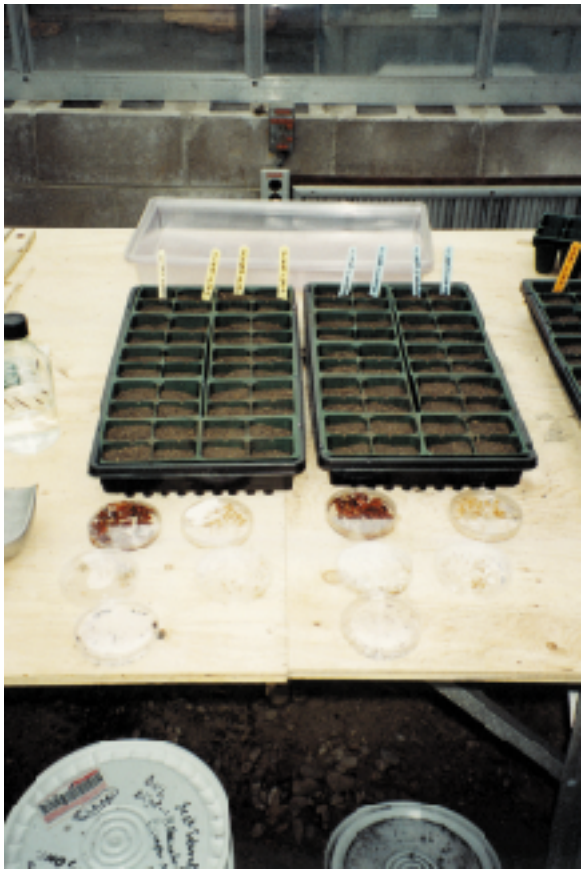

Photo 14: Germination Trial Set-up

Photo 15: Germination Trial at Day 7