# Characterizing the Influence of Clay Pads on Toluene Biogenesis in Peatlands

**Prepared for:**

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SOLSTICE

1 June 2017

Debbie Tainton
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Dear Ms. Tainton,

**Re: Characterizing the Influence of Clay Pads on Toluene Biogenesis in Peatlands**

Please find attached the final version of the report describing our research project investigating toluene levels below clay well pads. The report details the background, methodology, data, results, and conclusions, including tangible project outcomes and best practices recommendations for this project.

We trust that the attached report will communicate our investigation findings. Please contact the undersigned if you have any questions or concerns.

Sincerely,

**Solstice Canada Corp.**

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For her insight on soil analysis and remediation, project management, data management and technical review.

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**Anne McIntosh, Ph.D.**
University of Alberta, Augustana Campus

**Miles Tindal, M.Sc.**
Millennium EMS Solutions Ltd.

# Abstract

Solstice Canada Corp. (Solstice) investigated the influence of clay pads on toluene biogeneration in peatlands for The Petroleum Technology Alliance of Canada (PTAC). This investigation was motivated by elevated levels of toluene under clay pads that support petroleum extraction infrastructure and evidence that toluene can be produced through microbial processes. The results of an in-depth statistical analysis of past field level assessments showed that the clay caps significantly altered toluene levels. Through a comprehensive review of the scientific literature, the potential chemical and biological processes that encompass the toluene cycle were identified and documented as a comprehensive process. The synthesis of the statistical results and literature review revealed that toluene levels are dependent on site characteristics and suggested that toluene remediation criteria might best be established as benchmark comparisons to ‘background’. Solstice provides several Best Practices and toluene remediation recommendations informed by the knowledge gained. Additionally, recommendations for the experimental design of future research are provided.

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# Executive Summary

The Petroleum Technology Alliance of Canada (PTAC) commissioned Solstice Canada Corp. (Solstice) to investigate the influence of clay pads on toluene biogeneration in peatlands. Solstice performed an in-depth statistical analysis of a preexisting data set of routine salinity and hydrocarbon parameters from several field-level Environmental Site Assessments. In addition, Solstice investigated available literature to understand biogenic toluene generation and the implication of clay caps on these processes.

Toluene, an Alberta Tier 1 contaminant, has recently garnered attention from the petroleum and environmental industries. Elevated levels of toluene are frequently found under clay pads that support petroleum infrastructure in peatlands in Northern Alberta. These elevated toluene levels do not always follow patterns typical of petrogenic toluene. Additionally, there is scientific evidence that toluene can be produced biogenically under colder, anaerobic conditions, similar to those found at the clay pad sites. Solstice employed several statistical methods to evaluate the relationships between toluene and salinity parameters and site characteristics. The results of this analysis were synthesized with current scientific knowledge to create two toluene cycle schematics, one for sites capped with a well pad and one for uncapped sites in the study.

The results of the multivariate statistical analysis showed that at median pH conditions, the presence of a clay cap was associated with a significant increase in toluene level, by 0.46 mg/kg. Additionally, the soil samples under the clay pads had significantly higher pH (closer to neutral) and concentrations of the ionic constituents of gypsum, sulphate and calcium. Correlation analysis found that calcium, conductivity, pH, and sulphate had significant, negative associations with toluene levels. Through the literature review, sulphate was identified as a critical component of anaerobic biogenesis and degradation of toluene. Further, toluene can be broken down through aerobic microbial respiration. Phenylalanine, an amino acid component of humic acid, was identified as a potential precursor of toluene. Microbial anaerobic respiration breaks phenylalanine into toluene; this is inhibited by the presence of oxygen. Scientific literature indicates that the microbial driven processes in the toluene cycle flourish at neutral pH. The literature review results were synthesized into diagrams depicting the transport and transformation of toluene and related chemicals in peatlands.

Our study concluded that the clay pads appear to promote the production and accumulation of toluene. Potential mechanisms for this observation, identified through literature review, include increased supply of sulphate for anaerobic respiration and simultaneous blocking of oxygen from entering, and toluene from exiting the peatland system.

Our results indicated that at sample median pH, the presence of a well pad significantly increased toluene levels by 0.46 mg/kg. This suggests it is possible to detect differences from background samples and that remediation criteria might best be established as benchmark comparisons to ‘background’. ‘Background’ variability will make determination of a threshold level difficult in this, and potentially other peatland systems. Other factors in our multivariate analysis (pH, depth, frozen condition) suggest factors that could be controlled in sampling programs to better differentiate toluene differences originating from petroleum-induced effects. This analysis also found that anthropogenic activities, including clay pad development can influence toluene levels. Remediation options could differ significantly depending on the anthropogenic cause. In addition, the current toluene guideline of 0.12 mg/kg (AEP 2016a) was established for open water freshwater species not found in peatlands. We provide recommendations for management practices and future research that aim to inform a new remediation benchmark for toluene.

# Introduction

## Background

The appearance of elevated toluene in northern Alberta organic soils has recently garnered attention within the oil and gas industry. Through field-level Phase II site assessments in northern Alberta, Solstice Canada Corp. (Solstice) recorded numerous cases of elevated toluene levels beneath the clay pad of abandoned well-sites, which were not consistent with petrogenic sources. The toluene occurrences did not occur in a pattern indicative of natural gas seepage and did not co-occur with other petroleum hydrocarbons (e.g., benzene, ethylbenzene, xylene). This suggested a biogenic source of toluene. Similar instances have been discussed by members of the oil and gas industry, who also hypothesize these occurrences may be biogenic (Mayes and Luther 2015; Richards 2017).

Previous research strongly suggests that toluene can be produced biogenically under cold, anaerobic conditions similar to the condition experiences in northern Alberta peatlands. Toluene was found in the anoxic hypolimnion of lakes in Germany without indication of petrogenic influence (Juttner and Henatsch 1986). This toluene generation was reproduced in a lab (Juttner 1991) and the microbe responsible was identified (Fischer-Romero et al. 1996). Fischer-Romero et al. (1996) isolated the microbe and tested its environmental effects on toluene generation. Toluene formation occurred at pH levels of 6 and 7 but not 5.5 and 8 and growth occurred between 12-22 °C and was most optimized at 22 °C. Toluene is a biogenic intermediary product of microbial respiration (Jüttner & Henatsch 1986; Marczak et al. 2006). Jüttner & Henatsch (1986) theorized that complex carbon compounds such as carotene (an F4 hydrocarbon) are anaerobically broken down to toluene but the anaerobic conditions prevent further biodegradation.

Peatland systems have a unique chemical environment. Sphagnum moss utilizes cation exchange to intake ionic nutrients; this process promotes an acidic environment (Clymo 1963; Painter 1991; Rozbrojova & Michal 2008). In addition, there is evidence that hydrogen ions are generated during peat decomposition, lowering pH levels (Hemond 1980). Thus, peatlands have consistent, low pH conditions. (Aerts et al. 1990). The saturated conditions of peatlands also modulate the environment by creating low oxygen conditions (Lindsay 1979). Reduced conditions alter the speciation of elements and produce a chemical equilibrium different from upland soils. For example, bog ecosystems are known to be nutrient poor (Johnson & Damman 1991).

## Motivation

Toluene may be generated biogenically but it is toxic at very low concentrations to organisms at many trophic levels of fresh water ecosystems. For example, health effects have been identified in rainbow trout (*Oncorhynchus mykiss*) at levels starting around 0.02 mg/L. Further, amphibians such as the leopard frog (*Rana pipiens*) and the northwestern salamander (*Ambystoma gracile*) show chronic health effects at 0.39 mg/L, freshwater invertebrates like *Daphnia magna* show an $LC_{50}$ at 3.75 mg/L, and freshwater algae (*Selenastrum capricornutum*) show an $EC_{50}$ (reduction in growth) at 12.5 mg/L. (CCME 1999, 2004). The driving receptor for toluene remediation guidelines for a natural land use in organic soil is the protection of aquatic life (AEP 2016a). Unfortunately, the receptors identified in these guidelines are found in open water systems; receptors found in peatland ecosystems have not yet been identified.

Levels of toluene above the regulatory guideline are often found beneath the clay pad of abandoned well-sites built on peatlands (up to 26.7 mg/kg in this study). Alberta's Tier 1 Guidelines (AEP 2016a) are based on coarse mineral material, not representative of organic peatland soil, and set at 0.12 mg/kg. Currently, remediation is required to bring clay pad sites into regulatory compliance. Yet, it is possible that these elevated toluene levels are the result of biogenic processes. A lack of knowledge about how and why this biogenic toluene generation occurs makes establishing appropriate remediation guidelines difficult. This project aims to better understand the conditions and processes that result in elevated toluene levels under clay pads to inform appropriate remediation procedures. Remediation is costly and requires heavy equipment that can further damage sensitive ecosystems like peatlands. Efficient remediation of toluene levels is in the best interests of both peatland ecosystems and the petroleum industry.

## Preliminary Results

This project is an extension of previous research done by Solstice on data collected for Environmental Site Assessments (ESAs) of two groups of well-sites in northwest Alberta. This preliminary study found that toluene levels were higher and occurred at greater depths under well pads (capped) versus surrounding uncapped locations (See Appendix A). Additionally, sulphate had a positive correlation with toluene levels, and thus, was identified as a potential contributor to toluene generation. However, the Random Forest statistical models used in the analysis had high levels of prediction error that were attributed to small sample size. More detailed results from this analysis are included in Appendix A. This current research project was motivated by the availability of additional data from an industry collaborator. The results of this preliminary analysis provided direction for our analysis of the larger data set, as outlined below.

## Research Objectives

The overall research objective for this project was to characterize the influence of clay pads on toluene biogenesis in peatlands. The end goal of this objective was to determine background toluene levels to inform the adjustment of reclamation requirements for toluene. The analysis was organized into five sub objectives as follows:

1. Determine if the results from the preliminary analysis are supported by the larger data set.
    a. Determine if there are higher toluene levels below well pads (capped) versus surrounding areas (uncapped).
    b. Determine if there is a relationship between sulphate and toluene.
2. Investigate the influence of sulphate on toluene generation.
    a. Determine if there is a difference in sulphate levels between sites capped with a well pad and sites without a cap.
    b. Determine if the production of toluene through sulphate reduction has been documented in the literature.
3. Investigate the influence of other measured chemicals on toluene generation.
4. Determine if a background toluene benchmark can be determined from the data.
    a. Inform this decision with knowledge gained from investigation into the influences on toluene generation.
5. Describe the management implications of the results.

## Hypothesis

Generally, we predicted that biogenic toluene generation is common and wide spread in Northern Alberta peatlands, but the clay well pads are creating unique conditions that promote the increase in concentration of toluene (Figure 1). Toluene has a significant vapor pressure and low water solubility, which results in its tendency to float on water and disperse into the atmosphere (Environment Agency 2004). However, toluene adsorbs to soil organic matter; this can hinder migration in organic soils

(Environment Agency 2004). The clay borrow material that is used in well pad development is often over a meter thick and has been compacted by heavy equipment. The clay pad is believed to be creating a barrier to gas diffusion that is preventing oxygen from entering the peat system and toluene from leaving it. The lack of oxygen leads to the anoxic conditions favorable to toluene generation. In addition, toluene is buoyant in an aqueous environment and would naturally exit peat because of its high porosity (Landva & Phenney 1980). The clay pad may also be preventing the exit of toluene, causing an increase in concentration of biogenic toluene underneath it.

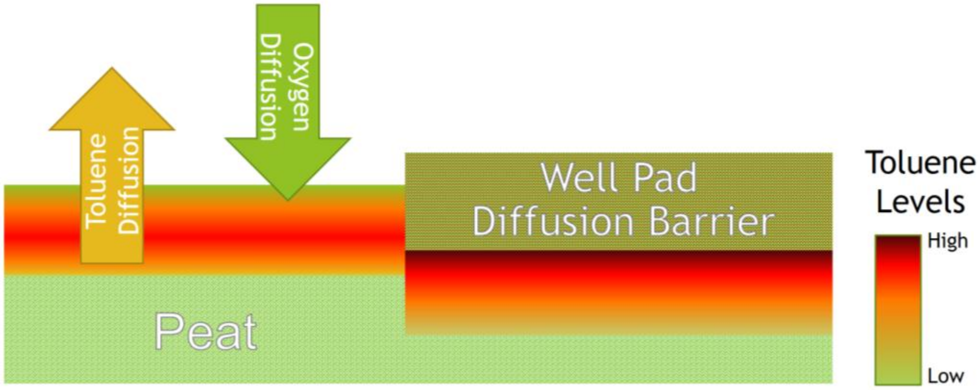

*Figure 1. Conceptual model for toluene diffusion in natural peatlands and under well pads.*

The clay pad material is also likely to be acting as a source of dissolved ions, such as sulphate and calcium. The preliminary analysis showed that sulphate levels had a strong, positive correlation with toluene levels. The addition of sulphate to nutrient poor ecosystems, like bogs, is likely increasing the activity of sulphate-reducing bacteria and leading to a corresponding increase in toluene biogenesis in proximity to the clay pad. This explanation formed the basis of our hypothesis, that the clay pad was promoting concentration of toluene. Testing this hypothesis required an understanding of toluene biogenesis, a process not well documented in the literature. As a first step, we developed a conceptual chemical model explaining toluene biogenesis under undisturbed (‘uncapped’) and ‘capped’ conditions.

