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Validation of Petroleum Hydrocarbon Stratified Remediation Subsoil Criteria

Final Report

B. Drozdowski and D. Degenhardt
Ecosystem Management
Alberta Innovates – Technology Futures
drozdowski@albertainnovates.ca
780-450-5230

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Executive Summary

This study set out to validate the current AESRD subsoil guidelines by providing empirical evidence that regional deep rooted crops such as canola (*Brassica napus*) and alfalfa (*Medicago sativa*) are not affected by critical PHC concentrations in subsoil under drought conditions when plants are forced to extract moisture from depths below 1.5 m. There were no significant differences in aboveground biomass for canola grown in fine textured subsoil contaminated with F2 and F3 hydrocarbons at levels near Alberta Tier 1 guideline concentrations. There was an effect of F2 and F3 hydrocarbons on canola yield in the second simulated growing season for the coarse textured soil, however by the 3rd and 4th simulated growing season, the yields between treatments were no longer significantly different for either soil texture type and the only overall treatment effect for above ground biomass was observed for canola grown in the coarse textured soil contaminated with F3 hydrocarbon. There were no belowground biomass treatment effects for canola grown in coarse textured subsoil contaminated with F2 and F3 hydrocarbons at levels near Alberta Tier 1 guideline concentrations, however, significant differences (p<0.05) were found belowground canola biomass in fine textured soil contaminated with F3 hydrocarbon.

The influences of F2 and F3 hydrocarbons on alfalfa biomass were not significant. The effect of F3 PHC on alfalfa aboveground biomass was observed in the first simulated growing season in the fine textured soil. There was no yield loss due to contaminated subsoil in the six subsequent harvests. Root biomass was reduced as a result of F3 PHC contaminated subsoil in the fine textured soils, in the lower section only. There was no significant reduction in the coarse root biomass in the lower sections as well as fine and coarse roots in the upper and mid sections for F2 and F3 treatments.

Based on the results of this study, there was no irreparable yield loss due to subsoil contaminated with F2 and F3 hydrocarbons at levels near Alberta Tier 1 guideline concentrations. Some yield losses were observed in the first and second simulated growing season for alfalfa and canola, respectively. However, the plants recovered in the subsequent simulated growing seasons.

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1.0 INTRODUCTION

Environmental contamination of petroleum hydrocarbons (PHC) is very common; in Canada alone it is estimated that there are more than 250,000 potential or actual terrestrial sites contaminated with PHCs (Canadian Council of Ministers of the Environment, 2008). Soil contamination occurs not only through accidental spills and during transport but also at petroleum extraction, refining and distribution facilities. Crude oil and petroleum products are mixtures of hydrocarbons with various physical-chemical properties and toxicity, thus making it impossible to develop soil reclamation and remediation guidelines based on total petroleum hydrocarbon (PHC) concentration (Canadian Council of Ministers of the Environment, 2008). As such, the Alberta Tier 1 Soil and Groundwater Remediation Guidelines have delineated specific guidelines for PHC fractions based on PHC groups consisting of four consecutive carbon number ranges (F1, C6 to C10; F2, >C10 to C16; F3, >C16 to C34; and F4, >34), benzene, toluene, ethylbenzene and xylenes (BTEX).

The PHC guidelines set out by Alberta Environment and Sustainable Resource Development (AESRD) for “surface soil” (up to 1.5 m depth) are more rigorous than “subsoil” (below 1.5 m depth) because the majority of biological activity as well as invertebrate and microbial populations are concentrated in the surface soil near the rooting zone where PHC can have the greatest toxic effects (Alberta Environment, 2010; Newman, 1988; Wallach, 1990). The critical PHC levels for the underlying “subsoil” have been arbitrarily set by AESRD based on the assumption that the surface soil criteria can be exceeded in the subsoil by a factor of two due to the lack of eco-contact at depth (Table 1). This concept is widely recognized as stratified soil remediation where surface soil criteria is used for the upper 1.5 m of the soil profile, and subsoil criteria is used for soils below 1.5 m (Alberta Environment, 2010). Alberta subsoil guidelines (Alberta Environment, 2010) utilize this concept as a generic guideline in one specific situation at the upstream oil and gas facilities; within 5 m from an oilfield wellhead on agricultural land and in natural areas. The PHC levels as specified in the current Alberta guidelines (Alberta Environment, 2010) for subsoil are considered arbitrarily safe as they are in general quite conservative, given a strictly limited area of subsoil criteria application and the critical concentrations, which are among the lowest in the world. However, some of the regional crops

such as canola (*Brassica napus*) and alfalfa (*Medicago sativa*) can develop root systems beyond the 1.5 m depth over one or several growing seasons, resulting in uncertainty regarding the phytotoxic effects of PHC on these crops, particularly under drought conditions when the majority of root water uptake occurs at depth (Dardanelli et al., 1997).

Table 1. Alberta Tier 1 PHC surface and subsoil guidelines for direct soil eco-contact in agricultural areas (Alberta Environment, 2010).

Soil Type	Depth (m)	PHC fraction (mg/kg)	
		F2	F3
Fine	<1.5	150	1,300
Coarse	<1.5	130	300
Fine	>1.5	300	2,600
Coarse	>1.5	160	600

There is a need to validate the current AESRD subsoil guidelines by providing empirical evidence that regional deep rooted crops are not affected by critical PHC concentrations in subsoil under drought conditions when the plants are forced to extract moisture from depths below 1.5 m.

A soil column study, funded by the Petroleum Technology Alliance of Canada (PTAC) was established to validate these guidelines and to determine the phytotoxic effects of PHC contaminated subsoil on deep rooted crops. The objective of the study was to determine if there are phytotoxic effects on canola and alfalfa grown in fine and coarse textured subsoil contaminated with F2 and F3 hydrocarbons at levels at, or above Alberta Tier 1 critical concentrations over at least four simulated growing seasons in the greenhouse. To measure phytotoxic effect on plant establishment and development, both the above and below-ground biomass of canola and alfalfa were evaluated to determine if there was a reduction in either, or both parameters, over the period of 14 months. In order to examine the effect of PHC on root biomass in the contaminated subsoil region (>1.5 m), this study utilized a bottom irrigation system that 'forced' the roots to grow in the lower, wetter contaminated zone.

2.0 MATERIALS AND METHODS

2.1 Experimental Design

A greenhouse trial was established to monitor the effects of critical levels of PHC fractions F2 and F3 on canola and alfalfa grown in 2 m long PVC columns (0.3 m in diameter) over four simulated growing seasons in coarse and fine soil. Although the experiment in the greenhouse contains 8 replicates of each combination of soil type (fine and coarse) (Figure 1), crop type (canola and alfalfa), and treatment (control, F2 and F3 contamination), no statistical comparisons are intended to be made between fine and coarse soils or between canola and alfalfa, rather each combination was analyzed separately using one way ANOVA to determine whether crop biomass differed between the treatments.

The experiment was conducted in a sunken greenhouse at the Alberta Innovates - Technology Futures (AITF) Vegreville facility. In order to optimize the greenhouse design, fine and coarse soil columns for a given type of soil contamination were exposed to the same water source. Watering boxes were constructed out of ½" pine plywood and polyliners were used to separate the 3 types of soil contamination (Plate 1) within each of the 8 watering boxes (Figure 1) to avoid any potential cross-contamination. From the point-of-view of the 4 statistical experiments, the columns were laid out in a randomized block design with ordering of the 3 types of soil contamination being random within each of the 8 watering boxes (blocks). The layout of the coarse and fine alfalfa and canola columns was also randomized within each compartment of the watering box (Figure 1). Red dotted lines indicate liner separations between watering boxes.

Plate 1. Watering boxes and PVC industrial plastic liners separating replicates.

Figure 1. Experimental design and layout in the sunken greenhouse at the AITF Vegreville Facility.

2.2 Soil Characteristics

Initial particle size analysis, pH and electrical conductivity were determined at AITF laboratories for the coarse and fine soils used in this experiment to ensure appropriate size classes and chemical characteristics (Table 2 and Plate 2). Detailed chemical and physical properties were determined by EXOVA labs in Edmonton (Tables 3 to 5).

Table 2. Initial soil analysis for particle size, pH and electrical conductivity.

Soil	Sat'n (%)	pH	E.C. (dS/m)	Sand (%)	Silt (%)	Clay (%)
Fine Soil	65.3	6.5	1.15	49	39	12
Coarse Soil	46.5	6.4	0.83	64	31	5

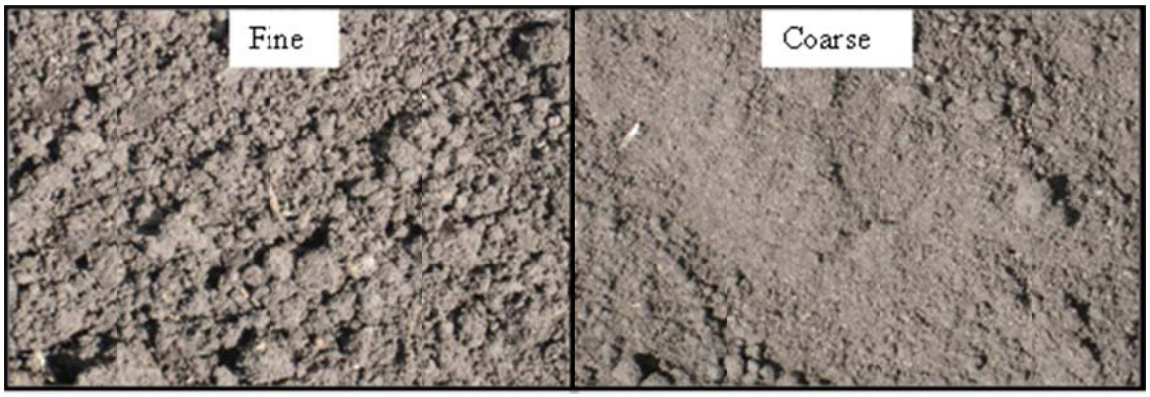

Plate 2. Soil used as growth medium in greenhouse trial.

