IMPACTS OF GEOTHERMAL HEATING ON GROUNDWATER QUALITY AND BTEX BIODEGRADATION

GINELLE KRYSTLE AZIZ

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Abstract

Benzene, toluene, ethylbenzene, and xylene (BTEX) are common groundwater contaminants that pose environmental and health risks. While bioremediation is a sustainable treatment method, low temperatures in Canada can limit microbial activity. This thesis investigates the coupling of bioremediation with geothermal heat pumps, as there is minimal research on the effects of geothermal heating on groundwater chemistry and microbial degradation in monocultures and a consortium. Laboratory experiments at 15, 28, and 40 °C evaluated BTEX degradation by *Bacillus infantis*, *Microbacterium esteraromaticum*, and a consortium. Groundwater parameters were monitored to assess temperature effects. A *Triticum aestivum* seed germination assay evaluated the soil toxicity post-treatment. Results showed that temperature enhanced microbial activity, with the consortium achieving 42% degradation at 28 °C. Remediated soils supported seed germination, though trace hydrocarbons remained bioavailable and reduced root growth. These findings emphasize integrating chemical and biological assessments to evaluate remediation effectiveness and support sustainable strategies for contaminated subsurface environments.

Dedication

I dedicate this thesis to the community that raised me and continues to shape and guide me along this journey of life.

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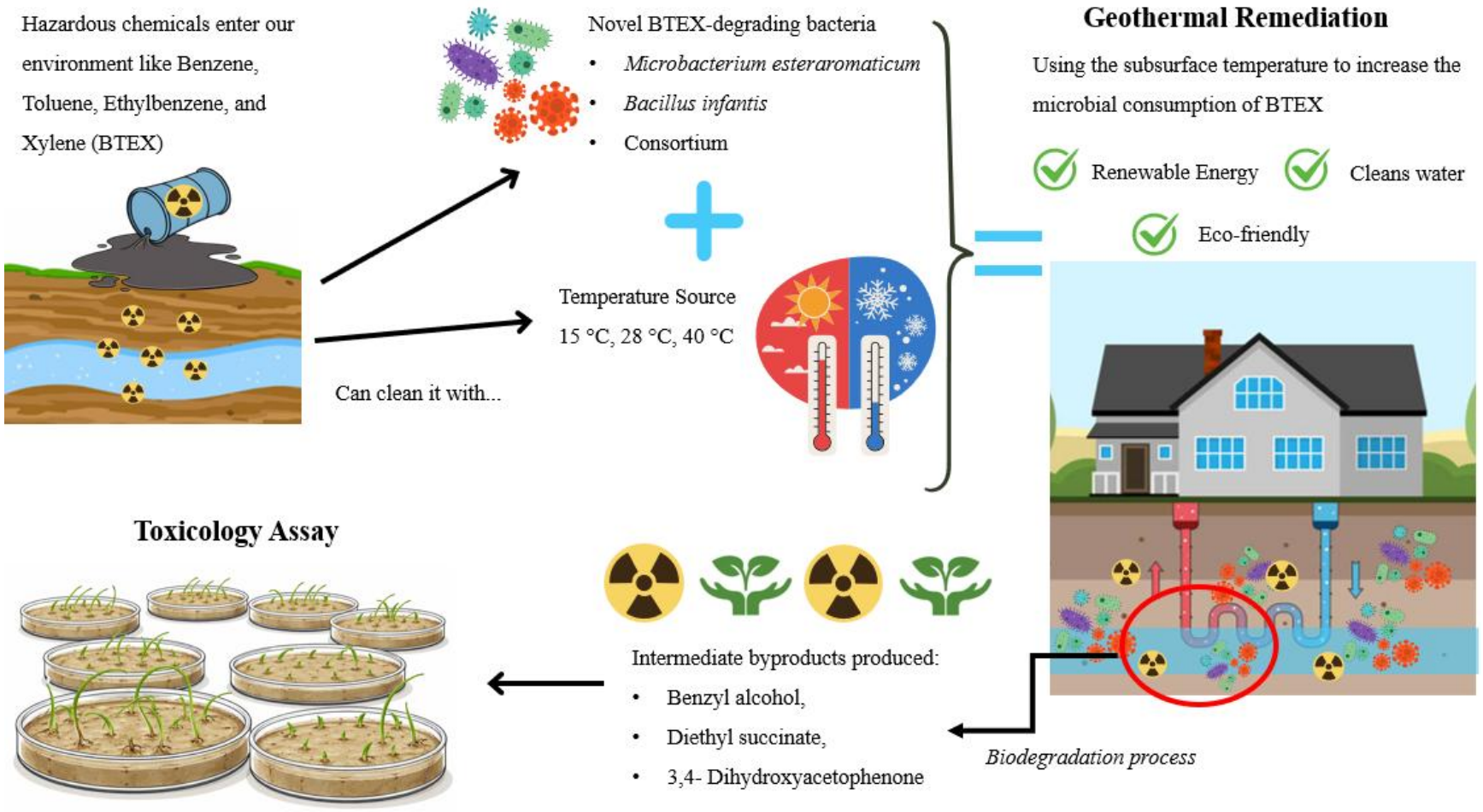