## Conceptual Chemical Model

Our conceptual chemical model (Figure 2) describes potential chemical processes that are occurring and affecting toluene levels in our study area. The source of toluene is anaerobic microbial respiration of organic matter, provided by peat. In our model, the terminal electron acceptor is sulphate. Sulphate’s reduced form is hydrogen sulphide ($HS^-$), which is both water soluble and can form a gas. These properties allow hydrogen sulphide to move into aerobic zones (if present) and be oxidized back to sulphate (Friedrich 1998), which is also water soluble and can mix and homogenize.

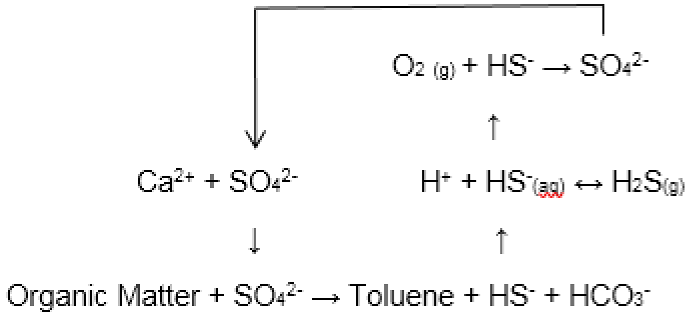

*Figure 2. Chemical conceptual model for toluene generation.*

# Methodology

## Data

This project capitalized on an opportunity to analyze a preexisting dataset. The composite data were initially collected for Environmental Site Assessments (ESAs) done by Solstice and an industry collaborator. The lab analyses for the Phase 2 Assessments resulted in measures for routine salinity parameters and CCME Petroleum Hydrocarbons. Combined, the chemical soil data were available from 54 well-site and remote sump pump ESAs, roughly within a 20-km radius of each other in a near continuous wetland region of northern Alberta. These investigations were completed in the winter months (January, February, March) in 2015. Typically, each ESA site included 10-15 boreholes that each had multiple soil sample points. The borehole locations were within the clay cap area (capped) and in the area surrounding the clay cap area (uncapped). The soil sampling was not initially designed to control for variability between or within natural systems but extensive data mining and appropriate statistical methods were employed to control for variability and allow accurate detection of differences.

The complete dataset was analyzed and compiled by Exova Canada Inc. (Exova) in a rich text format (RTF) and sent to Solstice as a single file. Exova used saturated paste extract techniques for analysis. The techniques used for analysis are important factors to consider when comparing results to guidelines or other data. For example, laboratory results from saturated paste extract techniques can be twice as high as field screening results for salt related chemical parameters (AEP 2001). The data were initially processed using Microsoft Access, to group the results by sample identification number. The resulting tabularized data were cross-checked with existing in-house data tables as a Quality Assurance and Quality Control measure. The collaborator’s data were cross-checked with data provided in the associated Phase 2 reports (also supplied to Solstice).

## Data Selection

### *Toluene Data*

To obtain biogenic toluene measures, the focus of analysis, we mined relevant information from the available soil chemistry data through a series of steps. The first was to focus on the data that included toluene measures; data points were eliminated that did not meet this criterion. The raw data included 6,518 sample points. Of these, 2,049 had toluene results.

### *Identification of Petroleum Hydrocarbon Impacted Samples*

Numerous samples were from locations with petroleum-based hydrocarbon impacts (petrogenic). These samples were discarded (N=413) by removing samples that showed combinations of carbon fractions that were consistent with petrogenic carbon distributions. This method was modeled after the results of Kelly-Hooper *et al.* (2013) that showed that peat exposed to crude oil had chromatograph results with elevated F2 and F3a carbon fractions. Samples that had elevated levels of known petrogenic sourced hydrocarbons (F1, F2, Benzene, Ethylbenzene, xylene) were discarded.

Quality assurance checks of split duplicate results showed that toluene and F3 hydrocarbons had average relative percent differences of 97% and 49%, respectively. We can assume that this variability was likely present in all samples, which could significantly influence results near the detection limit. Review of petroleum hydrocarbon (PHC) results identified isolated F1, F2, Benzene, Ethylbenzene, or Xylene occurrences that were only slightly above the detection limit. For example, an F2 PHC level of 51 mg/kg may co-occur with elevated toluene levels, but there was no indication of the characteristic PHC pattern on the corresponding chromatogram. Such isolated occurrences are likely to represent error and be inaccurate.

To adjust for isolated occurrences of an individual PHC, a threshold of the detection limit plus an error estimate of 20% was implemented for preliminary screening. The more conservative RPD for F3 hydrocarbons (49%) minus a safety factor, for potential sample heterogeneity, was used to produce the error estimate of 20%. Samples with isolated occurrences of individual PHCs were spot checked against their corresponding chromatogram to further evaluate if they were indicative of a PHC impact. The concentration threshold used for each of these parameters are given in Table 1.

***Table 1. Hydrocarbon thresholds for inclusion in dataset.***

| Hydrocarbon | Threshold for inclusion |
|---|---|
| F1 | 12 mg/kg |
| F2 | 60 mg/kg |
| Benzene | 0.006 mg/kg |
| Ethylbenzene | 0.012 mg/kg |
| Xylene | 0.036 mg/kg |

Once samples with elevated petrogenic sourced hydrocarbons were discarded, remaining samples that had elevated F3 or F4 levels were explored further to ensure that the hydrocarbons were biogenically sourced. If F3 levels were above 700 mg/kg or F4 levels were above 400 mg/kg, the chromatograms were reviewed to confirm that the signature was consistent with a natural peat signature. Chromatograms of samples from peat substrate with biogenic toluene have a distinct pattern. Biogenic chromatograms have a carbon distribution of discrete peaks clustered around C32 within the F3 hydrocarbon range and odd numbered peaks (C21, C23, C25, C27, C29) paired with a high carbon preference index (PFI = odd/even) (personal communication C. Swyngedouw, Exova May 28, 2015). Figure 3 shows an example of this "heartbeat pattern". Appendix B provides further details on the cluster around C32.

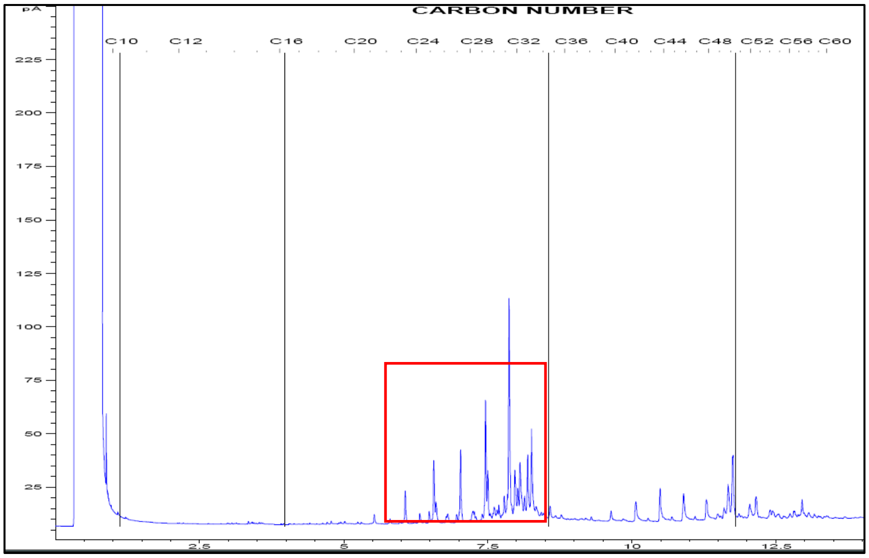

*Figure 3. Excerpt from a chromatogram with a pattern indicative of biogenic toluene generation.*

### *Elimination of Data Collected in Mineral Soil*

This project focused on the biogenic production of toluene in peatlands. As such, the data for mineral soils were excluded. Any samples with fine or coarse texture (i.e., non-organic) were removed from the sample data set. The soil texture was interpreted from three methodologies, in the following hierarchy:

- Non-organic samples were subject to a Sieve #200 (>75 micron) analysis. This analysis physically separated samples into fine and coarse mineral. Samples subject to this test with fine or course results were removed from the sample data set.
- The texture of the soil determined through hand texture from the borehole logs.
- Soil texture was estimated from the saturation percentages as per the methodology outlined by Boucher and Knafla (2014), adapted to peatland soils (see below).

Following Boucher and Knafla (2014), a relationship between saturation percentage and soil texture was established. Samples with saturation percent results greater than 95% were separated into quartiles. Five samples from each quartile were randomly selected and submitted for total organic carbon (TOC) analysis. The results of the TOC analysis were regressed against saturation percentage to establish their relationship (See Figure 5). From this relationship, organic texture was identified by a saturation percentage greater than 100%.

In a last check, the results from the three methodologies were compared to detect and reconcile differences. In instances where the results differed, the sieve results were favored over the borehole logs, which were favored over the soil texture estimated from the saturation percentages. Samples determined to be from mineral soil (N=1,220) were discarded.

### Toluene Analysis Data Set

After data cleaning, 416 toluene samples remained for analysis (Table 2). Approximately half (54%) of the samples were from a location capped with a well pad. The majority (89%) were from bog systems and had unfrozen soil (95%) at the time of sampling. Of 416 samples, 249 included detailed salt analysis results.

*Table 2. Breakdown of toluene dataset with available covariates.*

| | Total | | Solstice | | Collaborator | |
|---|---|---|---|---|---|---|
| Toluene Samples | N=416 | 100% | 306/416 | 74% | 110/416 | 26% |
| Detailed Salt Data | N=249 | 60% | 222/249 | 89% | 27/249 | 11% |
| Frozen | N=22 | 5% | 4/22 | 18% | 18/22 | 82% |
| Capped with Well Pad | N=226 | 54% | 148/226 | 65% | 78/226 | 35% |
| Bog | N=369 | 89% | 275/369 | 75% | 94/369 | 25% |

The lab analyses for the Phase 2 Assessments included measures for routine salinity. The following section outlines parameters included in our analysis, based on our conceptual chemical model, and known relationships with chemical parameters and toluene biogenesis. Their inclusion allowed us to characterize chemical differences between the capped and uncapped sites in terms of typically monitored chemical parameters.

### *Calcium*

Gypsum ($CaSO_42H_2O$) is known to naturally occur in the study area, within mineral soils (AEP 2001). Large (5cm diameter) gypsum crystals were discovered by Solstice while soil sampling near the study sites. This presents a possible source of calcium through leaching from the mineral soil to the peat, from either the locally sourced clay that constitutes the well pads or the parent material under the peat.

### *Sulphate*

The preliminary analysis identified a relationship between sulphate and toluene levels at study sites. We assumed in our conceptual chemical model that the source of toluene is from anaerobic microbial respiration of organic matter derived through the reduction of sulphate. Sulphate is present as salts in combination with calcium as gypsum in the study area. Sulphate plays a known role in microbial respiration in anaerobic conditions. Bacteria will reduce sulphate to sulphide as a source of energy when oxygen (or nitrates, manganese, or iron) is not available (Edwards et al. 1992; Rabus et al. 1993; Mitsch and Gosselink 2007). Activity of sulphate reducing bacteria (SRB) is limited by the availability of sulphate (Moestedt 2013), and low activity of SRBs may result in lower production of biogenic toluene. This suggests a correlation between sulphate and toluene may be visible in the data; therefore, the sulphate data were included in the analysis.

### *pH*

Site pH is an indicator of chemical condition, rather than a chemical component. As a reciprocal measure of the hydrogen ion activity in solution, it is a measure of acidic or basic conditions. Sphagnum moss species are known to use cation exchange (take up Ca, Na, etc. in exchange for H) to promote the acidic environments of peatlands (Clymo, 1983; Painter, 1991; Rozbrojova & Michal 2008).

### *Magnesium, Chloride, Potassium, Sodium*

These chemical ions can be present as naturally occurring salts. Magnesium sulphate, $MgSo_4$, has been observed in the study area by Solstice. These positive ions ($Mg^{2+}$, $Na^+$, $K^+$) can be taken up by sphagnum and exchanged for $H^+$ ions, reducing the pH of the system.

### *Electrical Conductivity*

A measure of the ability to conduct an electric current, conductivity indicates the abundance of ions in an aqueous solution. Electrical conductivity was included to identify trends in levels of ions that were not otherwise included in the dataset.

## Calculation of Descriptive Variables

To control for differences in conditions where samples originated and to better understand the influences on toluene generation, we calculated additional variables for our analysis that described site characteristics, other chemical compounds analyzed in the soil samples, and the depth at which the samples were collected.

### *Capped or Uncapped with Well Pad*

Categorical variables were included in the analysis to determine if there was a difference between samples collected under a well pad cap and those that were not. The borehole logs were reviewed to confirm the presence or absence of a clay pad cap on each borehole. The sites were labelled as capped (under a clay well pad) and uncapped (not under a clay well pad).

### *Depth Interval*

The preliminary study found differences in toluene levels by depth, suggesting that depth should be considered in analysis. To capture the depth at which the samples were collected, a continuous variable (“depth interval”) was created. This was used to control for the vertical distribution of toluene by inclusion in the multivariate analysis. The interval was defined as the distance between the midpoint of the sample depth and either the interface between peat material and well pad clay or peat and ice cover (in the absence of a clay pad). The borehole logs were consulted to determine the extent of the clay pad if applicable. The depth of the sample was taken from the depth recorded on the sample label at the time of sampling. This data were included in the Exova dataset.