Table 3. Physical and chemical characteristics of coarse and fine soil used in columns.

	Exchangeable Cations and Cation Exchange Capacity											Total C & N		Texture Class	
Texture Class	Ca	Mg	K	Na	Base Saturation %	Ca	Mg	Na	K	TEC	CEC	Carbon	Nitrogen	75 micron seive % retained	Texture Class
	mg/kg					meq/100g						Total %			
Coarse	3330	437	200	40	87	17	3.6	0.2	0.5	21	24	3.92	0.26	59.3	Coarse
Fine	4560	760	100	40	110	23	6.2	0.2	0.3	29	26	3.04	0.24	34	Fine

Table 4. Saturated paste extract data for the coarse and fine soil used in the columns.

Texture Class	pH	EC dS/m	SAR	Sat'n %	Ca		Mg		Na		K		Cl		SO_4		NO_3&NO_2-N	
					meq/L	mg/kg	meq/L	mg/kg	meq/L	mg/kg	meq/L	mg/kg	meq/L	mg/kg	meq/L	mg/kg	meq/L	mg/kg
Coarse	6.0	0.65	0.4	52	3.58	37.2	1.9	11.9	0.64	8	0.35	7	0.32	6	0.97	8.1	3.05	22.2
Fine	6.8	1.71	0.3	52	11.6	122	6.85	43.2	0.93	11	0.14	3	0.87	16	2.68	22.4	11.3	82.9

Table 5. Total elemental content of the column soils.

Texture Class	Hg	Al	Sb	As	Ba	Be	Bi	Cd	Cr	Ca	Co	Cu	Fe	Pb
	mg/kg													
Coarse	0.02	8160	<0.2	3.3	124	0.3	<0.5	0.18	12.5	5100	5.4	10	10600	5
Fine	0.02	12400	<0.2	4.9	142	0.6	<0.5	0.14	20.8	6200	7.5	13	17900	8.1

Texture Class	Mg	Mn	Mo	Ni	P	Se	Si	Ag	Sr	Th	Sn	Ti	V	Zn
	mg/kg													
Coarse	2200	329	<1	9.5	650	0.6	1460	0.2	40	0.1	1	262	23.8	47
Fine	3500	475	<1	16	530	0.8	1470	0.2	35	0.16	2	334	39.8	49

2.3 Soil Column Construction, Design and Installation

Soil columns were constructed by cutting 0.3 m diameter irrigation pipes to 2 m tall columns (Plate 3(a)). One replicate of each treatment contained a clear 30 cm Plexiglass insert at the interface of the clean and contaminated soil to view root development into the contaminated soil (Plate 3(b)). The columns were sealed at one end by inserting and gluing a fitted disk. The bottom 10 cm of each column was drilled with 0.15 cm (1/16 inch) holes (Plate 4(a)) and filled with 1.3 cm (½ inch) screened gravel to allow for water infiltration in the bottom 10 cm of the columns. Fibrous air filter material was placed on top of the gravel layer in each column to separate it from the soil and prevent clogging (Plate 4(b)). A total of 96 columns (8 replicates) were installed in the sunken greenhouse at the AITF Vegreville facility in July 2011 (Plate 5).

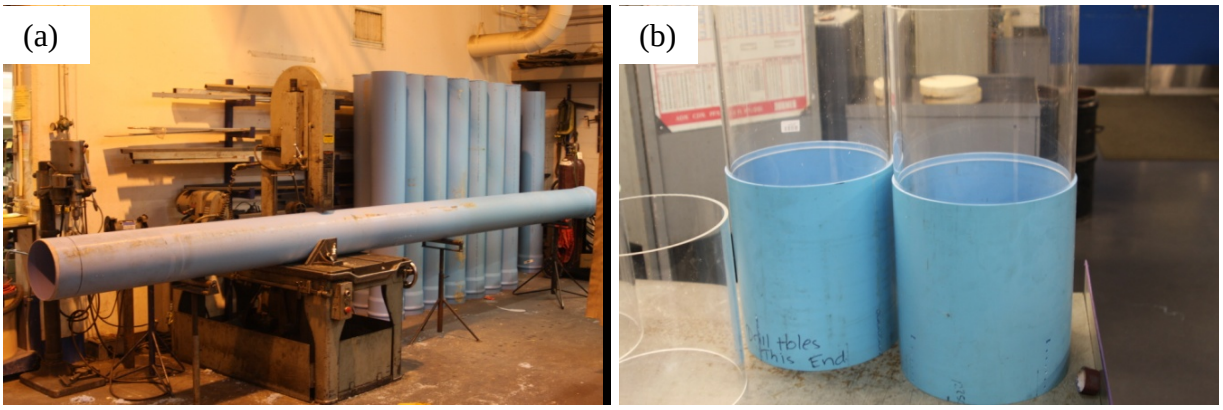

Plate 3. Irrigation pipes cut into (a) 2 m tall columns, some with (b) clear plexiglass inserts.

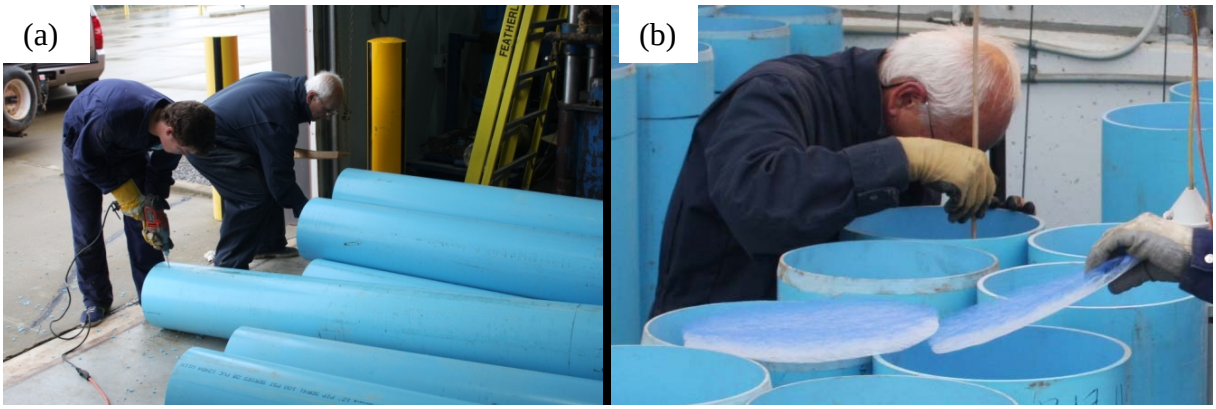

Plate 4. (a) Infiltration holes drilled in the bottom of each column and (b) inserted filters.

Plate 5. Soil columns installed in watering boxes in the sunken greenhouse in Vegreville.

2.4 Soil Contamination and Column Set-up

Appropriate PHC contaminants were obtained by separating the various fractions of oil within federated crude oil by distillation (ASTM D7169). In order to complete the F3 distillation an ASTM D5236 vacuum distillation was performed to reach 481°C. The following fractions were collected from the distillation:

Fraction #1: nC6 to nC10 (IBP -174°C)

Fraction #2: >nC10 to nC16 (IBP -174°C-287°C)

Fraction #3: >nC16 to nC34 (IBP -287°C-481°C)

Fraction #4: nC35+ (IBP -481°C+)

Density measurements (ASTM D4052 or D5002) of the F2 and F3 fractions were performed to obtain a proper mass balance and was used to quantify the amount of contaminate required for each soil:

F2 = 823.6 kg/m^3 @ 15°C (Calculated at 20°C = 820.1 kg/m3)

F3 = 868.8 kg/m^3 @ 30°C

This methodology ensured there would be no interference from other PHC fractions in the soil. Soil contamination was achieved by mixing clean coarse or fine soil with calculated amounts of F2 or F3 PHC to obtain critical concentrations. Subsoil remediation guideline values for agricultural land use and direct soil eco-contact F2 and F3 PHC levels were targeted (Table 1).

First the specified proportions of F2 or F3 oil were thoroughly mixed with approximately 10 kg of soil in a small industrial mixer (Plate 6) and then the contaminated soil was added to 250 kg of clean soil in a larger mixer (Plate 7) at a specified rate to match the F2 and F3 critical values as in Table 6.

Plate 6. Adding predetermined amount of F2/F3 PHC to 10 kg soil in small mixer.

Plate 7. Adding contaminated soil and collecting mixed soil in large mixer.

Table 6. Contaminated soil preparation and targeted contamination levels.