Publications

Gurpreet, Kaur, Ginelle Aziz, Richard Martel, Magdalena Krol, Satinder Kaur Brar (2026). Effects of cyclic temperature from geothermal heating on BTEX biodegradation in soil. *Submitted to the Journal of Contaminant Hydrology*

Conferences

Kaur G., Aziz G., Brar S.K., Krol M. (2025). Geothermal Remediation: Coupling of Geothermal Heating with Bioremediation for Enhanced BTEX Degradation. GAC MAC IAH-CNC 2025, May 11-14, 2025, University of Ottawa, Ottawa, Ontario, **Canada**.

Aziz G., Kaur G., Brar S.K., Krol M. (2025). Enhanced subsurface remediation of BTEX through geothermal heating and bioremediation. 60th CENTRAL Canadian Symposium on Water Quality Research, February 23-24, 2025, Toronto Metropolitan University, Toronto, Ontario, **Canada**.

Kaur G., Aziz G., Brar S.K., Krol M. (2024). Coupling geothermal heating with bioremediation for enhanced degradation of BTEX from subsurface. IWA World Water Congress & Exhibition, August 11-15, 2024, York University, Toronto, Ontario, **Canada**.

Aziz G., Kaur G., Brar S.K., Krol M. (2024). The Effect of Geothermal Cyclic Temperatures on BTEX Bioremediation in Groundwater. WaterWise: Graduate Research Event, August 27, 2025, York University, Toronto, Ontario, **Canada**.

Aziz G., Kaur G., Krol M., Brar S.K. (2023). The Effect of Cyclic Temperatures on Bioremediation of BTEX in Groundwater. 59th CENTRAL Canadian Symposium on Water Quality Research, April 9, 2024, York University, Toronto, Ontario, **Canada**.

Abbreviations

ATES - Aquifer thermal energy storage

BTES - Borehole thermal energy storage

BTEX – Benzene, Toluene, Ethylbenzene, and Xylene

CEPA - Canadian Environmental Protection Act, 1999

CFU – colony-forming units

COD – chemical oxygen demand

CVOCs - chlorinated volatile organic compounds

DO – Dissolved oxygen

IARC - International Agency for Research on Cancer

IC – Ion Chromatography

GC – Gas Chromatography

GHG – Greenhouse gas

GHP – geothermal heat pump

GSHPs - ground source heat pumps

GWT - groundwater temperature

LB - Lysogeny broth

LCA - life cycle assessment

MSM – Mineral salt media

PAH - polyaromatic hydrocarbons

SRB - sulphate-reducing bacteria

TS – Total solids

TSA – Tryptic soy agar

TSB – Tryptic soy broth

US EPA - U.S. Environmental Protection Agency

UTES - Underground thermal energy storage

VAC - volatile aromatic compounds

VOCs – Volatile organic compounds

VOHCs - volatile organic hydrocarbons

VS – volatile solids

Chapter 1: Introduction

1.1 Problem Scope

Water is vital to global health, serving as the backbone for all living organisms. Covering over 70% of the Earth's surface, water shapes the planet's climate, geography, and ecological balance. Despite its abundance, freshwater represents about 2.5% of the global water supply, with 30.1% of that fraction being groundwater, making its availability and quality crucial (Canada WaterPortal, 2024). According to the Treasury Board of Canada Secretariat, approximately 24,000 federally owned contaminated sites have been reported in Canada. Of these, around 3,000 sites involve groundwater contamination, with roughly 400 contaminated by BTEX compounds (benzene, toluene, ethylbenzene, and xylene) (Treasury Board of Canada Secretariat, 2024).