### *Ecosite Identification*

Samples were collected in bog and fen peatland communities. These ecosystems have different surface water hydrology that results in different chemical conditions and vegetation communities (Balasooriya et al. 2008). This would influence microbial community composition and activity, which could influence toluene biogenesis. To capture variation in the samples due to differences in the ecology of the sites, samples were classified as fen or bog by cross-referencing site coordinates with the Alberta Merged Wetland Inventory from the Alberta Environment and Parks (AEP 2016c). The wetland inventory information was downloaded as a shapefile from GeoDiscover Alberta. Some of borehole locations were not mapped as peatlands in the merged wetlands inventory dataset (potentially due to coarse resolution mapping). The ecosite types for these sites were interpreted from the closest wetland features and interpretation of aerial photography. A categorical variable for “Bog” was included to control and identify differences in toluene levels between bog and fen ecosystems.

### *Frozen/Unfrozen Samples*

Unavoidably, data were collected in winter during both the Solstice and Industry Contributor’s field programs, to facilitate safe access. Soil samples were collected from the top layer of frozen peat and from permafrost layers encountered at various depths. Therefore, a small portion of the soil samples (N=22) were frozen when they were collected, introducing a potential source of bias. To control for potential differences in frozen and unfrozen samples, a “frozen” categorical variable was added to the analysis. Frozen conditions could affect chemical measures in three different ways:

- Freezing of the soil alters the partitioning of solute adsorbed to the soil and this can change the equilibrium points at which solutes adsorb to the soil or become suspended in solution (Marion 1995). This suggests that the proportion of analyte in the environment that is adsorbed to the soil will be different in frozen and unfrozen samples.
- Frozen saturated soil is also less compressible than unfrozen saturated soil (Amiri et al. 2016). Therefore, the frozen samples could retain more moisture content during sampling than the unfrozen samples. Moisture is a key calculation value used to quantify the dilution effect on analyte extraction.
- Water expands when it freezes and can increase the pore volume of mineral soils by 9% (Ishikawa et al. 2014). This effect is likely to be even higher in peat soils. It is possible that when the frozen samples thaw during transport to the lab, greater head space is produced than unfrozen samples. This is important to analysis of analytes such as toluene, the focus of this investigation, since it can escape into the extra head space due to its relatively high vapor pressure.

Samples were identified as either frozen or unfrozen from the borehole logs, which detailed the location of samples relative to the extent of permafrost and the frozen top layer encountered during sampling.

## Statistical Methods

All statistical analyses were performed within the R statistical package (R Core Team 2016).

## Data Challenges

This study utilized a data set that resulted from ESAs conducted by different companies, and various field staff, as part of a larger Phase 2 program. Much of ESA sampling is performed to delineate the extent of chemical releases in context to guidelines (i.e., sampling did not follow a research design specific to this current study). Because of this opportunistic (rather than intentional) dataset, a large part of the analysis focused on finding appropriate statistical methods for a limited data set, and associated challenges:

- Chemical analyses have detection limits based on technical abilities and thus create censored data. Traditional statistical methods can produce biased results when applied to a censored data set. A detailed literature review was performed to support our statistical methodology. A summary can be found in Appendix C.
- With ESA data collection, the sampling of background conditions is generally secondary, especially for non-polar compounds like toluene. This leads to imbalances in the data, which systemically underrepresent background conditions. Most samples collected under well pads were not paired with sample locations in background areas. Imbalanced data can significantly affect a statistical method.
- The data were left-skewed (with many non-detect samples) and could not be corrected through logarithmic transformation (Figure 1A and 1B). Use of parametric statistics are strongly dependent on a normal distribution and skewed data can produce inaccurate results (Feng et al. 2014). As such, non-parametric statistics were a better fit for the data.

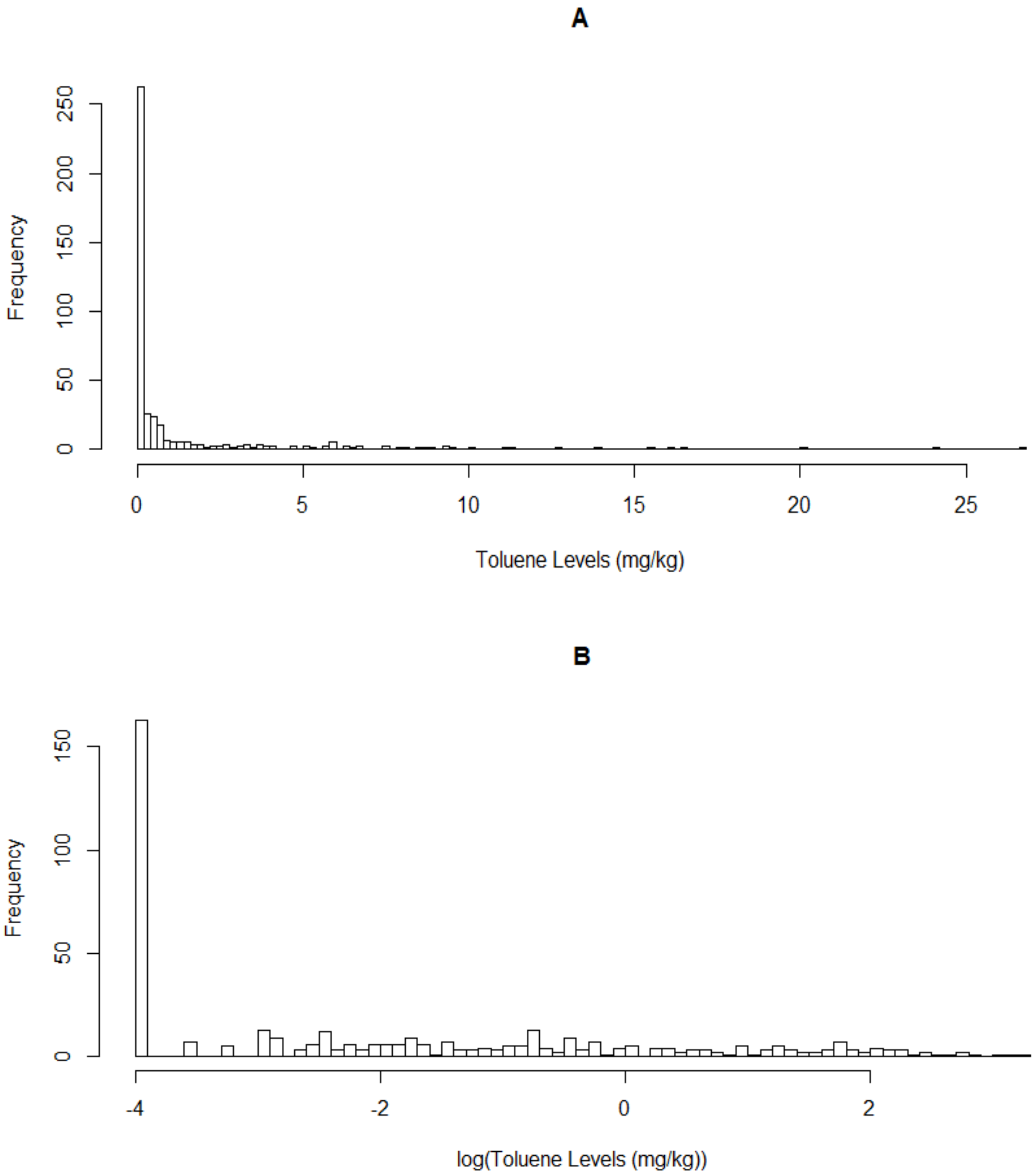

***Figure 4. Density plots for raw (A) and log-transformed (B) biogenic data.***

## Duplicate QAQC

High variability in lab results for organic soil is common due to a number of factors. For example, the volume of organic soil it takes to meet required mass levels for lab analysis is usually greater than the equipment can handle (Landva & Phenney 1980). As such, the samples must be diluted, adding an inherent source of error. To address these concerns, standard QAQC measures using relative percent duplicate (RPD) of split duplicate analyses were performed for the data in the analysis.

## Geographical Variation

The well-sites where the data were collected fell into natural clusters on the landscape, resulting in five distinct geographic groups of well-sites within a 20-km radius of each other. Although it was likely that these sample groups could be combined in the same analysis based on proximity, further testing was conducted to confirm suitability for pooling. Toluene results for each geographic group were compared using a non-parametric regression using the ‘np’ package available through the statistical package ‘R’ (R Core Team 2016). This is a nonparametric comparison analysis that is free of the limiting assumptions of parametric statistics and can accommodate comparison of categorical variables, like the five ESA groups of interest.

## Research Objectives Analysis

Statistical analyses used to address the five research objectives outlined in the introduction included non-parametric comparison tests, correlation analysis, multivariate analysis and other non-parametric analysis, as outlined below.

### *Differences in Two Sites*

We used the Mann-Whitney U rank sum test (a non-parametric test) to compare the capped and uncapped sites relative to toluene levels (Objective 1a), sulphate levels (Objective 2a), calcium and pH levels (Objective 3). In addition, differences in toluene levels between frozen and unfrozen sites, and ecosite types were investigated to determine if these covariates affected toluene levels (Objective 3). The Mann-Whitney U (Kruskal-Wallis) test uses ranking procedures and can accommodate non-normal, censored study data sets (Helsel 2012).

### *Correlation Analysis*

The correlation between toluene and the salinity parameters (Objective 3) was evaluated with the Kendall’s Tau test. This statistical method employs a ranking procedure and is non-parametric, which limits the bias resulting from the censored data (Helsel 2012). Correlations between the salinity parameters were evaluated with ordinary least squares linear regressions.

### *Multivariate Analysis*

As a biologic process, toluene biogenesis was anticipated to involve many factors. Multivariate statistical methods were employed to examine potential covariates and understand their influence on toluene levels (Objective 1, 2, 3). Specifically, we used maximum likelihood estimation (MLE) censored regression analysis. This method appropriately considers censored datasets to reduce bias (Helsel 2012) and can accommodate interactions between two explanatory variables. Multiple models were estimated using different combinations of explanatory variables. The explanatory variables included in the model selection were the previously described routine salinity parameters and descriptive variables (see “Data” section). Model selection was based on the inclusion of variables with statistically significant coefficients and limiting the number of residuals that failed the normal distribution assumption.

### *Non-Parametric Analysis*

In addition to the MLE regression, purely non-parametric methods were investigated to more accurately capture a small number of data points (approximately 10) that the MLE methods did not estimate well. Non-parametric regressions are not commonly used in environmental studies due to the larger data requirement for the models. To explore the potential for nonparametric analyses, a power test of toluene levels between sites was performed using the SIMR package within the statistical platform R. This method uses a nonparametric Monte Carlo simulation technique that has been cited as robust to “non-normal response variables” (Green and MacLeod 2016; Mumby 2002); however, it still uses an underlying definition of standard deviation from a linear, mixed effects model. The test returned a power of 77.6%, which suggests that there is a 77.6% chance that the sample replication is sufficient the show a difference in toluene levels between capped and uncapped sites. While this model is not ideal for this data, it does suggest that broad trends in the data available should be identifiable.

Initial review suggested several potential analytical methods:

1. Random Forest – with Under-Sampling
2. Random Forest – with Over-Sampling
3. Multi-Variate Regression Tree
4. Inference Tree
5. Cluster Analysis
6. Multi-Response Permutation Procedure (MRPP)
7. Linear Modeling with Mixed Effects
8. Parametric Non-Linear Modeling

Through research and initial analysis, kernel based regressions were deemed the most appropriate. These methods were run with the data with the 'np' package available in the statistical platform ‘R’, which is commonly used in the field of econometrics (Hayfield 2014). These methods were unable to identify any significant trends between toluene and the other variables in this analysis. More information on the methods, results and discussion of this part of the analysis are provided in Appendix D.

# Results

## TOC Analysis to Establish Relationship between Saturation Percentage and Carbon Content

TOC analysis showed that carbon content of the subsample ranged from 18% to 45.9%. Results of a regression between TOC and saturation percentage results showed a logarithmic relationship (Figure 5) with an $R^2$ of 0.67 ($p=0.001$). These results confirmed the relationship and suggested saturation percentage is a good proxy for TOC. From this relationship, organic texture was identified by a saturation percentage greater than 100%. Soils with saturation percentages between 80 and 100% may be organic soils, Ah horizons, or mineral horizons admixed with organic material.