Date	Soil Texture	PHC Fraction	Subsoil Guideline	Batch #	Predicted soil concentration based on PHC addition			
					ml of PHC added	PHC density g/ml	grams of PHC added	**Predicted PHC Concentration mg/kg**
July 15/2011	Fine	F2	300	1	100	0.8236	82	**309**
July 18/2011	Fine	F2	300	1	68.85	0.8236	57	**309**
July 18/2011	Fine	F2	300	2	68.85	0.8236	57	**309**
July 19/2011	Fine	F3	2600	1	700	0.8668	607	**3832**
July 19/2011	Fine	F3	2600	2	700	0.8668	607	**3832**
July 19/2011	Fine	F3	2600	3	700	0.8668	607	**3832**
July 19/2011	Fine	F3	2600	4	700	0.8668	607	**3832**
July 20/2011	Coarse	F3	600	1	150	0.8668	130	**678**
July 20/2011	Coarse	F3	600	2	150	0.8668	130	**678**
July 20/2011	Coarse	F3	600	3	150	0.8668	130	**678**
July 21/2011	Coarse	F2	160	1	65	0.8236	54	**268**
July 21/2011	Coarse	F2	160	2	65	0.8236	54	**268**
July 21/2011	Coarse	F2	160	3	65	0.8236	54	**268**

A bulk density of 1.2 g/cm^3 was targeted for calculating the required weight of soil required per soil column. Fine and coarse soil requirements were determined based on 145 cm of clean soil over 40 cm F2 contaminated soil, 40 cm F3 contaminated soil, or 40 cm control soil for a total of 185 cm soil added to each 30 cm diameter column (Figure 2). The soil was added to the columns using buckets that were calibrated to 10 kg (Plates 8 and 9). Targeted bulk densities were achieved by measuring the depth of the soil in a column and tamping to the proper height (Plate 8). A tally of buckets added was made to ensure that each treatment received the required amount of soil.

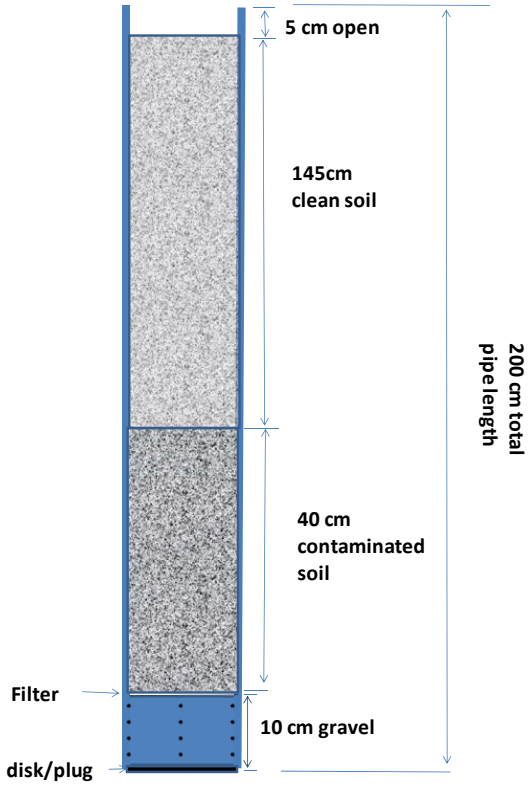

Figure 2. Soil column design and depths.

Plate 8. Calibrated bucket and tamping tool used to achieve targeted bulk density.

Plate 9. Adding soil to columns to achieve the required depth.

2.5 Seeding

The greenhouse experiment was conducted at 20°C from (6 am to 10 pm) and 15°C (10 pm to 6 am) under light conditions of L16:D8. A total of 10 plants of each canola (*Brassica napus* var. Barrier) and alfalfa (*Medicago sativa* var. PS 206 MF) were seeded in each column in August 2011 and thinned to 5 plants per column following germination and initial plant establishment. Canola plants were reseeded after each harvest and thinned to 5 plants per column after 2 weeks. Care was taken to ensure all replicates contained the same number of plants and growth stages were monitored (Plate 10).

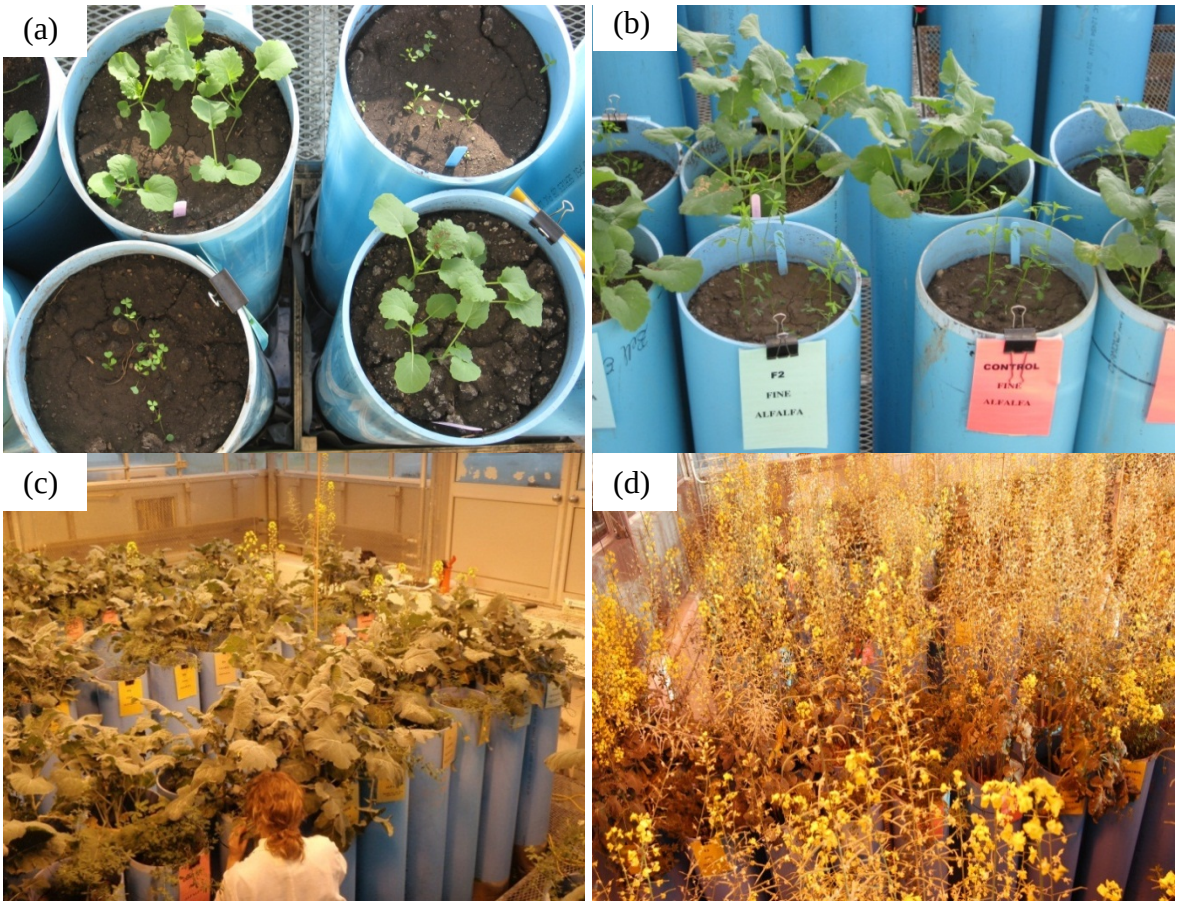

Plate 10. Growth stage of canola and alfalfa: a) seedlings taken on August 19, 2011; b) leaf development and stem elongation taken on September 5, 2011; c) inflorescence emergence (canola) and budding (alfalfa) taken on October 6, 2011 and d) flowering taken on November 16, 2011.

2.6 Watering

2.6.1 Proof of Concept Capillary Rise in Column

Prior to establishment of the column trial, a lab scale study was used to simulate the development of a water gradient using bottom irrigation (Plate 11). The lab study was designed similarly to the large column trial using 10 cm diameter clear Plexiglass columns with perforations and gravel pack to 5 cm and then filled with soil to 150 cm. The water box was then filled with water to a height of 7.5 cm (2.5 cm above the perforated gravel pack). The effect of capillary rise within the clear column was then measured on an hourly basis. The lab study results indicated that bottom irrigation would provide a moisture gradient well into the 50 cm height

(Figure 3). It was determined that adjustment and removal of the water height within the watering box would allow for moisture control within the columns for root penetration depth.

Plate 11. Lab study to evaluate the effect of bottom watering boxes and capillary rise.

