PLANT UPTAKE OF PETROLEUM HYDROCARBONS AND TRACE ELEMENTS-DERIVATION OF SOIL-TO-PLANT UPTAKE FACTORS

Gwen O'Sullivan

MOUNT ROYAL UNIVERSITY Department of Earth & Environmental Science, Mount Royal University
4825 Mount Royal Gate SW, Calgary, AB CanadaT3E 6K6

1. Introduction & Project Scope

Uptake of organic and inorganic contaminants into plants is an important route for assessing the exposure of humans and animals to toxic chemicals (1–5). Plants are the base of food webs and the largest component of the earth's biomass. Quantifying the uptake of contaminant by plants is essential for assessing human and ecological risk. Contamination of soil by inorganic and organic contaminants, including petroleum hydrocarbons (PHC) and trace metals, is a growing problem. The large number of sites and the extent of contamination make it a multibillion-dollar problem in Canada (6). Environmental risk assessment (ERA), including the application of environmental modeling, informs regulatory decisions in Canada. The Canadian Council of Ministers of the Environment (CCME) has developed a Canada-Wide Standard (CWS) with remedial guidelines and risk assessment framework for human and ecological exposure to PHCs (7). The CWS-PHC sets out generic remedial target levels (Tier 1), as well as a process for generating site-specific standards based on risk that are protective of human and ecological health (Tier 2 & 3). The risk posed is determined by both the hazardous property of the chemicals and by the nature of the exposure (8). Broadly, the ERA process involves identification of site-specific receptors (human and ecological), contaminants, and exposure pathways through which a receptor exposure can occur. One exposure pathway that is of particular importance to human and ecological receptors is ingestion of plant parts (for instance, crops, herbs, flowers and berries). However, an assumption in the derivation of CWS-PHC standards is that plants do not up-take PHCs from contaminated soils, suggesting that exposure to higher trophic levels is not a concern (9). Numerous studies however have reported plant up-take of PHCs, including aliphatic (10–12) and aromatic compounds (13–17), suggesting that this approach to risk assessment underestimates exposures and subsequent risks to ecological and human receptors (9). The uptake of organic and trace metal pollutants by plants at a contaminated site is therefore an important component of the ERA when determining the suitability of study areas for future development or existing use (18).

To evaluate exposure to contaminants in plants, environmental models may include plant uptake factors (PUFs). These factors are essentially a ratio of the contaminant concentration in plants to contaminant concentration in soil. There are very few PUFs available for PHCs and trace metals and are generally estimated from parameters such as the octanol/water partition coefficient (Kow) and not empirical data (19,20). PUFs for different organic chemicals vary considerably (2,18) due to the chemical's properties (e.g. Kow, chemical size), heterogonous soil (e.g. soil organics, pH), and plant properties (e.g. plant lipids) (4). The magnitude of PUF values can significantly affect the calculated mass of chemicals remaining in the soil pore water and subsequently the predicted concentrations in environmentally relevant compartments e.g. plant, leaching potential from soil etc. Often within exposure assessment tools, default parameter values may be chosen. These default values might be inappropriate for soil and plant types other than those for which they were developed (4,21). In the absence of default values, zero has also been applied in many environmental fate models e.g. PEARL, PELMO, PRIZM, RAIDER etc. thus removing plant uptake as a pathway for exposure. There are few studies testing the performance of plant uptake models against real world data, despite the fact that these tools are routinely used in human exposure assessment. There is a need for additional research to develop systematic experimental (lab and field) PUFs specific for chemicals, plants, and environmental conditions to provide more meaningful predictions of potential toxicity to humans and wildlife ingesting plants (5). In addition, changes to the CWS-PHC criteria have shifted the governing fractions of PHC from the F3 fraction (>nC16 to nC34) to the F2 fraction (>nC10 to nC16) for a number of the soils type, land and water use combinations. This decrease in target

PHC-F2 value, and increase in associated remediation cost of downstream sites, highlights the importance of fully understanding the fate and behavior of contaminants to better model ecological and human receptors and pathways. Using shared information, technology, resources, and with access to industry sites and equipment, the goals of the partnership are to:

1) Develop a plant uptake model, for CWS PHC-F2 fraction and salinity, for major plant functional types in Western Canada;
2) Assess the new model's performance against field data, from Western Canada, to test the statistical predictability that PUFs will correctly determine the concentration of PHC-F2 faction and trace metals in plants; and
3) Evaluate how the inclusion of the soil-plant pathway would influence risk assessment used in CWS-PHC Tier 3 across Canada.

2. Summary of Work Completed

The proposal we submitted initially for this project included both greenhouse and field studies with the expectation that we would apply to the NSERC Alliance Grant to provide financial support for the fieldwork. While we did apply we were unfortunately not successful in securing the grant so the scope of the project was reduced to element that were supported by PTAC e.g. greenhouse studies.