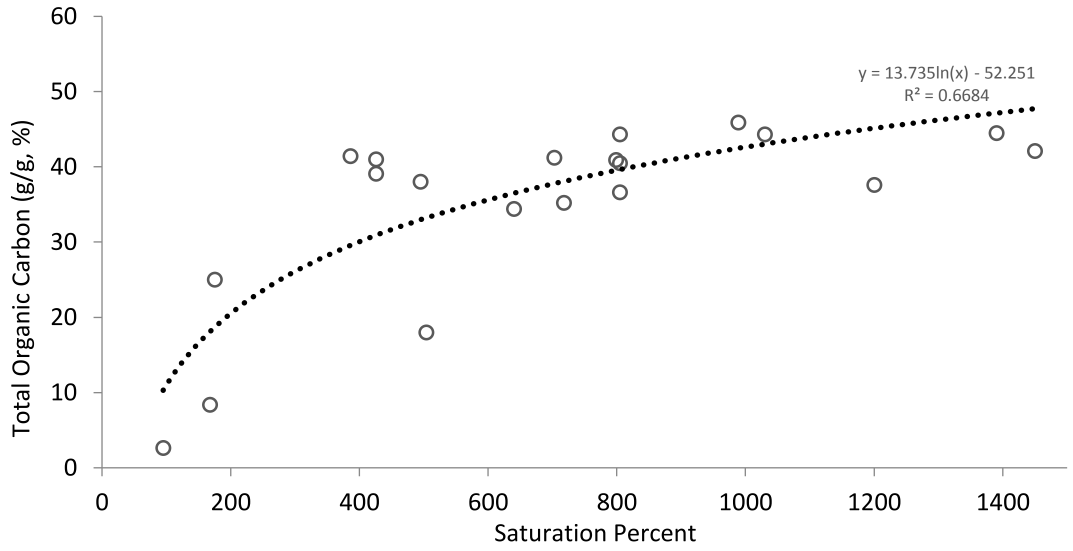

*Figure 5. Comparison of Total Organic Carbon to Saturation Percentage.*

## Duplicate Analysis

Standard QAQC measures using relative percent duplicate (RPD) of split duplicates supported the appearance of significant levels of error in lab results for toluene. CCME QAQC guidelines for split duplicates with solid matrixes recommend a maximum RPD of 75% to 100% (CCME 2016). Four out of 25 split duplicates showed an RPD between 75% and 100% and eleven greater than 100% (Figure 6).

Due to the increased uncertainty of analytical results that approach the laboratory reporting limit (LRL), a duplicate qualifier of twice the LRL is allowed by the CCME (CCME 2016). For example, the LRL for toluene is 0.02 mg/kg; therefore, duplicates with an absolute difference in toluene levels of less than 0.04 mg/kg are considered acceptable. There were two samples where the absolute difference in duplicates were less than 0.04 mg/kg; however, both samples were below an RPD of 50% and were already considered acceptable (Figure 6).

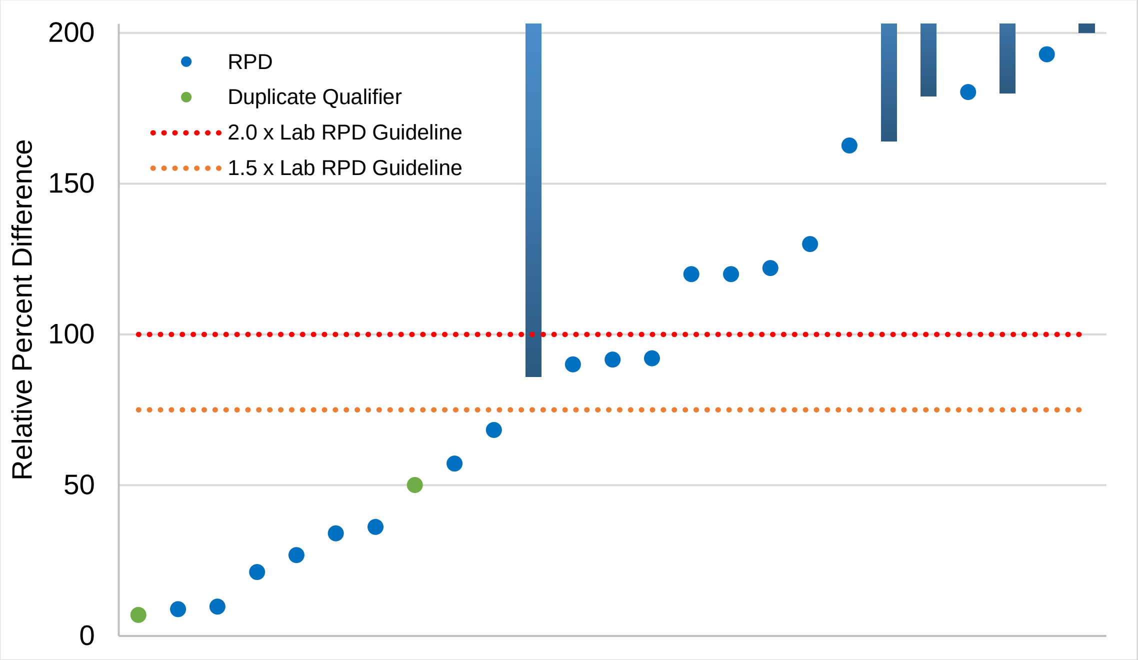

*Figure 6. RPD of split duplicate samples for toluene. Points represent samples where both results were above the detection limit, bars indicate samples where RPD potential begins because one sample was below the detection limit. Green points represent samples that are within the CCME Duplicate Qualifier guideline.*

Further comparison of RPD to the absolute toluene values generally confirms regulatory guidance that RPD increases as sample results approach the LRL (CCME 2016); however, there is still significant variability in this comparison which suggest the presence of unqualified error (Figure 7).

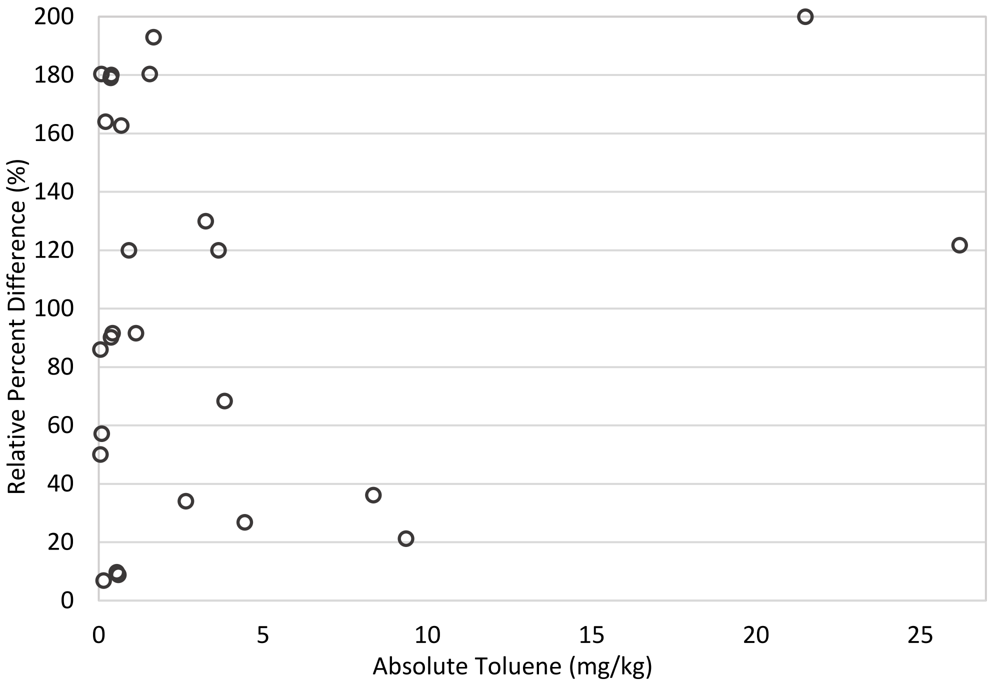

*Figure 7. Relative Percent Difference by absolute toluene values.*

Examination of the RPD for biogenic organic compounds (BOCs) in the F3 range had better repeatability results. BOCs present in the soil originate from fresh or decaying organic matter, but can typically be filtered out for PHC analysis using a silica gel cleanup step. Silica gel cleanup is effective for use with soil with ≥5% SOC (Kelly-Hooper et al. 2013). However, the peat material in this investigation had between 18% and 45.9% TOC. Results of the RPD for the F3 range showed 78% and 92% of RPD results from split duplicates were below 75% and 100%, respectively (Figure 8). These results suggested that analyses for F3 hydrocarbons have more repeatable results than toluene analyses.

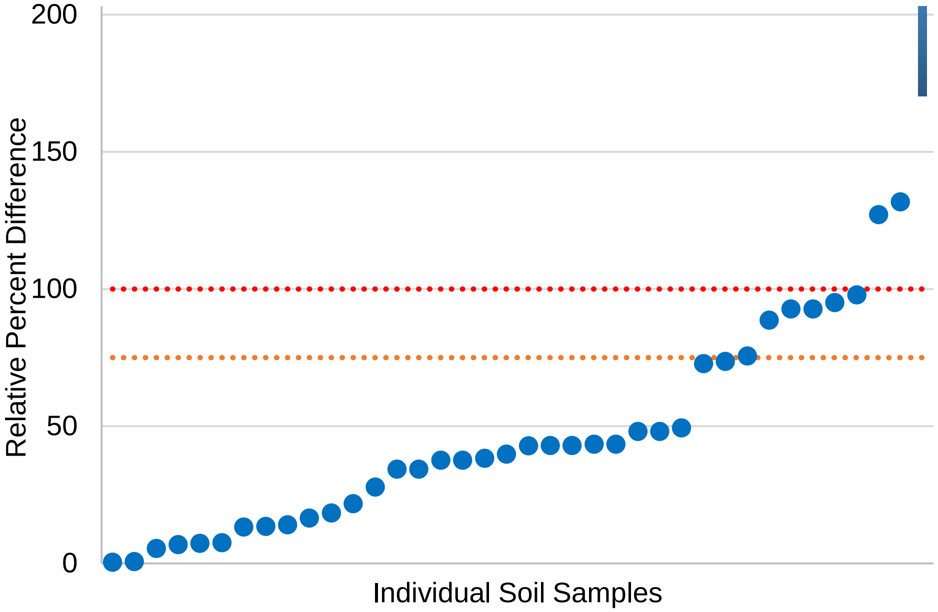

*Figure 8. RPD of split duplicate samples for F3 petroleum hydrocarbons. Points represent samples where both results were above the detection limit, bars indicate samples where RPD potential begins because one sample was below the detection limit.*

## Geographical Variation

The lack of statistically significant difference in toluene levels among geographical area (p =0.75; $R^2$=0.004) meant that the data among areas could be pooled for statistical analyses.

## Frozen Status and Ecosite

Mann-Whitney U tests between toluene levels in frozen and unfrozen sites and bog and fen sites failed to find a significant difference in sites (p=0.28 for frozen/unfrozen comparison and p=0.8 for the bog/fen comparison). The small number of frozen (N=22) and fen (N=47) sites could explain the lack of detectable difference in toluene levels. These variables were included in the multivariate analysis as a control measure, since they were a potential source of bias.

## Site Differences

### Toluene in Capped and Uncapped

The Mann-Whitney test between the toluene levels of the capped and uncapped sites showed no significant difference between them (p=0.73). However, the distributions of toluene for these two site types were slightly different (Figure 9). For example, the median toluene levels for both sites was 0.09 mg/kg; however, the 95th percentile for the uncapped and capped toluene levels were 6.12 mg/kg and 8.47 mg/kg, respectively. This suggests that while the central tendency of toluene levels is similar between sites, capped toluene levels had a wider range in values than did the uncapped sites.

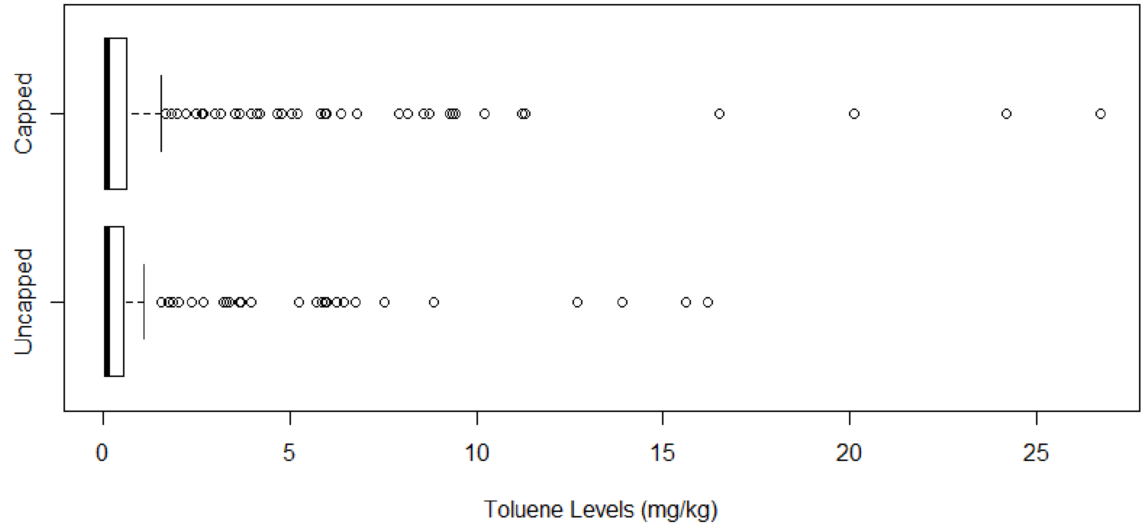

*Figure 9. Boxplots of toluene levels in the capped and uncapped sites.*

## Salinity Parameters in Capped and Uncapped

Summary statistics for the analyzed chemical parameters, grouped as capped or uncapped sites, are provided in Table 3 (total sample size, mean, median, and maximum and minimum values). Although calcium, chloride, potassium, magnesium, sodium and sulphate all had extremely high maximum values, they were right skewed distributions (i.e., mean was larger than the median), for both the capped and uncapped sites. Such distributions indicate more samples of low value, and based on the minimum and maximum values, indicate a broad ranges of salinity values at capped and uncapped sites. However, comparisons between the capped and uncapped sites showed that on average, the capped sites had higher pH ($p = 0.0003$) and electrical conductivity levels ($p = 6x10^{-6}$); as well as higher calcium ($p = 6x10^{-5}$), chloride ($p = 0.052$), magnesium ($p = 0.0008$), sodium ($p = 0.002$) and sulphate ($p = 5 \times 10^{-5}$) concentrations.