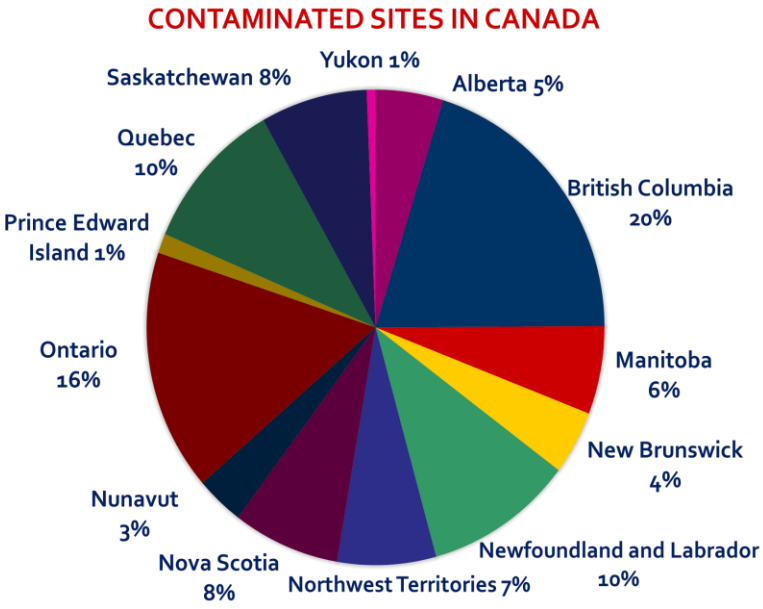


Figure 1.1. Distribution of the contaminated sites in Canada (Treasury Board of Canada Secretariat, 2024)

BTEX is a group of volatile organic compounds. These compounds are commonly found together in petroleum products such as gasoline, diesel, and crude oil, and are widely used in industrial processes, including solvent production and fuel distribution. Due to their widespread use, BTEX compounds are among the most frequently detected contaminants in soil and groundwater at industrial, commercial, and urban sites (Environment and Climate Change Canada & Health Canada, 2016; Environment Canada, 1984; Government of Canada et al., 1993; Government of Canada, 1992).

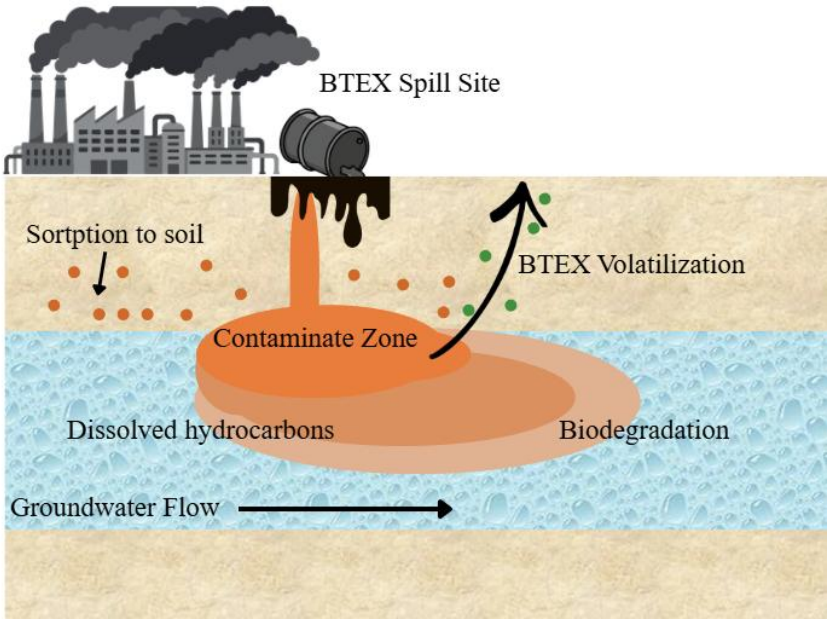


Figure 1.2. Conceptual model of BTEX contamination following a spill event, and how it transports

BTEX compounds typically enter the environment through leaking underground storage tanks and improper disposal of petroleum-based products. The average BTEX concentration in the subsurface can vary significantly depending on the specific location, depth, and contamination history. Once released, their relatively high mobility in both air and water allows them to migrate through soil and groundwater systems, posing significant environmental and human health risks, such as acute and chronic health effects, including impacts to the nervous and reproductive systems (Bolden et al., 2015a; Environment and Climate Change Canada & Health Canada, 2016; Environment Canada, 1984; Government of Canada et al., 1993; Government of Canada, 1992; B. Yu et al., 2022). A study at a chemical pesticide industrial site in China reported soil concentrations from 0.05 to 142 mg/kg for benzene, 0.05 to 315 mg/kg for toluene, 0.05 to 889 mg/kg for ethylbenzene, and 0.05 to 2800 mg/kg for xylenes. Groundwater concentrations were also reported with ranges from 0.7 to 340,000 µg/L for benzene, 0.9 to 4070 µg/L for toluene, 0.5 to 1900 µg/L for ethylbenzene, 1.6 to 6000 µg/L for m-&p-xylene, and 0.6 to 1500 µg/L for o-xylene, exceeding the allowable environmental limits (Huang et al., 2024). Depending on the site and the duration of contamination, concentrations vary.