The objectives of the greenhouse plant studies were to evaluate the uptake of PHCs and trace metal in plants and determine contaminant mass balance, metabolic turnover and species-dependent soil-to-plant UFs. A series of greenhouse studies were completed including hydroponics and short and long-term studies in soil. The following sections outline the work completed in each of these studies.

2.1. Plant Species Selection

Plant species for the studies were selected to ensure variation in functional types, availability and ease to work with in the laboratory, growth characteristics (e.g., speed of growth, time to flowering) and importantly their presence in Alberta ecosystems (Table 1). A series of experiments were completed including 9-day hydroponics (metals), 9-days soil (metals), 3 month- metals & PHC and the plant species used in each are summarized in Table 1.

Table 1 Plant species and functional type, typical range in Alberta, and studies used in

Functional Type	Common Name	Species	Range	Studies
Grass	Barley	*Hordeum vulgare*	Peace River Region, Central AB, and Southern/Southeastern AB	Hydroponics, Soil-9 day, Soils-3 month, Soils-PHC
Grass	Italian ryegrass	*Lolium multiflorum*	Peace River Region, Central AB, and Southern AB (irrigation)	Hydroponics, Soil-9 day, Soils-3 month, Soils-PHC
Grass	Corn (Maize)	*Zea mays*	Southern region of AB	Hydroponics, Soil-9 day, Soils-3 month, Soils-PHC

Forb	Yarrow	*Achillea millefolium*	Throughout AB	Hydroponics, Soils-3 month, Soils-PHC
Legume	Pea plant	*Pisum sativum*	Peace River Region, Central and Southern AB	Hydroponics, Soil-9 day, Soils-3 month, Soils-PHC

2.2. Greenhouse Studies

2.2.1. Hydroponics – Metals 9 Day Uptake Study

Table 2 summaries the design of the hydroponic 9-day study for 3 plant species and 4 trace metals (chromium, copper, nickel, and lead) at 5 times CCME guidelines. For each plant species separate hydroponic studies were set up for individual metals. In brief, seeds were germinated in perlite (Figure 1) to BBCH 12 (two leaf unfolded stage), transferred to nutrient solution, and preconditioned to BBCH 13 (3 leaves unfolded stage). They were then transferred to the test solution and grown for 9 days in a growth chamber under controlled light (16 hrs. light/8 hrs. dark) and temperature (23°C ± 2°C).

Table 2 Summary of experimental design, qa/qc controls and number of replicates for each hydroponic study for each plant species and target metals e.g. chromium, copper, nickel, lead

Experiment	Plant	Test Solution	Nutrient Solution	Replicates
Spike – Type 1	Yes	Yes	Yes	4
Spike – Type 2	No	Yes	Yes	2
Blank – Type 1	Yes	No	Yes	2
Blank – Type 2	No	Yes	Yes	2
Blank- Type 3	No	No	Yes	2

On day 0, 1, 2, 3, 6, 7 and 9, aliquots of the test solution were sampled for chemical analysis. In addition, rate of evapotranspiration, pH, O_2 saturation, and measurement of shoot and leaf heights were recorded. On day 9 plants were removed from the test solutions and roots gently washed to recover any compounds associated to the roots. Plants were weighed (fresh mass) and then separated into fractions (roots and shoots). Plant materials were digested and analyzed, along with the aliquots of the test solutions, using inductively coupled mass spectrometry (ICP-MS) for trace metals (Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Ba, Ti, Pb, Th, U).

In total 1,296 samples (plant organ and growth solution) were analyzed for the 9-day hydroponics study (Table 3). Following analysis of samples, data was processed to correct for mass and dilution factors. Plots of growth, evaporation, transpiration, and concentrations in solution and plant organs have been created. Data from this study, including plant uptake factors (PUF), will be used to develop plant-specific models (PSM) to quantify PUF for PHC-F2 and trace metals.

Table 3 Number of samples analyzed by matrix type (plant organ and solution) for each plant species

Plant Species	# Root & Shoot Samples	# Liquid Analysis
Barley	96	336
Corn (Maize)	96	336
Pea plant	96	336
Total # samples	288	1,008

Figure 1 Pictures of *Pisum sativum* during various stages of the 9-day hydroponics study