*Table 3. Summary statistics for the chemical parameters in the analysis.*

| Parameter | Sat % | Ca (mg/kg) | pH | Cl (mg/kg) | EC (dS/m) | K (mg/kg) | Mg (mg/kg) | Na (mg/kg) | $SO_4$ (mg/kg) | Depth Interval (m) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Capped Sites** | | | | | | | | | | |
| **N=** | 131 | 126 | 130 | 126 | 126 | 126 | 126 | 126 | 126 | 226 |
| **Mean** | 732 | 486 | 5.4 | 135 | 0.6 | 21.8 | 90.3 | 96.5 | 1371 | 1.2 |
| **Median** | 690 | 344 | 5.6 | 103 | 0.44 | 17.5 | 63 | 64 | 920 | 1.1 |
| **Maximum** | 1850 | 3330 | 7.5 | 1280 | 2.8 | 106 | 755 | 774 | 11000 | 3.6 |
| **Minimum** | 107 | 54.2 | 3.2 | 24 | 0.09 | 4 | 20.6 | 14 | 25 | 0.075 |
| **Uncapped Sites** | | | | | | | | | | |
| **N=** | 126 | 123 | 135 | 123 | 123 | 123 | 123 | 123 | 123 | 190 |
| **Mean** | 826 | 321 | 4.95 | 122 | 0.4 | 20.9 | 70.1 | 79.5 | 883.3 | 1.5 |
| **Median** | 798 | 248 | 5.1 | 91 | 0.27 | 14 | 43.9 | 48 | 552 | 1.25 |
| **Maximum** | 1980 | 2090 | 7.6 | 2560 | 2.39 | 120 | 667 | 1480 | 7730 | 5.1 |
| **Minimum** | 122 | 29.4 | 3 | 16 | 0.06 | 5 | 13 | 11 | 22 | 0.075 |

### *Gypsum (Calcium and Sulphate)*

Gypsum is not a routine salinity parameter but its dissolved ion constituents, calcium and sulphate, are. Dissolved calcium ranged from 29.4 mg/kg to 3330 mg/kg in this study sample, with a median of 284 and mean of 404 mg/kg (N=249). Dissolved sulphate ranged from 22 to 11,000 mg/kg with a median of 690 and mean of 1130 mg/kg.

Figures 10 and 11 show the distribution of dissolved sulphate and calcium levels, respectively, within the capped and uncapped sites. Mann-Whitney U tests comparing sulphate (p=0.00005) and calcium (p=0.00006) levels within the capped and uncapped sites indicated that they differed. This supported our hypothesis that the clay caps are a source of gypsum and its component ions, sulphate and calcium.

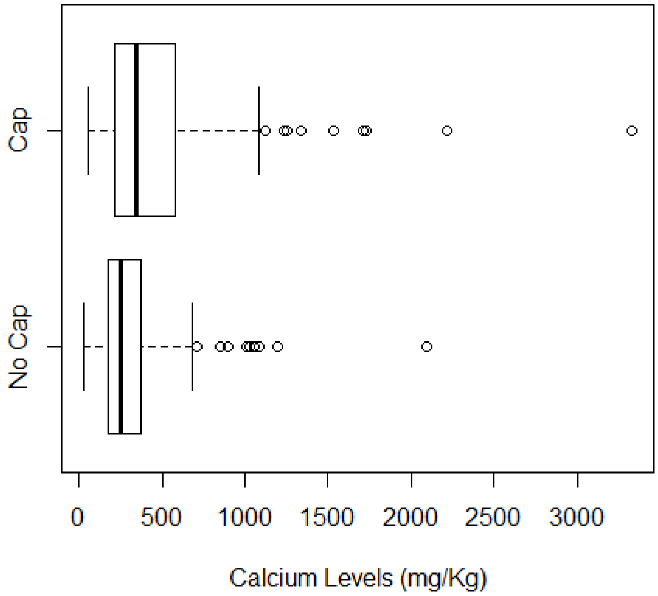

*Figure 10. Calcium levels in capped and uncapped sites.*

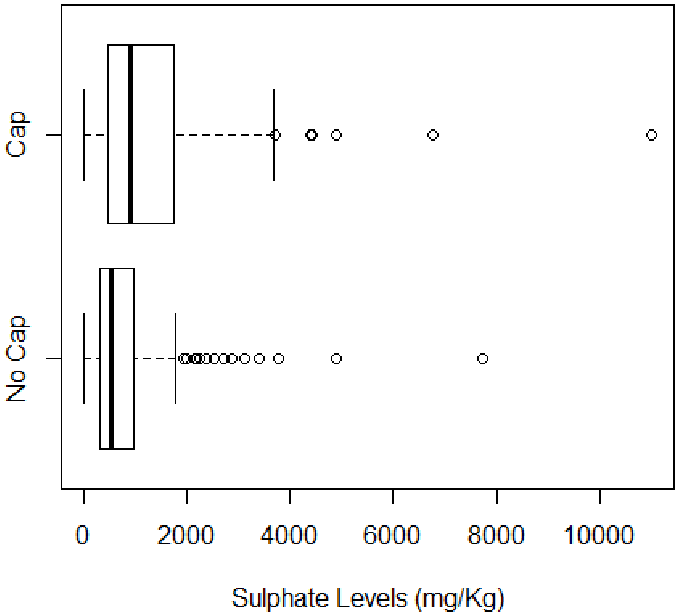

*Figure 11. Sulphate levels in capped and uncapped sites.*

### *pH*

The range of pH in our samples was 3 to 7.6, with a median of 5.2 and mean of 5.3. The capped sites had higher pH levels than the uncapped ones (Figure 12; Mann-Whitney U test (p=0.0003)). The effect of the interaction between pH and capped status was a significant component of the multivariate analysis, in addition to the independent effect of both cap and pH.

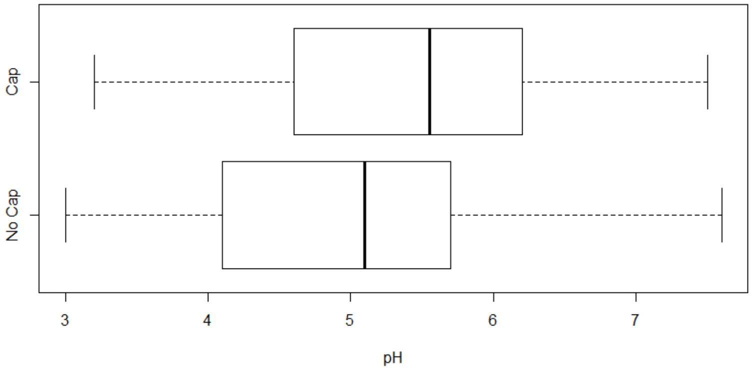

*Figure 12. Boxplot showing the distribution of pH for the capped and uncapped sites.*

## Correlation Analysis

The Kendall's Tau coefficients indicated that calcium, conductivity, pH, and sulphate all had a significant, negative correlation with toluene levels (Table 4).

*Table 4. Kendall's Tau coefficients showing the statistical significance of correlations between Toluene and the salinity parameters. *p<0.1, **p<0.05, ***p<0.01*

| | **Toluene** |
|---|---|
| **Ca** | -0.33*** |
| **Cl** | -0.10 |
| **EC** | -0.17** |
| **K** | 0.05 |
| **Na** | 0.13 |
| **pH** | -0.38*** |
| **SO4** | -0.28*** |

## Cation Exchange

Cation exchange was identified as a possibly biologic process that could influence the pH conditions of sphagnum dominated sites. To investigate if cation exchange was occurring, the correlation between pH and dissolved calcium was assessed.

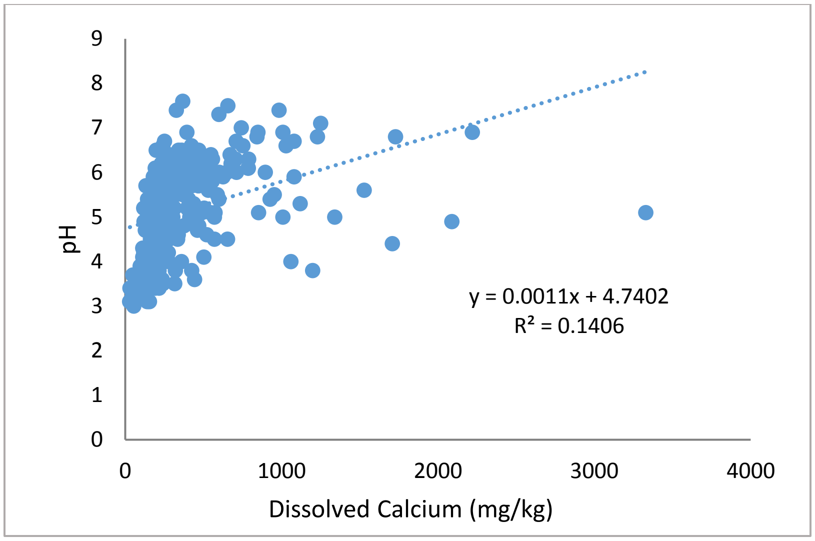

*Figure 13. Scatterplot of correlation between pH and calcium for capped and uncapped sites.*

The correlations between pH and calcium were not strong. A linear regression for capped and uncapped sites combined (Figure 13) indicated a weak, positive correlation between pH and dissolved calcium (slope of 0.001, $R^2$ of 0.14). This relationship could be an indication of cation exchange facilitation from sphagnum. If sphagnum is taking up cations in exchange for H ions, the correlation should be stronger in the uncapped sites because sphagnum under the cap is presumed dead. However, in our data set, the correlation was stronger in capped sites (Figures 14 and 15).

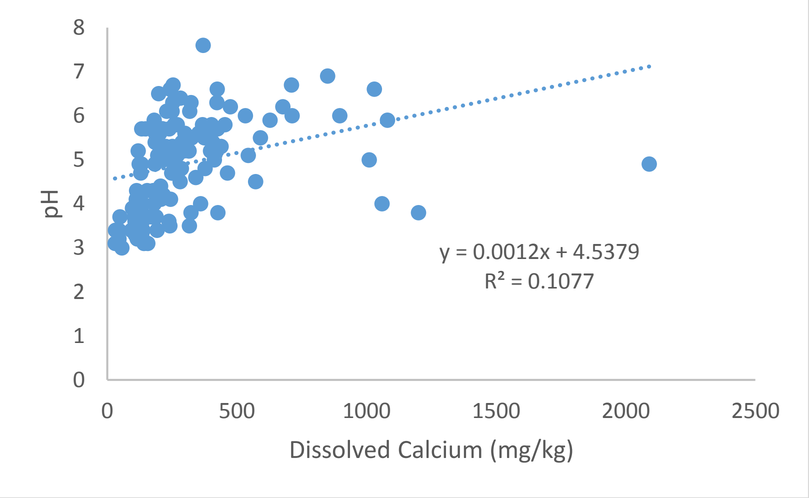

*Figure 14. Scatterplot of correlation between pH and calcium for uncapped sites.*

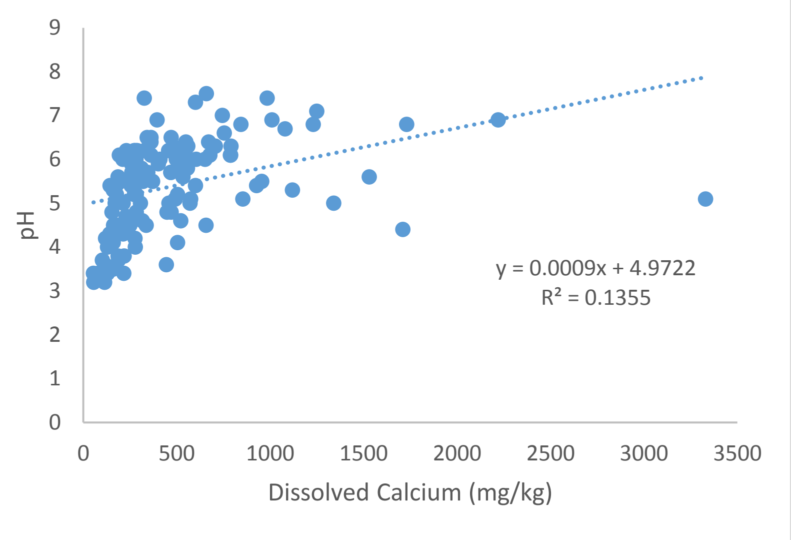

*Figure 15. Scatterplot of correlation between pH and calcium for the capped sites.*

## Multivariate Results

The MLE censored regression results (Table 5) helped characterize the relationship between toluene (dependent variable) and the seven explanatory variables. The frozen and ecosite (Bog) categorical variables were included in the multivariate analysis as a control measure because of the evidence of bias from literature (Marion 1995; Balasooriya et al. 2008; Ishikawa et al. 2014; Amiri et al. 2016), even though they did not significantly contribute to the model ($p>0.05$).