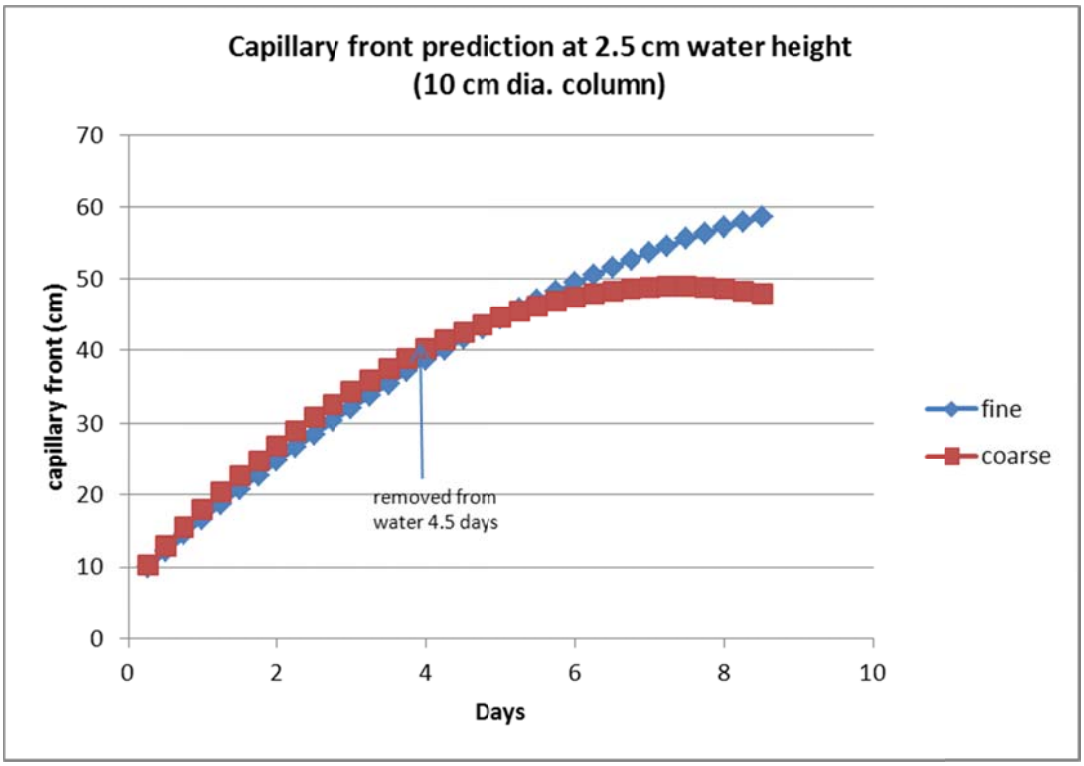

Figure 3. Lab simulation study on capillary front prediction for the fine and coarse textured soils.

2.6.2 Column Study Water Gradient

The large columns were placed in lined boxes in the sunken greenhouse to allow for water height adjustments to create a gradient in plant available soil water in the columns. The columns were designed so that water was supplied at the bottom of each column through a small diameter hole pattern drilled to a 10-cm height. The purpose of bottom irrigation was to create a vertical depth gradient in plant available soil water in the columns to ensure roots were growing as deep as possible. A 10 cm thick layer of gravel was placed at the bottom of each soil column to allow free movement of water. The water level in all watering boxes was brought to a height of 25 cm (15 cm above the perforated gravel layer) at the beginning of the experiment to create enough hydraulic head to ensure water was moving up into the columns and was reduced to 12 cm on October 6, 2011. Lowering the water created a vertical moisture gradient along the column, which was used to promote deep root growth. The capillary water supplied at the bottom is the primary source of moisture for each replicate throughout the duration of the experiment, however some watering from the top was applied for germination for the canola plants.

2.7 Pest control

Biocontrol *A. cucumeris,* a thrips predatory mite was used throughout the experiment to control the population of thrips. Other pest control methods included using "banker plants", planting pots of barley along sides of alfalfa as thrips are more attracted to barley. Despite the valiant effort of using biocontrol and banker plants, canola and alfalfa were also susceptible to other greenhouse pests such as aphids, spider mites and diamond back moths as well as powdery mildew in canola. Hence, chemical pesticides were used on a regular basis to effectively control the population of insect and diseases from damaging the crops. The following pesticides were applied to control aphids, diamondback moths, powdery mildew and thrips: Nova 40 (for powdery mildew), Success (for diamondback moths and thrips), Deltagard (for aphids) and Intercept (for aphids). Despite efforts made to control the aphids with frequent pesticide application, severe infestations were detected in early November, 2011 and end of August, 2012. Both canola and alfalfa were harvested in those instances to prevent further damage by the pests.

2.8 Aboveground biomass harvest

There were a total of four aboveground canola biomass harvests and seven aboveground alfalfa biomass harvests from August 2011 to August 2012 (Table 5). At each harvest, approximately 75 to 80% of alfalfa plants had open flowers with no seed pods and approximately 75% of canola seed pods had reached final size. After each harvest, canola was reseeded and thinned down to 5 plants after 2 weeks. The canola biomass data was normalized using a natural log transformation and then analyzed using an analysis of variance with treatment (F2, F3, and control) and harvest as fixed effects and block as the random effect. The interaction between treatment and harvest was also evaluated. If the model revealed statistical significance ($P \leq 0.05$), Tukey-Kramer adjusted comparisons were used to determine if pairwise differences existed between treatments and/or harvests. The normally distributed alfalfa biomass data was analyzed using a repeated measures analysis of variance to evaluate differences between the control and treatments (F2 and F3) while controlling for differences between the harvests. If the model revealed statistical significance ($P \leq 0.05$), Tukey-Kramer adjusted comparisons were used to determine if pairwise differences existed between treatments and/or harvests.

Pictures from each harvest are chronologically displayed in the Appendix attached to this document.

Table 7. Dates of aboveground biomass harvest for canola and alfalfa in 2011 and 2012.

Aboveground biomass harvest	Date of harvest
1st Canola harvest	Nov 11, 2011
2nd Canola harvest	March 28, 2012
3rd Canola harvest	June 12, 2012
4th Canola harvest	Aug 20, 2012
1st Alfalfa harvest	Dec 6, 2011
2nd Alfalfa harvest	Feb 10, 2012
3rd Alfalfa harvest	March 28, 2012
4th Alfalfa harvest	May 7, 2012
5th Alfalfa harvest	June 12, 2012
6th Alfalfa harvest	July 26, 2012
7th Alfalfa harvest	Aug 20, 2012

2.9 Below-ground biomass harvest

2.9.1 Column Extraction

From September 17th to 29th, columns were extracted out of the greenhouse using clamps and a hoist attached to a telescopic forklift (Plate 12). The columns were cut horizontally to preserve the intactness of the cores (Plate 12). The entire soil core was divided into three sections (Figure 4). The first section was approximately 50 cm long starting from the bottom of the column and contained the contaminated section. The second and third sections were each approximately 60 cm long. The length of the soil within each section of the column was recorded. Two soil samples were collected using a 3-inch (7.62 cm) soil corer from each section at the designated lengths (Figure 4) and were combined into one composite sample and stored at 4°C until root washing (Plate 13). For the alfalfa treatments, coarse roots (>2 mm) were also manually picked out from each section by hand and weighed separately for total coarse root biomass.

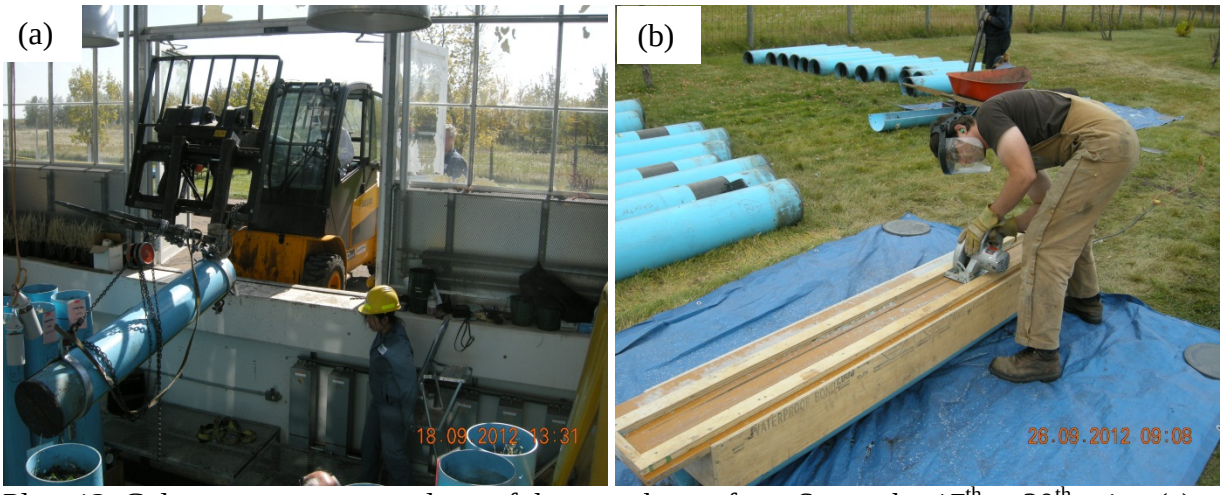

Plate 12. Columns were extracted out of the greenhouse from September17th to 30th using (a) clamps and a hoist attached to a telescopic forklift. The columns were (b) cut open length wise with a circular saw.

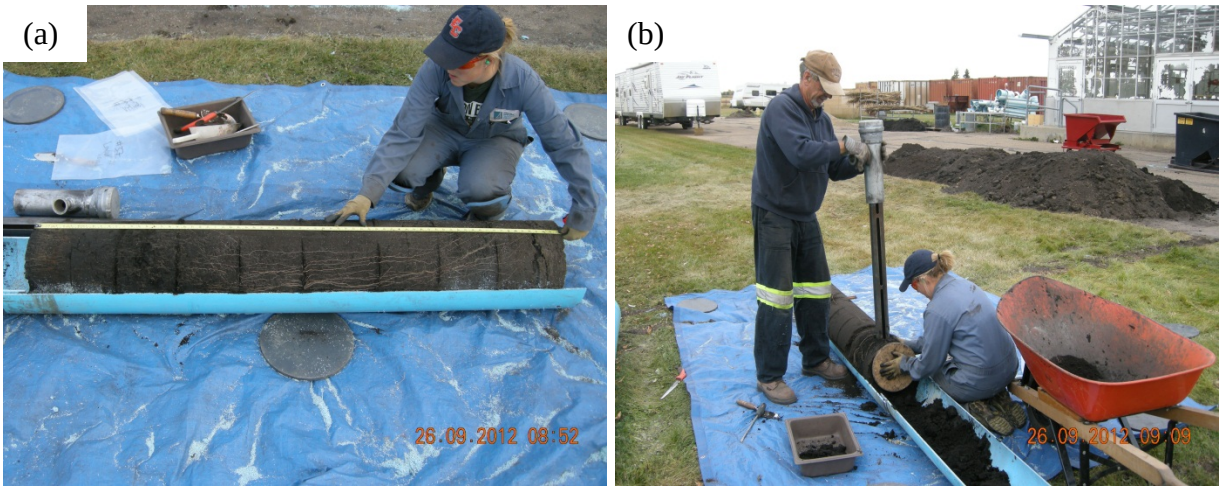

Plate 13. Each column was (a) divided into upper, mid and lower sections and (b) two soil samples were collected from each section using a 3 inch soil corer.