2.2.2. Soils – Metals 9 Day Uptake Study

Table 4 summaries the design of the soils 9-day metal study. Five plant species (Barley, Corn, Italian rye, Pea, and Yarrow) and 4 metals (chromium, copper, nickel, and lead) at 5 times CCME guidelines were used in this experiment. Soils were spiked by repeated wetting of a batch of soil with a target metal solution. The volumetric soil moisture content was calculated and use to guide the amount of solution to be applied at each wetting. Soils were gentling mixed and air dried in between wetting. Three cycles of wetting were used. As before, seeds were germinated in perlite to BBCH 12 (two leaf unfolded stage) at which stage they were transferred to individual pots with 100-250g of soil (dependent on plant species) and grown for 9 days in a growth chamber under controlled light (16 hrs. light/8 hrs. dark) and temperature (23°C ± 2°C) (Figure 2). Samples of soil and plants were taken at day 0 and day 9. In addition, rate of evapotranspiration, and measurement of shoot and leaf heights were recorded on day 0, 2, 5,7, and 9. Soil samples collected were dried, ground, and digested in duplicate. Digests were analyzed by ICP-MS for the same suit of metals as the hydroponics study.

Table 4 Summary of experimental design, qa/qc controls and number of replicates for the soil 9- day study for each plant species (Barley, Corn, Italian rye, Pea, Yarrow) and target metals (chromium, copper, nickel, lead)

Experiment	Plant	Spiked Soil	Blank Soil	Replicates
Spike – Type 1	Yes	Yes	No	3
Spike – Type 2	No	Yes	No	2
Blank – Type 1	Yes	No	Yes	2

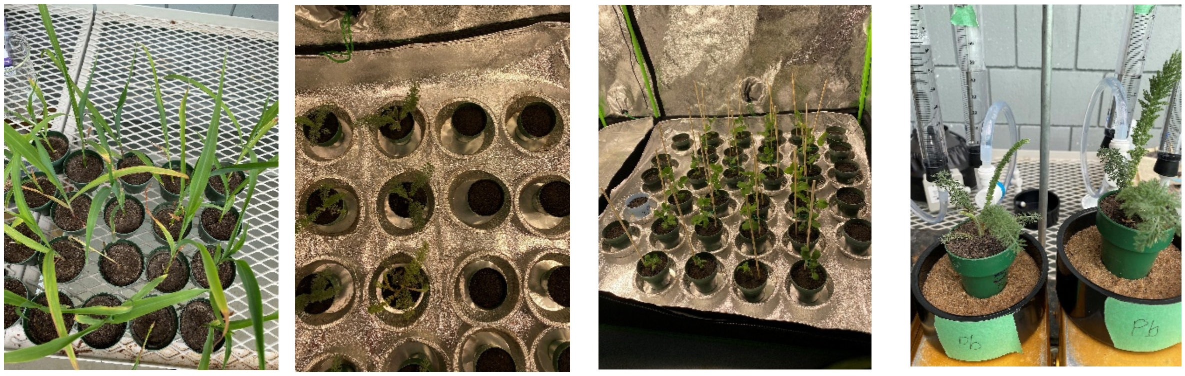

Figure 2 Pictures of Corn, Yarrow, and Pea during various stages of the 9-day metals soil study

In total 360 samples (plant organ and soils) were analyzed for the 9-day soil study (Table 5). Following analysis of samples, data was processed to correct for mass and dilution factors. Plots of growth, evaporation, transpiration, and concentrations in solution and plant organs have been created. Data from this study, including plant uptake factors (PUF), will be used to develop plant-specific models (PSM) to quantify PUF for PHC-F2 and trace metals.

Table 5 Number of samples analyzed by matrix type (plant organ and soil) for each plant species for the 9-day soil metal study

Plant Species	**# Root & Shoot Samples**	**# Soils Analysis**
Barley	24	48
Corn (Maize)	24	48
Italian Rye	24	48
Pea plant	24	48
Yarrow	24	48
Total # samples	**120**	**240**

2.2.3. Soils – Metals 8-week Uptake Study (longevity study)

The design of the soils metal longevity study (8 weeks) is similar to the set up for the 9-day study (Table 4) except that the plants were placed in larger pots to allow root growth. Four plant species (Barley, Corn, Italian rye, Pea) and 4 metals (chromium, copper, nickel, and lead) at 5 times CCME guidelines were used in this experiment. In brief, seeds were germinated in perlite to BBCH 12 (two leaf unfolded stage) at which stage they were transferred to individual pots with between 400-1000g of soil and grown for 8 weeks in a growth chamber under controlled light (16 hrs. light/8 hrs. dark) and temperature (23°C ± 2°C)(Figure 3). Samples of soil and plants were taken at day 0, 9, 14, 35, and 49. In addition, rate of evapotranspiration, and measurement of shoot and leaf heights were recorded every 2-3 days. Soil samples collected were dried, ground, and digested in duplicate. Digests were analyzed by ICP-MS for the same suit of metals as the hydroponics study. In total 720 samples (plant organs and soils) were analyzed for the 8-week soil study (Table 5).