*Table 5. Results from the MLE regression relating toluene to several measures of site condition. Significant P-values highlighted in bold.*

| Explanatory Variable | Coefficient | Standard Error | P-value |
|---|---|---|---|
| Intercept | 4.89 | 1.48 | **0.0009** |
| Cap | 5.33 | 1.91 | **0.005** |
| pH | -0.66 | 0.26 | **0.01** |
| Cap*pH | -0.87 | 0.36 | **0.02** |
| Depth Interval | -0.62 | 0.23 | **0.007** |
| Frozen | -1.00 | 0.92 | 0.28 |
| Bog | 0.56 | 0.63 | 0.38 |

The MLE results can be interpreted in equation form:

(Equation 1)

$$Toluene_{\left(\frac{mg}{kg}\right)} = 4.89 + 5.33(cap) - 0.66\,(pH) - 0.87\,(cap * pH) - 0.62(Depth\ Interval) - 1(frozen) + 0.56(bog)$$

The coefficients for the explanatory variables (Table 5 and Equation 1) are the quantified effect of the explanatory variable on toluene when all other variables are held constant within our sample. For example, an increase in pH of 1 was associated with a 0.66 mg/kg decrease in toluene. In addition, the coefficients indicated that pH, depth interval and the clay cap / pH multiplicative interaction all had significant ($p<0.05$), negative trends with toluene levels for our study system. The intercept (4.89) is the "base" toluene level without the incorporated effect of any of the explanatory variables. The salt ions (calcium, sulphate, magnesium, sodium, potassium, chloride) and electrical conductivity were not statistically significant additions to the model and therefore, not included.

The significant coefficients for the clay cap (Cap) and cap / pH multiplicative interaction terms indicate that the clay cap had a significant impact on toluene levels. The effect was reliant on site pH conditions. The relationship between the clay cap and toluene can be further clarified by looking at the change in toluene resulting from the presence of a clay cap (cap = 1 in Equation 1). Equation 2 defines the impact of the clay cap and is a derivation of Equation 1 above.

(Equation 2)

$$\Delta Toluene_{\left(\frac{mg}{kg}\right)} \Big/ {}_{\Delta Cap} = 5.33 - 0.87 \times (pH)$$

From Equation 2, we can predict the impact of a clay cap on toluene levels at different pH levels present at the capped sites in our sample. At the sample median pH level, 5.6, the impact of the cap on toluene is 5.33 – (0.87 x 5.6) = 0.46 mg/kg. The range of toluene impact is 2.55 to -1.20 mg/kg resulting from a clay cap at the minimum (3.2) to maximum (7.5) sample pH levels, respectively. Table 6 below shows the predicted toluene levels at median site conditions for the capped and uncapped sites. At median site conditions, the toluene level for capped sites is 18% higher than at uncapped.

*Table 6. Predicted toluene levels for a capped and uncapped site at median site conditions.*

| | **Toluene Prediction by Median Site Conditions** | |
|---|---|---|
| **Explanatory Variable** | **Capped** | **Uncapped** |
| Intercept | 4.89 | 4.89 |
| Cap | 5.33 x 1 = 5.33 | 5.33 x 0 = 0 |
| pH | -0.66 x 5.6 = -3.70 | -0.66 x 5.1 = -3.37 |
| Cap*pH | -0.87 x 1 x 5.6 = -4.87 | -0.87 x 0 x 5.1 = 0 |
| Depth Interval | -0.62 x 1.1 = -0.68 | -0.62 x 1.25 = -0.78 |
| Frozen | -1.00 x 0 = 0 | -1.00 x 0 = 0 |
| Bog | 0.56 x 1 = 0.56 | 0.56 x 1 = 0.56 |
| **Total Toluene (mg/kg)** | **1.53 mg/kg** | **1.30 mg/kg** |

The residuals of the model (Figure 16) indicated that a small number of (N~10, ~4%) toluene observations were not captured well with the model. The large residuals corresponded with large outlier observations of toluene in our sample. There was no clear pattern of association between these extreme values and any of our other measured parameters.

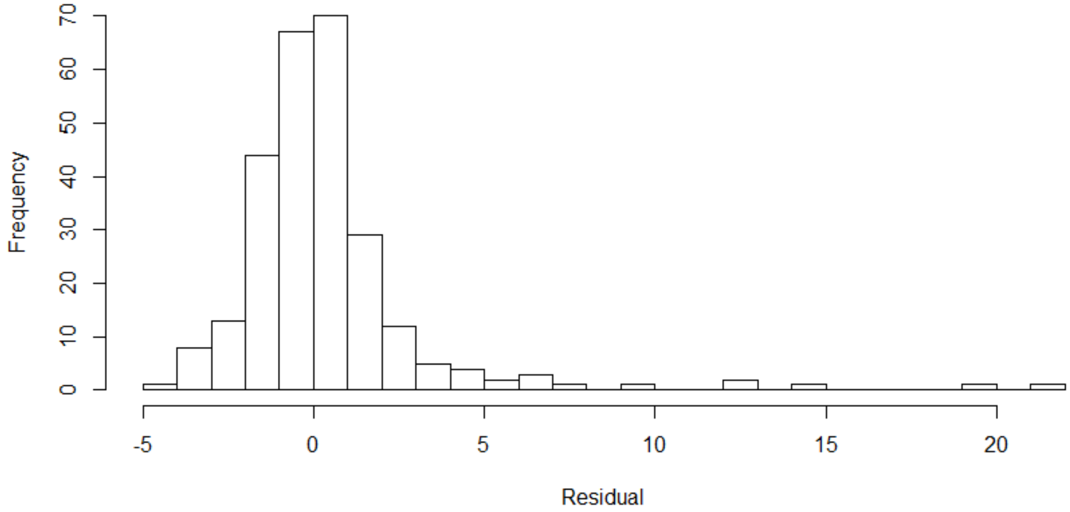

*Figure 16. Histogram of the residuals from the MLE regression.*

# Discussion

## Updated Chemical Models

Consideration of our study results and a supplemental literature review led to updated toluene cycles that depict potential underlying toluene processes for the capped (Figure 17) and uncapped (Figure 18) sites. These cycles synthesize the underlying chemical and biological functions identified in the literature and are supported by the toluene results of this study.

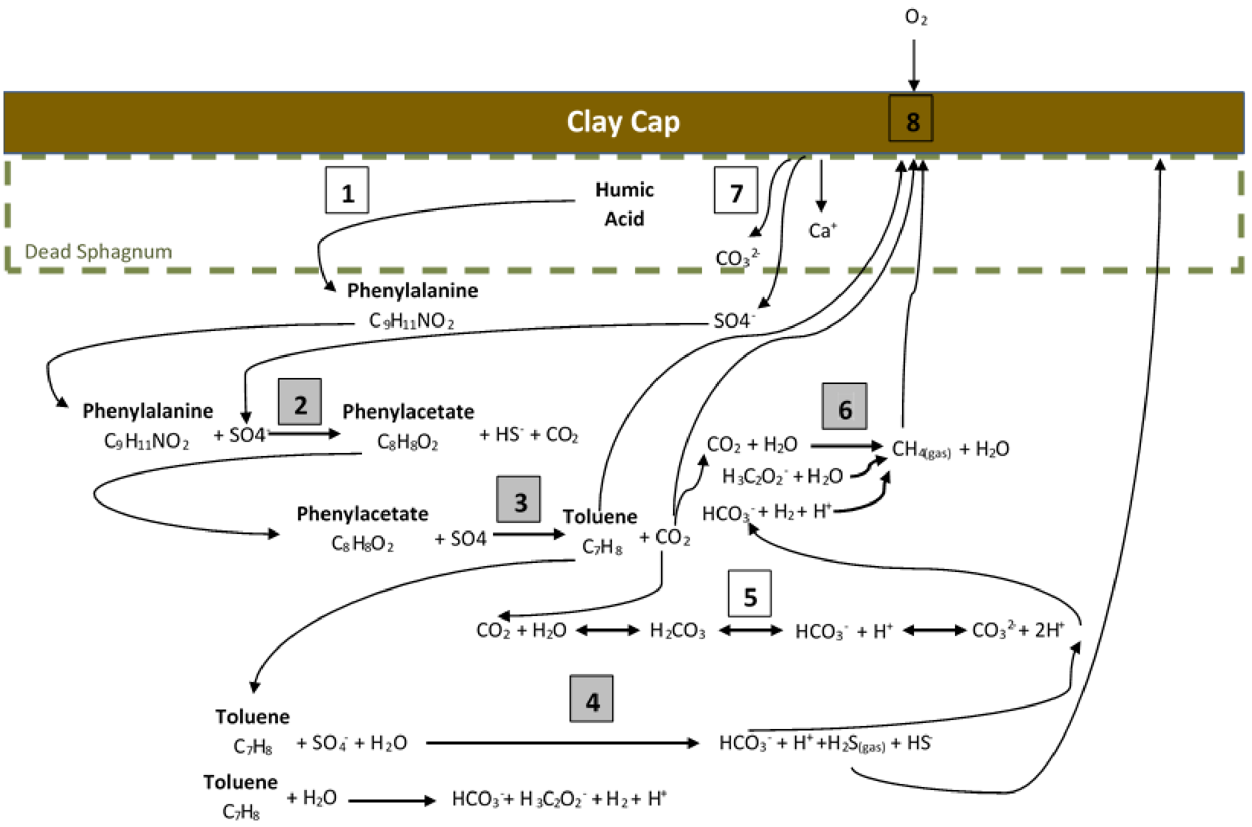

*Figure 17. Updated Conceptual model for Toluene generation for the capped populations. Numbered processes in grey boxes are biologically driven.*

Revisiting the literature after the analysis provided several additional insights that enhanced our understanding of toluene generation and degradation. Biological degradation of toluene can occur aerobically or anaerobically under nitrate reduction, sulfate reduction, iron reduction, perchlorate reduction, and methanogenic conditions (Lovley and Lonergan 1990; Edwards et al. 1992; Rabus et al. 1993; Edwards and Grbic-Galic 1994; Zhou et al. 1995; Kasai et al. 2005). Noh et al. (2003) investigated the degradation of toluene by sulphur reducing bacteria and found that optimal conditions for degradation were at a pH range of 6-8 and temperature of 30-37 °C. The presence of ethylbenzene or xylene did not affect degradation rates but benzene presence slowed it down (Noh et al. 2003). Sivagurunation et al. (2003) found that humic acid extracted from peat slowed down the aerobic degradation of toluene by one microbe (*Pseudomonas fluorescens*). In anaerobic sludge digestion, the optimal conditions for toluene degradation were a pH range of 7-7.2 (Mrowiec et al. 2005). These insights suggested that both biological and chemical processes might influence toluene degradation at capped and uncapped sites, with reactions dependent on oxygen levels, pH and temperature. The process steps identified in Figures 16 and 17 break down these interactions into steps mediated either biologically, or chemically.

The numbered processes in Figure 17 are described in more detail below:

**1)** Phenylalanine is an amino acid component of humic acid that is separated through hydrolysis (Damian et al. 1985). Toluene can be produced from phenylalanine (Fischer-Romero et al. 1996).

2) Through anaerobic bacterial respiration, phenylalanine can be oxidized to phenylacetate (Fischer-Romero et al. 1996).
3) Through anaerobic bacterial respiration, phenylacetate can be broken down to toluene. This process is inhibited by oxygen presence (Zargar et al. 2016).
4) Through anaerobic bacterial respiration, toluene can be broken down into bicarbonate and bisulphide (Rabus et al. 1993; Beller et al. 1996; Harms et al. 1999). Anaerobic bacterial respiration of toluene can also happen through denitrification, iron reduction, and methanogenic consortia (Heider et al. 1999). Sulphate reduction processes would dominate in northern Alberta peatlands due to its relatively higher concentration compared to alternative anaerobic electron acceptors and higher energy yield compared to methanogenisis (Heider et al. 1999).
5) This depicts the bicarbonate equilibrium and incorporates the conversion between the many dissolved forms of carbon dioxide in aquatic environments. The form present depends on the pH of the system. For example, in more acidic conditions (more H+ ions available), the equilibrium will compensate and most carbon will be present in the forms on the left side of the equilibrium (Dodds and Whiles 2010).
6) Through anaerobic bacterial respiration, methane can be produced through the reduction of carbon dioxide or other organic compounds. This is a common pathway for organic matter breakdown in anaerobic wetlands when other electron acceptors have been depleted (Mitsch and Gosselink 2007).
7) The clay cap is a potential source of gypsum, which dissociated to calcium and sulphate ions in solution. This is supported by the presence of higher calcium and sulphate concentrations in the capped sites and observations of gypsum crystals in the area. In addition, the local clay for the study site is expected to be a source of carbonate ions as the bedrock is of marine origin (Prior et al. 2013).
8) The clay cap acts as a barrier to diffusion. This affects both influx of oxygen into the system and outflux of toluene, carbon dioxide, methane and hydrogen sulphide.

In the uncapped situation (Figure 18) the conceptual model differs from that for the capped site in several ways. The absence of the clay cap allows oxygen to diffuse into the system, creating an aerobic zone (shown in blue). The availability of oxygen allows aerobic processes 9 and 10 to occur. Additionally, the absence of the clay cap allows sunlight to reach the sphagnum community. Living sphagnum (represented by the green box) facilitates Process 11 (photosynthesis). The clay cap at the capped sites would kill the sphagnum under it, preventing active photosynthesis and cation exchange.