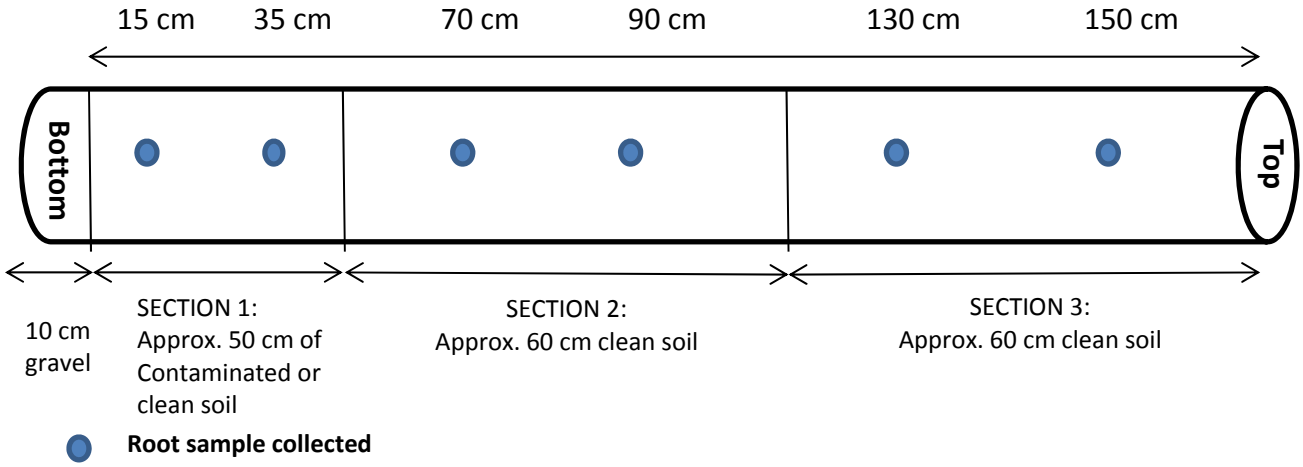

Figure 4. Sampling scheme for root biomass determination.

2.10 Post experiment soil sampling

A composite soil sample was randomly collected in the lower section (contaminated zone) from the columns with the red circles in Figure 5. These soil samples were submitted to ALS Environmental for F2 and F3 PHC analysis. The remaining contaminated soils were stored and disposed of appropriately.

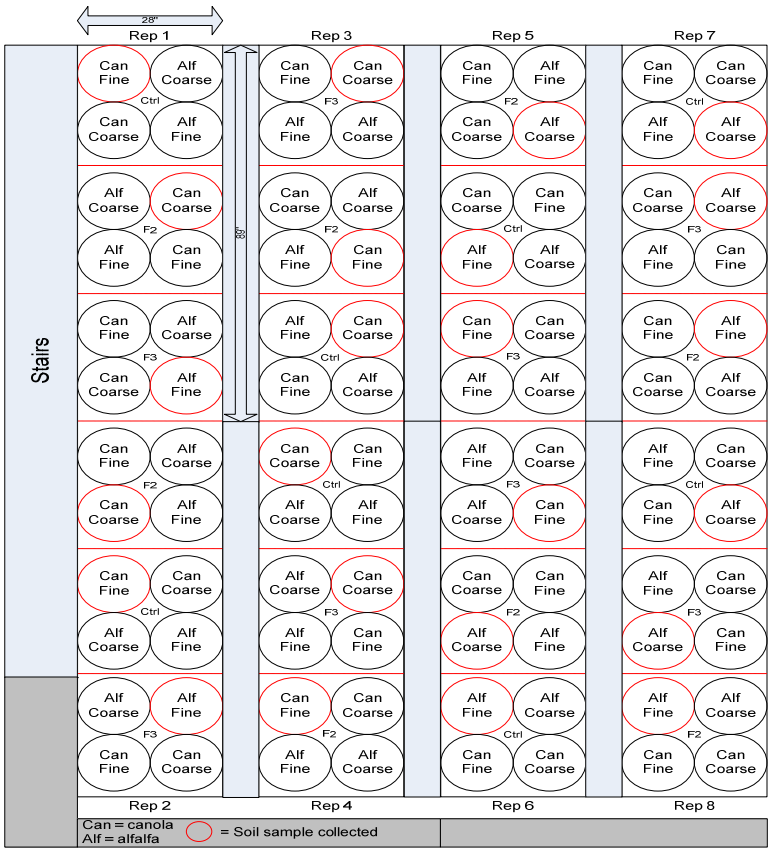

Figure 5. Soil sampling diagram for petroleum hydrocarbon levels post experiment.

2.11 Root washing

2.11.1 Alfalfa roots

Approximately 2.8 L of soil was collected from each section per column. The soil collected from the upper and mid sections were typically dry, therefore the samples were first sieved through ~1 mm diameter mesh (Plate 14). The fine roots (< 2mm diameter) were removed from the soil using tweezers. The coarse roots (> 2mm diameter) from the entire section were removed from the soil by hand during root sampling and were also washed and oven drying at 40°C (Plate 14). Mortar and pestle were used to gently break up clumps of the soil. The lower sections were generally wet and therefore the fine roots were collected from washing the soil under a gentle stream of water through a 0.5 mm diameter sieve (Plate 14). The coarse roots were manually removed from the soil and were also washed and oven dried (Plate 16).

Plate 14. The soils from the (a) upper and lower sections were dry sieved and fine roots were removed with tweezers. The soils from the lower sections were (b) washed under water and roots were collected using a sieve. The coarse roots were (c) removed from each section during root sampling and (d) washed separately.

2.11.2 Canola roots

Unlike Alfalfa, there were no coarse roots in the canola treatments. For the upper and mid-sections, the entire soil sample was sieved through a fine mesh (~ 1mm diameter) (Plate 15). Clumps of soils were gently broken up using a mortar and pestle. Fine roots (<2 mm in diameter) were picked out with tweezers, and the roots were washed prior to oven drying at 40°C (Plate 3). The samples from lower sections were similarly dealt with as the alfalfa samples. The samples were washed under a gentle steam of water through a 0.5 mm sieve. The fine roots were removed using tweezers and oven dried (Plate 16).

(a)

(a)

Plate 15. Root samples from each section of the column were washed separately. The fine roots from the lower sections were washed under water with a sieve (a) while the roots in the mid (b) and upper (c) sections were picked out by tweezers. The coarse roots (c) were hand picked out and washed separately.

(a)

(a)

Plate 16. Both the (a) fine and coarse roots from alfalfa and (b) fine roots from canola were dried and weighed separately by section.

3.0 RESULTS AND DISCUSSIONS

3.1 Aboveground biomass

3.1.1 Canola

The total aboveground plant biomass for each of the canola harvests is presented in Table 9. Overall, for canola biomass in coarse soil, there were significant treatment and harvest effects (p=0.0350 and p<0.0001, respectively). The interaction between treatment and harvest was not significant (p=0.0809). Both the F2 and F3 treatment in the coarse soil yielded significantly less

biomass than the control in the 2nd harvest. Although the yields from the various treatments in the 3rd or 4th harvest were not statistically different, the F3 treatment in the coarse soil yielded less aboveground biomass compared to the control. Overall, F3 had significantly lower canola biomass than the control in the coarse soil (p=0.0280). No significant treatment effects were observed for F2 PHC in coarse textured soil.

Although the F3 treatment in the fine soil yielded less biomass than the control in the first harvest, overall for fine soil in terms of the canola biomass, there was no significant treatment effect or interaction between treatment and harvest (p=0.5061 and p=0.0640, respectively). There was a significant different between harvests in terms of the canola biomass (p<0.0001). Harvest 2 had significantly lower canola biomass than harvests 1, 3, and 4 (all p<0.0001). Harvest 4 had significantly lower canola biomass than harvest 1 and 3 (both p<0.0001). Harvest 3 had significantly lower biomass than harvest 1 (p<0.0001). Note that the differences between harvests were consistent for both the coarse and fine soil in terms of canola biomass. The variation in harvest biomass was largely due to the seasonal effect (i.e. daylight and UV intensity between summer and winter) as well as loss of yield as a result of insect and disease infestation.

Table 8. Aboveground plant biomass means and standard deviation (in brackets) for canola harvests conducted in 2011 and 2012 for all 8 replicates.