Table 6 Number of samples analyzed by matrix type (plant organ and soil) for each plant species for the 8-week soil metals study

Plant Species	# Root & Shoot Samples	# Soils Analysis
Barley	120	60
Corn (Maize)	120	60
Italian Rye	120	60
Pea plant	120	60
Total # samples	**480**	**240**

Plots of growth, evaporation, transpiration, and concentrations in solution and plant organs have been created. Data from this study, including plant uptake factors (PUF), will be used to develop plant-specific models (PSM) to quantify PUF for PHC-F2 and trace metals.

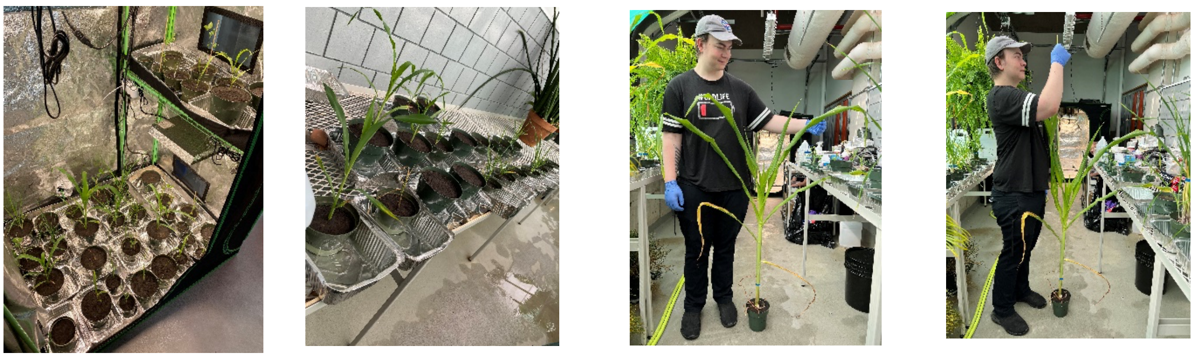

Figure 3 Pictures of plants during various stages of the 9-week metals soil study

2.2.4. Soil – PHCs 9-Day Uptake Study

Table 7 summaries the design of the soils PHC 9-day study. Five plant species (Barley, Corn, Italian rye, Pea, and Yarrow) and PHC-F2 impacted soil at 10mg/kg were used in this experiment. The impacted soil was created by spiking perlite with diesel, mixing this with 5kg of soil, and aging for 30 days. Seeds were germinated in perlite to BBCH 12 (two leaf unfolded stage) at which stage they were transferred to individual pots with 100-250g of soil (dependent on plant species) and grown for 9 days in a growth chamber under controlled light (16 hrs. light/8 hrs. dark) and temperature (23°C ± 2°C).

Samples of soil and plants were taken at day 0 and day 9. In addition, rate of evapotranspiration, and measurement of shoot and leaf heights were recorded on day 0, 2, 5,7, and 9. Soil samples collected were dried, ground, and solvent extracted using accelerated solvent extract. Extracts were evaporated to 1ml and PHC analyzed using multidimensional gas chromatography time of flight mass spectrometer (GCxGC-TOFMS). In total 72 samples (plant organs, soils, qa/qc) were analyzed for the 9-day PHC soil study (Table 5).

Table 7 Summary of experimental design, qa/qc controls and number of replicates for the 9- day PHC soil study for each plant species (Barley, Corn, Italian rye, Pea, Yarrow) and PHC-F2

Experiment	Plant	Spiked Soil	Blank Soil	Replicates
Spike – Type 1	Yes	Yes	No	3
Spike – Type 2	No	Yes	No	3
Blank – Type 1	Yes	No	Yes	4

Table 8 Number of samples analyzed by matrix type (plant organ and soil) for each plant species for the 8-week soil metals study

Plant Species	# Root & Shoot Samples	# Soils Analysis
Barley	8	6
Corn (Maize)	8	6
Italian Rye	8	6
Pea plant	8	6
Extract Material	‘--	9
Total # samples	**32**	**33**

Plots of growth, evaporation, transpiration, and concentrations in solution and plant organs have been created. Data from this study, including plant uptake factors (PUF), will be used to develop plant-specific models (PSM) to quantify PUF for PHC-F2 and trace metals.

3. Dissemination of Findings

Preliminary results of the data have been presented at the following conferences:

- Remediation Technologies Symposium 2022 - October 12, 2022: Plant Uptake of Metals and PHCs: Advancing ERA
- Remediation Technologies Symposium 2022 East- May 30, 2023 - Plant Uptake of Metals and PHCs: Advancing ERA

Data analysis of the results is on-going and will be used to develop publications to be submitted to peer review journals e.g. Journal of Environmental Exposure & Assessment.

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