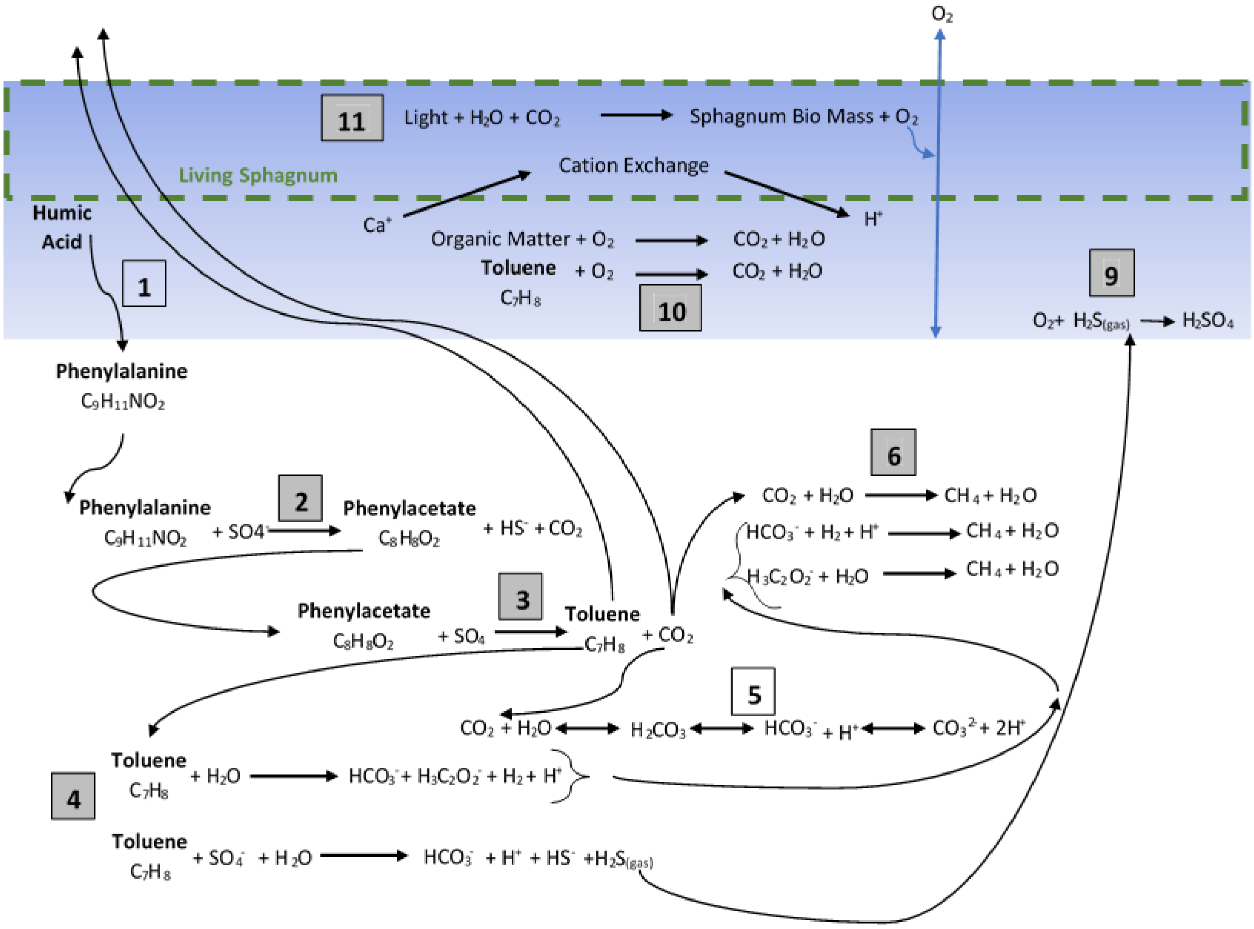

*Figure 18. Updated conceptual model for the uncapped populations. Numbered processes in grey are biologically driven.*

An explanation of how the numbered processes differ between the capped and uncapped models follows:

**1)** The hydrolysis of phenylalanine should remain largely unchanged between the two models.

**2) 3)** and **4)** These anaerobic processes, facilitated by bacteria, are inhibited by the presence of oxygen (Fischer-Romero et al. 1996; Zargar et al. 2016) and therefore, would only occur in the anaerobic zone. These processes require sulphate as an electron acceptor. In the capped site model, the clay cap acted as a supply of ions, including sulphate. In the absence of this sulphate source in the uncapped site, these processes are expected to happen at a lower rate. However, the uncapped sites in our study still had sulphate available so the pathways are not absent in the uncapped site model. Toluene degradation through other processes, like the proton reducing pathway shown in process 4, might play a bigger role when less sulphate is present.

**5)** The bicarbonate equilibrium would be affected in many ways. Toluene production might be retarded because of the presence of oxygen and lower levels of sulphate. Therefore, lower carbon dioxide outputs from process 2 and 3 would be expected. Additionally, lower toluene production could result in less toluene breakdown, reducing the output from process 4. Aerobic respiration (process 10) would increase $CO_2$ output. The clay cap is also suspected to be a source of carbonate to the system. This is strongly supported by the results; pH levels were significantly higher in capped sites.

**6)** Our research indicated that capped sites had significantly less sulphate. Organic matter breakdown through processes that utilize electron acceptors other than sulphate, like methanogenesis, might increase.

**9)** With the addition of oxygen to the system, reduced hydrogen sulphide gas could be re-oxygenated to sulphate, once it reaches the aerobic zone. Hydrogen sulphide is water soluble and can form a gas. These properties allow hydrogen sulphide to potentially move into aerobic zones and be oxidized back to sulphate (Friedrich 1998).

**10)** Oxygen is the highest energy yield electron acceptor (Mitsch and Gosselink 2007). Aerobic respiration can be expected to dominate the organic matter breakdown processes in the aerobic zone of the system. In addition, toluene can be broken down aerobically (Sivagurunation et al. 2003; Kasai 2005). This process is specified in the aerobic respiration process.

**11)** Living sphagnum also performs photosynthesis. This process uses $CO_2$ to build biomass. Typically, in peatland systems, the production of biomass occurs at a faster rate than organic matter breakdown, which leads to the storage of organic matter (Mitsch and Gosselink 2007; AEP 2015a). This is expected to be the case in uncapped sites. Capped sites would be dominated by organic matter breakdown, because the sphagnum has been killed due to lack of access to sunlight.

## Research Objectives

The conceptual model helped us to explain the results of our analysis, relative to each research objective.

### Toluene Level Differences Between Capped and Uncapped Sites (Objective 1A)

The rank sum population comparisons (Mann Whitney U) did not find a significant difference in toluene levels. However, within the multivariate MLE, the clay pad caps were a significant contributor to the accumulation of toluene. This indicated that the difference was observed but toluene levels were also impacted by other parameters that obscured the pattern. The MLE censored regression evaluated the effect of the pad and found that the impact of the clay cap varied by pH level. At median sample pH levels, the presence of a clay cap was associated with a 0.46 mg/kg increase in toluene.

The conceptual models (Figures 17 and 18) provide a potential mechanism to explain the observations of higher toluene levels in the capped sites. The clay cap appeared to supply sulphate ions to the system. Sulphate is a required component of one of the known toluene generation pathways. The additional sulphate encourages more toluene production. Toluene is mobile and buoyant and would be expected to move upward toward the surface. The clay cap could be preventing the exit of toluene through this channel, increasing concentrations. In addition, the aerobic zone within the uncapped sites would allow for aerobic toluene degradation. Aerobic processes with oxygen terminal electron acceptors have higher energy yields (Mitsch and Gosselink 2007). Therefore, such processes can be expected to be quicker and more efficient than anaerobic toluene degradation.

### Relationship Between Sulphate and Toluene (Objective 1B, Objective 2)

Sulphate levels were higher in the capped sites than uncapped, suggesting an influx of sulphate ions from the clay cap material. In addition, sulphate had a significant negative correlation with toluene (Table 4). This could reflect the relationship between sulphate and toluene generation - as sulphate is reduced or "used up", toluene is generated. However, sulphate was not a significant predictor of toluene in the MLE regression (Table 5). The literature review (and resulting conceptual models) revealed that sulphate can play a role in the anaerobic production and degradation of toluene (Processes 1, 2, 3 and 4 in the conceptual models). This complicated relationship between toluene and sulphate could explain why a consistent trend was not detected. The analysis and detection of sulphate was further complicated by the mobility of its reduced form, hydrogen sulphide ($HS^-$), which is water soluble and can form a gas. These properties allow hydrogen sulphide to potentially move into aerobic zones and be oxidized back to sulphate (Friedrich 1998). Sulphate is also water soluble and can mix and homogenize. The properties of sulphur in reduced and oxidized forms may obscure the relationship between sulphate and toluene.

## Influence of other Measured Chemicals on Toluene Generation (Objective 3)

### *Salt Ions*

There were multiple indications from the results that the clay cap was a source of ions to the system. There were significantly higher levels of calcium and sulphate in the capped sites (Figures 10 and 11). The literature review identified sulphate as an important factor for toluene but calcium was not. The significant trend between calcium and electrical conductivity with toluene (Table 4) was likely an effect of the relationship between sulphate, electrical conductivity and calcium. The literature review did identify the importance of calcium in sphagnum cation exchange, but we did not find strong evidence that this process was prevalent in our dataset.

The electrical conductivity (EC) range was 0.06-2.8 for all sites (Table 3). This range falls within the good or fair level of the Alberta Tier 1 Salt Remediation Guidelines (AEP 2016). EC was not a significant contributor in the MLE (Table 5). The capped sites consistently had higher salt ions and EC than the uncapped sites (Table 3). However, chloride, potassium, and sodium were not correlated to toluene (Table 4) and did not have a significant contribution to the MLE model (Table 5). The results and literature review did not indicate that these ions were important for toluene production in our system.

### *PH*

The pH ranged from 3 to 7.6 at the study sites and the median pH for the uncapped and capped sites was 5.1 and 5.6, respectively (Table 3). According to the Alberta Wetland Classification System (AEP 2015b), the pH ranges for extreme rich fens are higher than 7, moderate rich fens have a pH of 5.5-7, poor fens tend to have pH levels below 5.5 but can be as low as 3.5, and bog pH levels are usually below 4.5. The majority of study sites were in bogs (89%, Table 2). The pH range was higher than expected for bog systems (especially for the capped sites), but within the range of typical pH levels for fen and bog systems.

The significantly higher pH levels at the capped sites (Figure 12) were likely the result of carbonate ions and disturbance caused by the clay cap. The bedrock geology for the study area is the Shaftesbury Formation, consisting of mudstone and carbonate compound concretions of marine origin (Prior et al. 2013). Likely, the clay is buffering the pH of the capped sites at a level closer to neutral. Bogs have natural low pH conditions because of dissolved organic matter, cation exchange and separation from ground water (AEP 2015a). The disturbance caused by the clay cap is likely disrupting the normal processes that produce low pH levels in bog systems.

The literature that has been published thus far suggests that optimal conditions for microbial activity for toluene generation and degradation is near neutral pH and in warm temperatures (Fischer-Romero et al. 1996; Noh et al. 2003; Mrowiec et al. 2005). However, biogenic toluene generation was known to occur at temperatures as low as 12 °C (Fischer-Romero et al. 1996). Our results were collected in temperatures much lower than 12 °C but the temperature when the toluene was generated is unknown since our samples lacked temporal variation. Granted, our results showed that pH had a significant, negative correlation with toluene, indicating that sites with higher pH had lower toluene. Additionally, pH was a significant contributor to the MLE model with a negative coefficient (Table 5). This could indicate that toluene degradation is more sensitive to pH conditions than toluene generation. More likely, this trend is a consequence of more complicated underlying relationships that were unobservable because of the lack of temporal and temperature controls in the study. Study results clearly indicate that the clay pads are influencing pH and toluene levels, but the effect of the pH change on the biological community and resulting toluene is not clear.

### *Depth Interval*

The depth interval was a significant contribution to the MLE (Table 5). It indicated that sites with larger depth intervals tended to have lower toluene levels, holding all else constant. This supports our conceptual model for the capped sites that indicated that the clay cap blocks the escape of toluene causing accumulation directly under the pad because of its buoyancy in water.

## Toluene Benchmark (Objective 4)

A benchmark for toluene levels in uncapped sites could not be generated from this data set. There are several reasons this was not possible. First, there was a lack of temperature and temporal control in the study, factors that were revealed to be important from literature review. In addition, the data provided by our industry collaborator did not increase the sample size by as much as was hoped; it was only 26% of our dataset (Table 2). Statistical analysis for censored data, like toluene concentrations, is complicated and higher sample sizes can help reduce variability effects. A larger dataset could produce more straightforward results from the non-parametric methods that resulted in inconclusive results (Appendix D). Additionally, the distribution of the residuals for the MLE model (Figure 16) indicated that the salinity parameters did not adequately explain the extremely high toluene observations. This implies that there are other variables at play that were not accounted for in our dataset.