Canola Harvest	Date of harvest	Mean Aboveground Plant Biomass (g)					
		Coarse Soil			Fine Soil		
		Control	F2 PHC	F3 PHC	Control	F2 PHC	F3 PHC
Harvest 1	11/11/11	170 (55.5)	185.6 (40.1)	165.9 (42.1)	172.7[a] (49.6)	181.6[a] (37.4)	122.7[b] (30.0)
Harvest 2	03/28/12	26.7[a] (9.6)	17.5[b] (4.5)	16.1[b] (5.8)	16.1 (8.6)	21.4 (9.5)	20.8 (4.5)
Harvest 3	06/12/12	104.1 (20.0)	107.11 (14.2)	95.4 (18.7)	72.2 (14.9)	74.2 (14.1)	81.3 (16.8)
Harvest 4	08/20/12	51.0 (14.63)	41.7 (12.9)	44.9 (12.9)	37.5 (11.5)	37.2 (19.6)	38.3 (10.9)
Average yield (std.dev.)		88.0 (64)	91.2 (72)	80.6 (66)	73.2 (69)	78.2 (72)	65.5 (46)

[a-b] Means in a column followed by the same letter are not significantly different according to the Fisher's least significant difference (LSD) test (P = 0.05).

3.1.2 Alfalfa

There were more alfalfa harvests than canola harvests because alfalfa is a perennial crop and thus re-seeding was not required after each harvest and hence less time was needed to reach similar maturity level as canola. The total aboveground plant biomass for each of the alfalfa harvests is

tabulated in Table 10. In terms of the alfalfa biomass for coarse soil, there was no significant treatment effect or treatment by harvest interaction (p=0.5869 and p=0.5744, respectively).

For fine soil, there was no significant overall treatment effect for the alfalfa biomass (p=0.4884), however there was a significant harvest and treatment by harvest interaction (p<0.0001 and p=0.0006, respectively). As was observed in the canola crops, there was a significant decrease in alfalfa biomass in the first stimulated growing season in the F3 treatment for the fine textured soil (p<0.0001). There were no other significant treatment differences. Harvest 1 had significantly lower biomass than all the other harvests (all p<0.0001). Harvest 7 had significantly lower biomass than harvests 2, 3, 4, 5, and 6 (all p<0.0010). Harvests 5 and 6 had significantly lower alfalfa biomass than harvests 2 and 3 (all p<0.02). Finally harvest 4 had significantly lower alfalfa biomass than harvest 3 (p<0.0001). In the 6th harvest (on July 26th), the control yielded less biomass than the F3 treatment. However, this anomaly was related a thrips infestation that affected the control treatments more than the F3 treatments.

Compared to the canola harvest yields, the standard deviations for the average alfalfa yield based on seven harvests were much smaller, indicating less variation in biomass between each harvest. This is not surprising given that alfalfa is a hardier plant than canola especially in the greenhouse, partly due to the leguminous nature and its perennial life cycle and it also has fewer disease and insect issues compared to canola.

Table 9. Aboveground plant biomass means and standard deviation (in brackets) for alfalfa harvests conducted in 2011 and 2012 for all 8 replicates.

Alfalfa Harvest	Date of harvest	Mean Aboveground Plant Biomass (g)					
		Coarse Soil			Fine Soil		
		Control	F2 PHC	F3 PHC	Control	F2 PHC	F3 PHC
1st harvest	12/16/11	30.5 (17.8)	26.4 (11.1)	26.9 (16.0)	30.0[a] (3.9)	27.0[a] (7.3)	10.8[b] (3.9)
2nd harvest	02/10/12	68.8 (28.5)	65.0 (22.9)	68.3 (28.9)	69.4 (19.5)	74.9 (17.8)	52.4 (23.0)
3rd harvest	03/28/12	94.3 (37.9)	86.9 (28.7)	87.0 (25.0)	74.3 (7.1)	76.5 (16.0)	67.4 (22.5)
4th harvest	05/07/12	85.4 (28.2)	79.3 (13.2)	88.4 (16.4)	52.6 (8.4)	57.1 (8.9)	53.3 (22.6)
5th harvest	06/12/12	67.3 (17.6)	64.7 (12.3)	63.6 (9.1)	43.1 (5.5)	52.4 (16.5)	50.1 (10.3)
6th harvest	07/26/12	66.2 (14.8)	56.9 (9.3)	59.0 (14.6)	40.7[a] (7.8)	49.7[a] (15.6)	58.2[b] (7.7)
7th harvest	08/20/12	49.7 (10.4)	42.5 (13.6)	47.3 (10.2)	33.8 (7.4)	33.8 (4.5)	42.0 (7.8)
Average yield (std.dev.)		66 (21)	61 (21)	63.1 (22)	49.1 (17)	53.1 (19)	47.7 (18)

[a-b] Means in a column followed by the same letter are not significantly different according to the Fisher's least significant difference (LSD) test (P = 0.05).

3.2 Below-ground biomass

3.2.1 Canola

To fully understand the effect of PHC on the crop health, it is important to evaluate the effect of PHC on the below-ground biomass, especially the fine root portion because they comprise the majority of the root surface area and root length responsible for water and nutrient uptake (Zobel et al., 2007). The total fine root biomass for each section, calculated based on the amount of fine roots collected from the two 3 inch (7.62 cm) soil core samples, are tabulated in Table 11. Statistics were conducted on the total root biomass and not the root biomass from the two 3-inch soil cores. The only significant difference was found in the lower sections for the fine textured soil between the control and F3 treatments. Overall, there was more root biomass in the lower section than the mid and upper sections in all treatments for both soil types, however the standard deviations for the lower sections were very high. This large variability in root biomass between replicates, especially in the lower section was observed during root sampling (Figure 7). Given the large variability in aboveground biomass (Table 9), it is likely that some reps were less affected by disease and insect and thus were able to produce more above- and belowground biomass when compared to those more affected. Contrary to the above-ground biomass data, there was more below-ground biomass in the fine texture soil than coarse textured soil (Figure 6). This observation may be a response to the water-stress condition that the canola

plants were subjected to. Canola plants have the ability to change root distribution with depth to exploit water deeper in soil profiles, an important mechanism to avoid drought stress (Liu et al., 2010).

Canola have a taproot system, which under bottom irrigation conditions can 'force' the roots to grow in the lower, wetter contaminated sub-soil zone. Thus, this study represents the worst case scenario for examining the effect of PHC at guideline levels on deep rooting agricultural crop species. Not surprisingly, the effect of PHC on below-ground biomass in canola was observed only in the lower section where the contaminated soils were located. Furthermore, the decrease in below-ground biomass was very much limited to the F3 treatments in fine textured soil.

Table 10. Below ground root biomass means and standard deviation (in brackets) for canola.

Crop /Root Type	Section	Mean Root Biomass and Standard Deviation (g)					
		Coarse Soil			Fine Soil		
		Control	F2 PHC	F3 PHC	Control	F2 PHC	F3 PHC
Canola Fine Roots*	Upper	3.4 (3.7)	2.4 (1.0)	3.8 (3.4)	4.2 (2.0)	3.7 (1.8)	3.4 (2.3)
	Mid	1.9 (1.0)	3.0 (1.0)	2.3 (1.1)	2.3 (0.6)	3.0 (1.0)	2.5 (1.2)
	Lower	6.1 (6.1)	6.0 (5.1)	7.9 (7.4)	9.3[a] (3.0)	9.2[a] (5.0)	3.7[b] (3.4)
	TOTAL	11.4	11.4	14.0	15.8	15.9	9.6

* Fine root biomass data for each section was extrapolated from the two 3 inch soil core samples.
[a-b] Means in a column followed by the same letter are not significantly different according to the Fisher's least significant difference (LSD) test ($P = 0.05$).

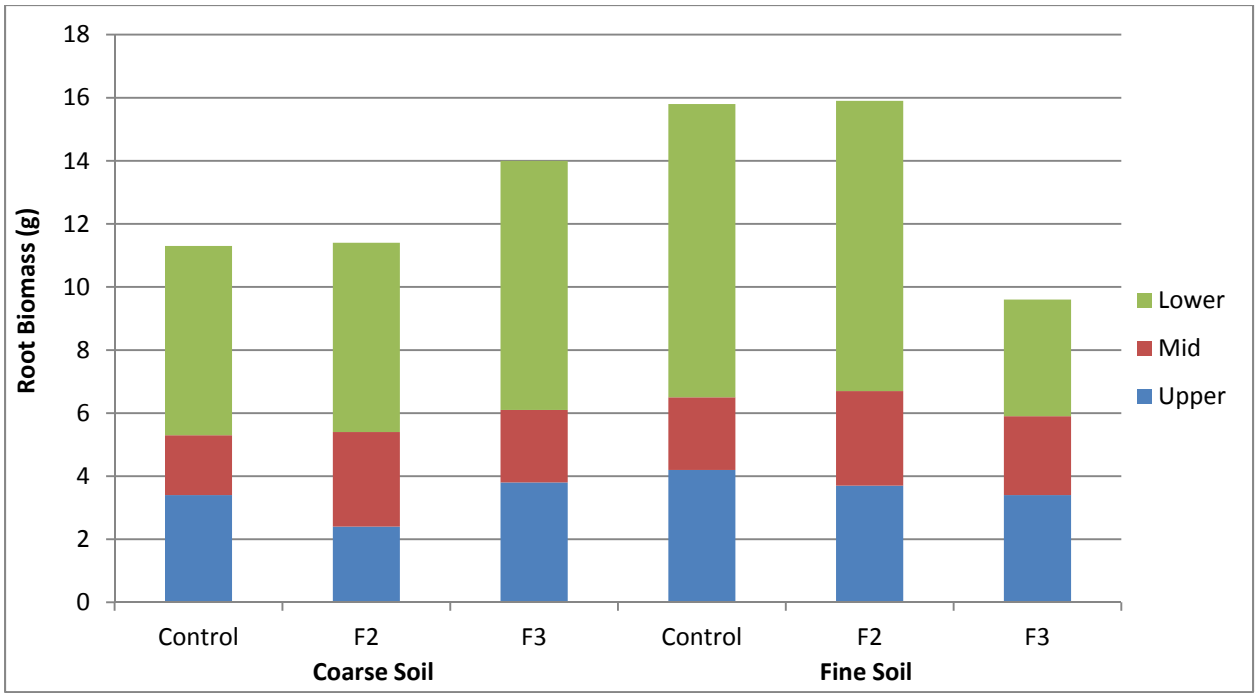

Figure 6. Total canola fine root biomass for control, F2 and F3 treatments in coarse and fine textured soil extrapolated from biomass collected in soil cores.

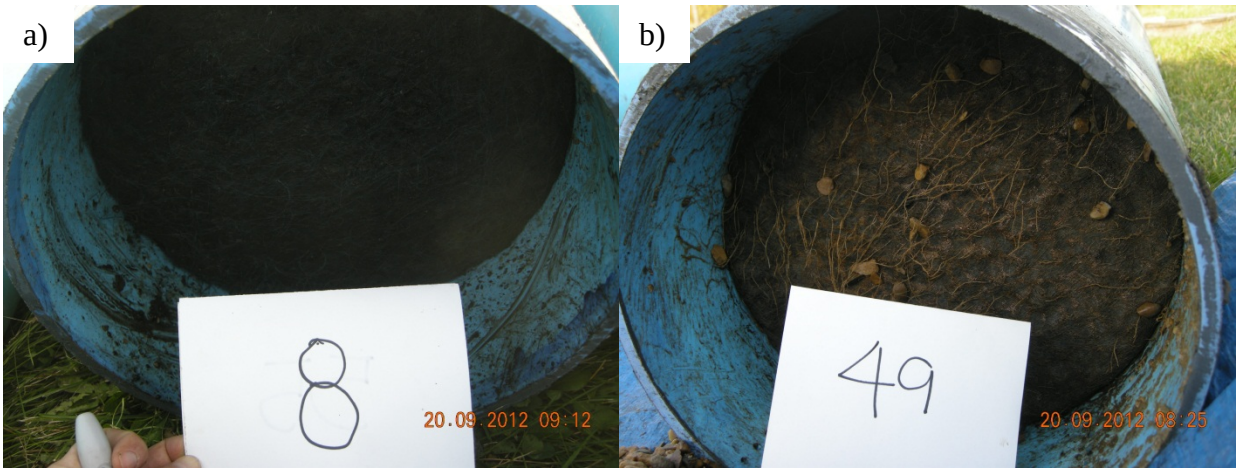

Figure 7. Fine root growth in F2 PHC canola fine soil treatments in a) rep 1 and rep 5.

3.2.2 Alfalfa

Total fine roots (<2 mm) biomass, calculated from the two 3 inch (7.62 cm) soil cores in each section and the total coarse roots (>2 mm) are tabulated for the different treatments in both fine and coarse soil in Table 9. The majority of the treatments were not significantly different from the control. The only significant difference in fine root biomass was found in the lower sections for the fine soil type between the control and F2 as well as the control and F3 treatments. There

were no significant differences in coarse root biomass between the treatments. Overall, there was a greater portion of fine root biomass found in the lower section than the mid and upper section for all treatments (Figure 8). There was also more preferential fine root growth along the walls of the columns in the alfalfa treatments than the canola treatments (Figure 9). Contrarily, there is greater portion of coarse roots found in the upper section than mid and lower sections for all treatments. Similar to the root biomass data for canola, there is greater root biomass (both coarse and fine roots) recovered in the fine texture soil than coarse textured soil (Figure 8), this was most likely a response to the water stress conditions that the plants were subjected to.

Table 11. Below ground root biomass means and standard deviation (in brackets) for alfalfa.

Crop/ Root Type	Section	Mean Root Biomass and Standard Deviation (g)					
		Coarse Soil			Fine Soil		
		Control	F2 PHC	F3 PHC	Control	F2 PHC	F3 PHC
Alfalfa Fine Roots*	Upper	4.1 (1.7)	3.5 (1.2)	4.2 (2.3)	4.2 (1.4)	2.6 (1.7)	5.4 (2.4)
	Mid	2.6 (0.7)	3.0 (1.3)	3.0 (1.0)	2.4 (1.5)	3.3 (2.1)	3.7 (1.3)
	Lower	14 (11)	12 (5.0)	16 (11)	44[a] (23)	20[b] (14)	21[b] (21)
	TOTAL	20.7	18.5	23.2	50.6	25.9	30.1
Alfalfa Coarse Roots	Upper	40 (14)	43 (10)	41 (12)	44 (12)	47 (6.0)	42 (12)
	Mid	18 (7.0)	24 (15)	15 (5.0)	16 (4.6)	17 (4.7)	14 (2.5)
	Lower	4.8 (2.6)	7.7 (5.0)	5.5 (2.0)	7.1 (2.2)	6.3 (2.4)	5.4 (1.8)
	TOTAL	62.8	74.7	61.5	67.1	70.3	61.4

* Fine root biomass data for each section was extrapolated from the two 3 inch soil core samples.
[a-b] Means in a column followed by the same letter are not significantly different according to the Fisher's least significant difference (LSD) test ($P = 0.05$).

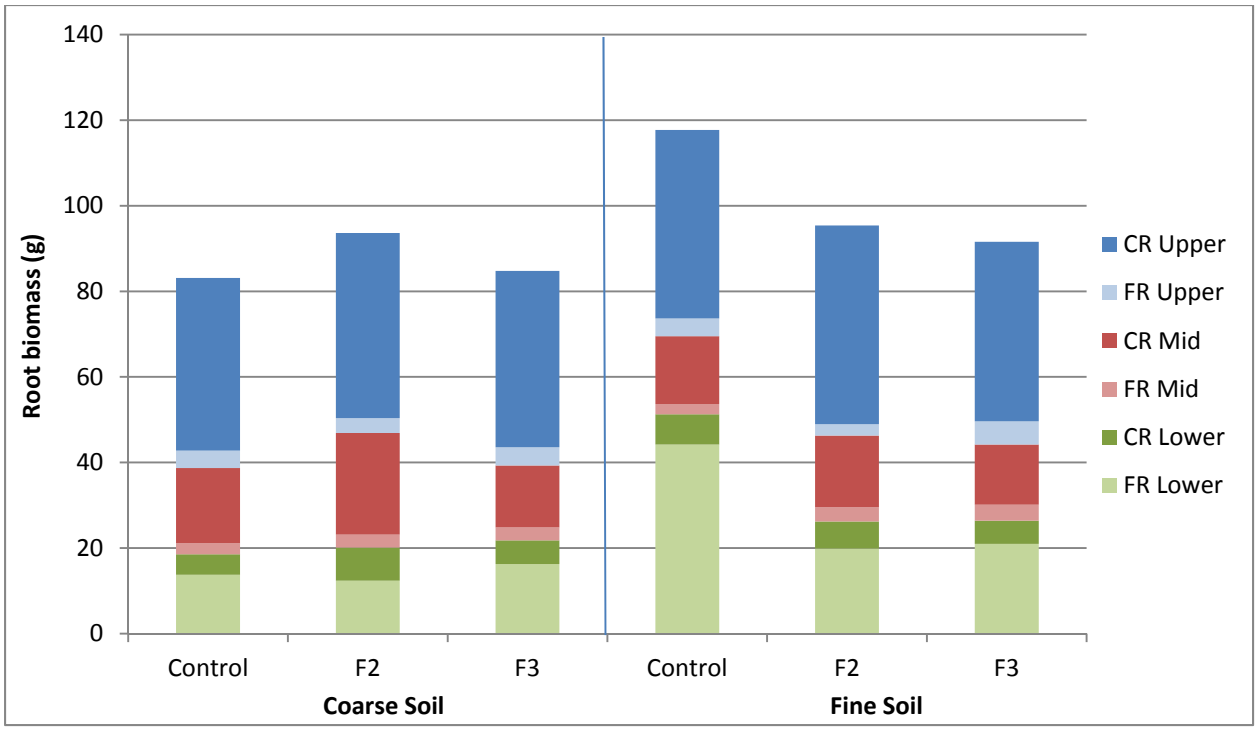

Figure 8. Total alfalfa root biomass including coarse roots (CR) and fine roots (FR) for Control, F2 and F3 treatments in coarse and fine textured soil. Fine roots biomass was calculated from soil cores.