The scientific literature indicates that warm temperatures are important for toluene generation and degradation (Fischer-Romero et al. 1996; Noh et al. 2003; Mrowiec et al. 2005). This suggests that the winter toluene levels observed in our study could be relic toluene that was generated in summer. It is likely that toluene concentrations vary throughout the year. An additional complication due to the timing of this study is that during winter months, ice cover likely seals the uncapped sites from the atmosphere, like the function of clay caps. Likely, the difference in toluene levels between the sites is more pronounced when there is no ice cover. Without temporal measures of toluene that cover a full year (including both warmer growing season and winter months), a complete picture of the underlying processes affecting toluene levels and the full range of toluene concentrations was not possible.

A further complication was that there was no way to guarantee that the uncapped sites in this dataset were unaffected by the clay cap. The physical space for sampling was limited by safety concerns for sampling personnel and the peatland systems themselves. Thus, the uncapped samples were often close to the capped sites. Therefore, some interaction was possible. Peat has high hydrologic conductivity and toluene is mobile in the environment. This brings into question the validity of our uncapped sites as representative samples of true background conditions. Toluene split duplicate analysis indicated that the toluene measures were not consistent, but the source of error was unclear. Error could relate to the nature of toluene distribution in the environment, sampling complications, or lab analysis. Through our background research, many sources of potential error were identified. Our best practices recommendations offer suggestions to control these issues.

Despite the limitations of this combined dataset, our results did indicate that the presence of well caps significantly increased toluene levels by 0.46 mg/kg at median pH levels. This suggests it is possible to detect differences from background samples and that remediation criteria might best be established as benchmark comparisons to 'background'. 'Background' variability (especially in pH level) will make determination of a threshold level difficult in this, and potentially other peatland systems. In addition, other factors held constant in our multivariate analysis (depth, frozen condition, ecosite) suggest factors that could be controlled in sampling programs to better differentiate toluene differences. This analysis also found that anthropogenic activities, including clay pad development can influence toluene levels, in addition to petroleum induced effects. Remediation options could differ significantly depending on the anthropogenic cause. This difference is contextually significant when the current toluene guideline of 0.12 mg/kg (AEP 2016a) is considered. The guideline was established for open water freshwater species, not found in peatlands. The guideline may not be contextually relevant, or reflect true toxicological risk.

## Conclusions

This study made great strides in understanding how biogenic toluene is generated and why clay caps promote toluene accumulation. The overall conclusion was that generally, our hypothesized conceptual model was correct; the presence of a clay cap was associated with a significant increase in toluene level by 0.46 mg/kg at average pH conditions. In addition, this effect of the clay cap on toluene levels was significantly dependent on site pH level. This and the significant correlation between toluene and sample depth indicated that site conditions are important predictors of toluene levels. The results of this study suggest it is possible to detect toluene differences between sites with differing conditions and that remediation criteria might best be established as benchmark comparisons to 'background'. The literature review conducted as a part of this study identified several biological and chemical mechanisms that may be contributing to the observed difference in toluene levels. Through the literature review, specific toluene generation pathways were identified. Additionally, we substantially contributed to our initial conceptual model of toluene generation by adding the known pathways of toluene degradation, including through methanogenesis, anaerobic oxidation of sulphate, and aerobic respiration (for the uncapped sites). Furthermore, the addition of the carbonate cycle accounted for the significantly higher pH levels observed in our sites capped with clay.

The differences between the anaerobic and aerobic pathways, captured in our capped and uncapped schematics suggest complex biological and chemical interactions that will require additional study to support establishment of evidence-based remediation criteria. The ability to detect a difference between the two type of sites, likely attributable to 'anthropogenic' processes related to the clay cap, offers both promise of establishing better criteria for remediation, and a broader consideration of causes of increased toluene levels.

### Projection of Tangible Project Outcomes

We have synthesized available literature into toluene cycle schematics. The toluene cycle schematics (Figures 17 and 18) are tools that members of the oil and gas industry can use to understand the biogenic and chemical processes that result in toluene accumulation under well pads. These models can inform changes to industry practices that can help alleviate the presence of toluene. We have noted recommendations formulated from knowledge gained during this analysis in the following recommendations section.

## Recommendations

- The results of our literature review indicated that the production and degradation of toluene is a biologically facilitated process. In our literature study, sulphate was identified as an important factor for toluene generation, but was difficult to differentiate through statistical analysis, possibly because both biogenesis and biodegradation processes are occurring coincidentally. It is evident that when clay caps are constructed, particularly in areas such as this, where sulphate content of the clay will be high, sulphate could influence both processes. However, as we have also demonstrated, the generation of toluene is not fully understood. The knowledge that sulphate reduction is used to produce toluene is new (Zardar et al. 2016). It is possible that there are other unknown pathways for toluene production. Further research of the variables contributing to toluene biogenesis, and biodegradation, as suggested below, will clarify these processes and help establish valid remediation criteria.
- Other nutrients (nitrate and iron manganese) can be used as electron acceptors in toluene breakdown (Heider et al. 1999). This suggests that these chemicals could lower toluene levels, and may offer remediation solutions. Again, further research is required to develop remediation options appropriate for peatlands.
- The clay pad also created a physical barrier that contributed to toluene levels in our MLE model. This suggests that physical removal of the pad to promote aerobic degradation could reduce toluene levels without any further remediation. The full or partial removal of the clay to allow oxygen to penetrate to the organic matter underneath could remediate toluene levels without other intervention. The extent of cap removal required may depend on the sulphate content of the clay, a factor that requires further investigation.
- Capped sites had higher pH when compared to uncapped sites, but differences in toluene levels could only be detected through multivariate analysis. Multivariate analysis also indicated that toluene levels were negatively correlated with pH. However, the scientific literature suggests a positive correlation between pH and toluene, related to biogenesis through microbial activity (Fischer-Romero et al. 1996). Higher pH can also spur microbial degradation (Noh et al. 2003, Mrowiec et al. 2005). It may be that concurrent toluene biogenesis and degradation are occurring, obscuring relationships, as with sulphate results above. The overall effect of pH on toluene concentrations should be determined with further research. More details on a future study are included in the “Application” section below.

## Best Practices Recommendations

This analysis enhanced our understanding of the limitations of data collection and analysis, as well as toluene biogenesis and biodegradation. These insights lead to two best practices recommendations outlined below.

### Peatland-based Guidelines

A challenge facing professionals working on remediation in peatlands is the absence of guidelines developed specifically for this ecosystem type. In the absence of such guidelines, surrogates are used, such as coarse soils (Alberta Tier 1 Guidelines) and aquatic life risk receptors for open water ecosystems

(CCME Guidelines for Protection of Aquatic Life). Our work suggests that chemical and biotic processes may differ in peatlands, relative to these surrogates, such that the risk assumed in these criteria is not appropriately captured, and thus mitigated. The natural variation in cations and anions is not yet well understood, which makes interpretation of results, such as those seen in this study difficult to interpret. Further, peatland systems are likely more complex that these surrogate systems, due to the interactions of water, plant life and parent materials. Risk-based criteria developed specifically for peatlands, and supported with relevant contextual research are a critical gap in the remediation toolbox.

### Complications Associated with Frozen Samples

The freezing of soil is known to change the chemical and physical properties. Freezing of the soil changes the equilibrium points at which solutes adsorb to the organic soil or become suspended in solution (Marion 1995). Frozen, saturated organic soil is also less compressible than unfrozen, saturated samples (Amiri et al. 2016). This suggests frozen samples would retain more of the water phase by volume than unfrozen samples. Moisture content is critical in calculations that quantify the dilution effect on analyte extraction. Additionally, water expands when it freezes and can increase the pore volume of mineral soils by 9% (Ishikawa, et al. 2014), and is likely even higher in peat soils. It is possible that when the frozen samples thaw during transport to the lab, consistently greater head space is produced than unfrozen samples. This is important to analytes such as toluene, the focus of this investigation, which can escape into the extra head space due to its relatively high vapor pressure. Toluene also has high ecotoxicity at very low levels (CCME 1999, 2004); therefore, even small changes can be ecologically significant.

These properties suggest that the collection of both frozen and unfrozen soils or the freezing or thawing of soils during collection or transit introduces a source of error. To address this in our own study, the MLE regression controlled for the frozen status of soils when collected. These concerns have been recognized by Alberta Agriculture and Forestry. Best management practices for the soil sampling recommended by Alberta Agriculture and Forestry (2004) state, “Frozen and water-logged soils should not be sampled because of the difficulty in obtaining a representative sample.” However, peatland systems are very difficult and dangerous to sample when they aren’t frozen. Common practice is to complete this work in the winter. Clearly a solution that would update this standard is needed. Additional study that quantifies the variability introduced by freezing and thawing and influence of original sample condition (frozen/thawed) would help to develop more practical guidelines.

### Complications with Sampling Locations

Further uncertainty in the delineation of potential well pad influences is created by spatial restrictions placed on field sampling. Well pads built on peat are assessed during the winter due to safety concerns with the operation of heavy equipment on lease roads and well pads. Ecologically, it is not desirable to operate heavy equipment during summer in sensitive ecosystems like bogs and fens. Significant effort is required for snow removal not only to facilitate equipment access but also to increase the freezing depth into the soil for road development. Logistically, this limits the area that can cost-effectively be cleared and sampled. By necessity, snow clearing for the collection of background is usually limited to the clay pad area and a buffer.

Peat material can contain up to 98% pore-space (Landva & Phenney 1980), which significantly increases its hydraulic conductivity. Recent work by Chemistry Matters suggests that shallow groundwater flow may carry biogenic toluene from bogs to adjacent peatlands (e.g., fens) (Richards 2017). Collecting “background” samples from close proximity to the well pad creates the potential for sample contamination, if they are within the well pad's radius of influence for the diffusion of toluene and other mobile chemicals. This could lead to over-estimates of background toluene levels and decrease the ability of statistics to show a difference between sites. Careful site selection for background samples, that takes these factors into consideration, can control for such variables.

Delineating differences between background biogenic toluene concentrations and those under the well pad are complicated by common industry practices such as pump-off and the placement of background sampling locations. Pump-off is the accepted industry practice of pumping the clear free-board liquid from sumps into the surrounding area. This liquid is typically rich in dissolved ions derived from drilling mud additives and is likely to have a significant influence on nutrient poor ecosystems, like bogs. Nutrient additions such as these are likely to change baseline microbial activity. Pump-off areas are typically not recorded and are not visually apparent during site visits, especially when site visits occur in the winter. This adds uncertainty as to whether background sampling locations are truly representative. Again, a clearer understanding of natural variation of cations and anions in peatlands would help in differentiating such anthropogenic effects.

## Application

### Future Study

A detailed observational study should be undertaken to confirm and quantify the rate of the different chemical processes identified in our conceptual models and detect any additional relevant pathways. This study should observe toluene levels at one or more well pad sites through multiple strategic sample locations that span the well pad cap and adjacent reference area across a range of distances away from the well pad. Ideally, the timing of these observations would span an entire year to capture seasonality effects. In addition, there are several covariates that should be measured that relate to the chemical and biological processes identified in our conceptual models. Variables that should be included in a future study and the rational for their inclusion are given in Table 7.

*Table 7. Variables that should be further studied in relation to toluene generation.*

| Variables to include or manipulate | Purpose |
|---|---|
| Temperature | Identify effect of temperature on toluene cycle in the system of concern |
| Temporal (seasonality, age of disturbance) | Identify how toluene concentrations change throughout year and age of site. Inform whether toluene criterion should be dependent on season |
| Proximity to well pad | Identify where pad effects are mitigated and what true background toluene levels are |
| Redox Potential | Inform whether the system is dominantly aerobic or anaerobic and which electron acceptor is active |
| Sulphur as sulphate and sulphide | Quantify toluene generation and degradation through sulphate reduction |
| pH | Identify effect of pH on toluene cycle in the system of concern |
| Carbon as carbonate, carbon dioxide, dissolved organic carbon, methane | Quantify toluene degradation through methanogenesis, identify carbonate and carbon dioxide effects on pH in the system |

| | |
|---|---|
| Nitrogen, Manganese, Iron | Identify additional anaerobic toluene respiration pathways |
| Microbe composition | Identify what specific microbes are participating in the toluene cycle in the systems of concern as many microbes can perform these functions and literature tends to focus on one species per finding |

The study should capture seasonality effects on capped systems, as our analyses were limited to observations at one point in time during the winter. Temperature should be an experimental variable as lab analysis has indicated that warm temperatures spur toluene production and degradation but the balance between these two processes in our study system is unknown. Other potential electron acceptors for anaerobic respiration of toluene (nitrogen, manganese, iron) were not available in our dataset but are good candidates for inclusion in future studies. Anaerobic generation of toluene through the reduction of these electron acceptors has not been confirmed in the literature but the monitoring of them in an observational study or manipulation in an experiment would help alleviate this knowledge gap. Measuring redox potential could also inform which of these electron acceptors are being used. In particular, an understanding of the bacteria linked to specific stages in toluene biogenesis and biodegradation and the conditions that influence their productivity would be helpful for remediation. A key variable that should be assessed is proximity to the well pad in terms of the migration of toluene away from the well pad. A study that assesses the effect of distance from the well pad on ‘background’ levels of toluene and other chemical parameters involved in biogenesis and degradation would inform future sampling protocol, by identifying the zone of influence around the well pad. It could also help determine typical background levels. Because season could influence migration from the well pad, both summer and winter sampling would be important.

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