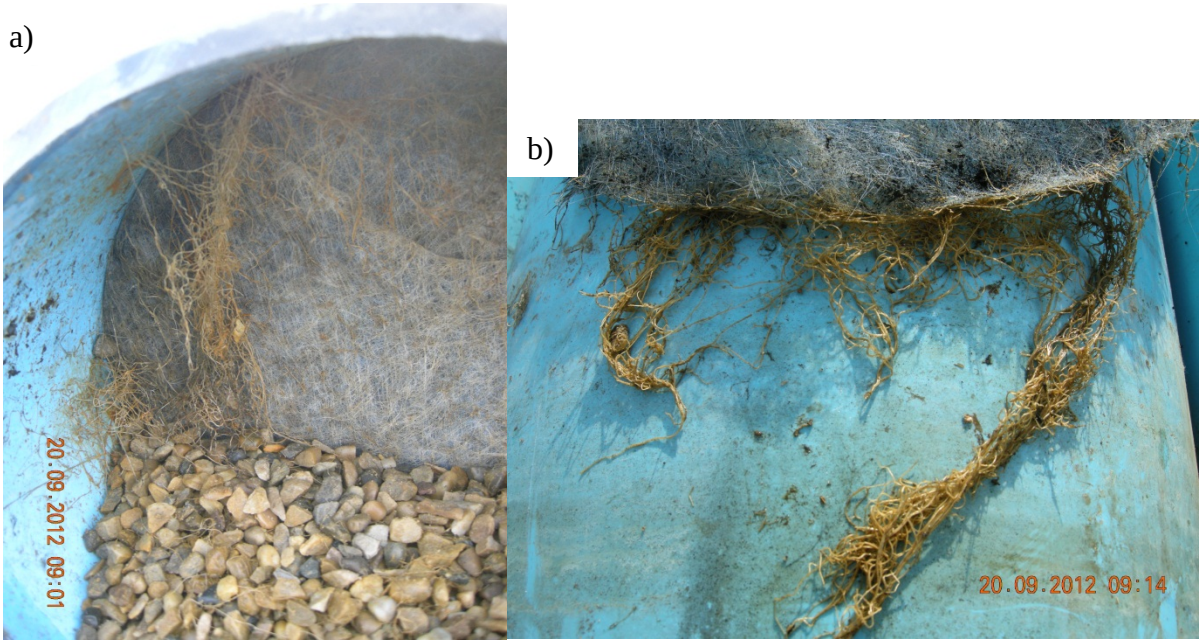

Figure 9. Preferential root growth along the walls of the column in F3 PHC alfalfa coarse texture soil treatment a) rep 1 and b) rep 3.

3.3 PHC level in the soil post experiment

The soil samples from the lower section (the contaminated zone) collected during below-ground biomass sampling were submitted to EXOVA Laboratory (in Edmonton) to determine the concentration for each PHC fraction. The PHC fractions were extracted using the shakeout method in 50:50 hexane and acetone with silica gel cleanup; the extracts were analyzed according to the Reference Method for the Canada-wide Standard for Petroleum Hydrocarbons in Soil – Tier 1 Standard (Canadian Council of Ministers of the Environment, 2001). EXOVA reported that all F2 fractions in both coarse and fine textured contaminated soil were below the method detection limit of 50 mg/kg (Table 10). The F2 concentrations post-experiment has decreased significantly compared to the initial F2 concentrations. Some microbial degradation as well as leaching of the F2 PHC to the water below is expected. The F3 concentration from both coarse and fine soils also came back less than the initial F3 levels analyzed at the beginning of the study (Table 10). The measured F3 concentrations ranged from 1270 to 2380 mg/kg in the fine textured soil, while the F3 concentration was measured at 136 mg/kg in the coarse textured soil. Similar to F2 PHC, some losses of the F3 PHC is expected through microbial degradation, however the value reported for the F3 concentration post experiment is questionable. With the presence of fine and coarse (alfalfa only) roots in the subsoil (lower) sections and a substantial decrease in both the F2 and F3 PHC concentrations post-experiment, it is reasonable to assume that F2 and F3 PHC were biodegraded through the stimulation of microorganisms in the bulk soil as well as the rhizosphere. The impact of PHC on above and below-ground biomass will likely lessen as PHC dissipates with time and as roots establish in the contaminated subsoil region.

Table 12. Measured petroleum hydrocarbon concentrations from the spiked soil used in this study before and after the experiment.

Sampling Date	Soil Texture	PHC Fraction	Subsoil Guideline	Batch	Targeted	Measured PHC[a]	Measured PHC[b]	Measured PHC[c]
					mg/kg	mg/kg	mg/kg	mg/kg
July 18/2011	Fine	F2	300	1-1	309	149	-	-
July 18/2011	Fine	F2	300	1-2	309	74	-	-
July 18/2011	Fine	F2	300	2-2	309	114	-	-
Oct 4/2011	Fine	F2	300	1-1 (Rep7)	309	-	561[d]	-
Oct 4/2011	Fine	F2	300	2-2 (Rep1)	309	-	80[d]	-
July 21/2011	Coarse	F2	160	1-3	268	63	-	-
July 21/2011	Coarse	F2	160	2-3	268	50	-	-
July 21/2011	Coarse	F2	160	3-3	268	50	-	-
Oct 4/2011	Coarse	F2	160	1-3 (Rep 7)	268	-	215[d]	-
Oct 4/2011	Coarse	F2	160	3-3 (Rep 2)	268	-	66[d]	-
July 19/2011	Fine	F3	2600	1-4	3832	1670	4810	-
July 19/2011	Fine	F3	2600	2-4	3832	1790	2810	-
July 19/2011	Fine	F3	2600	3-4	3832	2030	3800	-
July 19/2011	Fine	F3	2600	4-4	3832	2220	2900	-
July 20/2011	Coarse	F3	600	1-3	678	557	669	-
July 20/2011	Coarse	F3	600	2-3	678	416	739	-
July 20/2011	Coarse	F3	600	3-3	678	342	612	-
Sept 30/2012	Fine	F2	300	Rep 7	309	-	-	<50
Sept 30/2012	Fine	F2	300	Rep 2	309	-	-	<50
Sept 30/2012	Fine	F2	300	Rep 3	309	-	-	<50
Sept 30/2012	Fine	F2	300	Rep 8	309	-	-	<50
Sept 30/2012	Coarse	F2	160	Rep 5	268	-	-	<50
Sept 30/2012	Coarse	F2	160	Rep8	268	-	-	54
Sept 30/2012	Coarse	F2	160	Rep 1	268	-	-	<50
Sept 30/2012	Fine	F3	2600	Rep 1	3832	-	-	1270
Sept 30/2012	Fine	F3	2600	Rep 8	3832	-	-	2380[e]
Sept 30/2012	Fine	F3	2600	Rep 8	3832	-	-	1250[f]
Sept 30/2012	Fine	F3	2600	Rep 5	3832	-	-	1810
Sept 30/2012	Fine	F3	2600	Rep 6	3832	-	-	1440
Sept 30/2012	Coarse	F3	600	Rep 3	678	-	-	136

[a]PHC analyzed by ALS.

[b]PHC analyzed by AITF Trace Organics Laboratory.

[c]PHC analyzed by EXOVA.

[d]These samples were collected from the contaminated zone through holes drilled in the PVC pipes.

[e,f] Both of these samples were collected from the same column. [e]was collected at the lower 15 cm and [f]was collected from the 15-30 cm region.

4.0 CONCLUSIONS

This study represents the worst case scenario for examining the effect of PHC at guideline levels on deep rooting agricultural crop species. Both canola and alfalfa have a taproot system, which under drought or moisture limiting conditions can extend into the sub-soil zone.

This study demonstrated that there were no significant overall treatment effects (F2 or F3) in coarse or fine textured soil for alfalfa and no significant overall treatment effects (F2 or F3) in fine textured soil for canola. However, in the first simulated growing season, there was an effect of the contaminated F3 subsoil on aboveground biomass for both canola and alfalfa in the fine textured soil. No significant effects were observed in the fine textured soil in the subsequent harvests. The effect of PHC on the root biomass was observed in the fine root fraction in fine textured soils only. F2 and F3 PHC impacted the alfalfa fine root biomass whereas only the F3 PHC impacted the canola fine root biomass.

There was an overall significant F3 treatment effect in coarse textured soil for canola aboveground biomass. Effects of contaminated F2 and F3 subsoil were observed in the canola coarse soil treatments in the second simulated growing season, however statistical analysis concluded the only significant effect was for the F3 treatment. Overall conclusions for each soil type, F2 and F3 contamination and plant species include:

- **F2 contaminated COARSE textured soil** – no treatment effect on above and below-ground biomass for alfalfa or canola
- **F2 contaminated FINE textured soil** – no treatment effect on above ground biomass for alfalfa or canola; significant treatment effect on alfalfa fine root biomass; no effect on alfalfa coarse root biomass or canola fine root biomass
- **F3 contaminated COARSE texture soil** – overall treatment effect on above ground biomass for canola; no treatment effect on aboveground biomass for alfalfa; no treatment effect on below ground biomass for alfalfa and canola
- **F3 contaminated FINE textured soil** – no treatment effect on aboveground biomass for alfalfa and canola; significant treatment effect for alfalfa fine root biomass and canola fine root biomass; no effect on alfalfa coarse root biomass

The current PHC levels for the underlying “subsoil” was arbitrarily set by AESRD based on the assumption that the surface soil criteria can be exceeded in the subsoil by a factor of two due to the lack of eco-contact at depth. Results from this study suggest there may not be a phytotoxic effect on alfalfa under the current F2 PHC subsoil guidelines for both fine and coarse textured soil. Based on the results of this study, there was no irreparable yield loss due to subsoil

contaminated with F2 and F3 hydrocarbons at levels near Alberta Tier 1 guideline concentrations, therefore the significance of the PHC effect on fine root biomass is uncertain. Results may differ under field conditions where roots are exposed to soils contaminated by weathered hydrocarbons as opposed to freshly spiked soils under greenhouse conditions where direct exposure to fresh hydrocarbons is different than what roots would experience on a wellsite.

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