A variety of remediation technologies exist to address BTEX contamination. Biological methods, such as bioremediation, utilize nature's counterparts to degrade pollutants. Bioremediation is often considered the favoured remediation approach due to its cost-effectiveness and minimal environmental disruption, however, there are limitations (Bala et al., 2022; Sharma, 2021). In Canada, the shallow subsurface (below 20 m) temperature is consistently around 10 °C throughout the year (Ferguson & Woodbury, 2004; Grasby

et al., 2012). Since temperatures below 28 ºC can limit microbial growth, an external temperature source is often used. Some conventional thermal treatments include electrical resistance heating, steam injection, radiofrequency, and thermal conduction heating (Kaur et al., 2021; Krol et al., 2011; Parkinson et al., 2004). While these thermally enhanced bioremediation methods are effective at removing contaminants, they require substantial external energy to maintain the elevated temperature. For example, a chlorinated solvent-contaminated site operated a steam injection system for 12 months, using 48*109 kJ, which equates to supplying electricity for 1 year to 1.5 single-detached homes (Aydinalp Koksal et al., 2015; Parkinson et al., 2004). An alternative is to use renewable energy, such as geothermal heat pumps (GHPs). Geothermal heating loops can be installed in the subsurface and connected to a pumping system to extract or inject heat as seasonal conditions change. GHPs can be used for heating and cooling of buildings, and GHPs are reported to save about 60% in energy costs compared to electric baseboard heating and uses 50% less energy than natural gas furnaces (Canada Energy Regulator, 2018). If GHPs are used at contaminated sites, the injected heat can be used to enhance bioremediation efficiency.

To date, minimal literature has been reported on the coupling of bioremediation with GHPs to degrade hydrocarbons and on their effects on groundwater chemistry. This thesis investigates the effects of temperature on the biodegradation of BTEX, and on groundwater quality parameters. Constant temperature experiments with monocultures and a consortium were conducted to investigate the bacterial growth and BTEX degradation patterns at 15 °C, 28 °C, and 40 °C. Temperature effects on groundwater pH, dissolved oxygen, and anions were observed. Additionally, studies have shown that the bioremediation process can produce intermediate byproducts during the breakdown of BTEX contaminants, such as benzyl alcohol, benzoyl-CoA, (S)-1-phenylethanol, and methyl benzyl succinate (Hernández-Ospina et al., 2024). To investigate these possible toxic byproducts, a seed germination toxicology study was conducted. The study used native *Triticum aestivum* (wheat) seeds from Ontario, Canada, which is a major crop, covering more than 18 million acres across Canada, and is commonly used in ecotoxicity studies of petroleum-contaminated sites (M. K. Banks & Schultz, 2005; Du et al., 2025; Miri et al., 2022; Statistics Canada, 2025). Overall, the groundwater remediation experiment and the toxicology study were performed to investigate the potential environmental impacts of enhanced bioremediation and to design a holistic approach to geothermal bioremediation.

1.2 Research Gaps

The coupling of GHPs, bioremediation, and their prospective environmental impacts are not well-understood. This gap prevents the thermal enhancement of bioremediation using a renewable energy source while providing heating and cooling for buildings. Both GHPs and bioremediation technologies are well

understood in existing literature. However, experimental data on the coupling of the two technologies and their respective effects on BTEX contamination and groundwater quality are very limited. We hypothesize that mesophilic subsurface temperatures achievable with GHP operation (≈20–35 °C) will enhance aerobic BTEX biodegradation while causing measurable but manageable shifts in groundwater chemistry. Thus, the investigation of geothermal heating in the subsurface, on contaminant transport and groundwater properties, is essential for optimizing the coupling of these two technologies for a possible remediation strategy in Canada.

1.3 Research Objectives

Considering the significance of possible temperature effects on groundwater quality, the primary objective of this research was to investigate the effects of temperature on groundwater, on the biodegradation of BTEX in groundwater, and to examine the post-bioremediation effects of BTEX in soil, all under controlled laboratory conditions.

To achieve the primary research goals, the following objectives were established

1. Investigate and analyze the effects of temperature on groundwater properties under constant temperatures, and how it affects BTEX biodegradation with known microbial strains *Bacillus infantis*, *Microbacterium esteraromaticum,* and a consortium – Chapter 3
2. Conduct a toxicology assay to study the germination and growth parameters of *Triticum aestivum* on BTEX-remediated soil – Chapter 4

1.4 Dissertation Layout

Chapter 1 introduces the study by outlining its specific objectives. It also presents the research motivation, identifies existing research gaps, and provides an overview of the thesis structure.

Chapter 2 is a comprehensive review of the existing literature and is structured into two distinct sections. The first section addresses the existence of BTEX in the environment, remediation techniques, and geothermal heat pumps (GHPs). The second section reviews the literature on seed germination bioassays of petroleum hydrocarbon-contaminated soil. This section dives into a variety of plant species used for toxicology assays on contaminated and remediated soil, the different methodologies used, and the toxic intermediates produced during remediation.

Chapter 3 addresses objective 1 by outlining the materials, analytical methods and instruments used in the temperature-effect study. The chapter further presents the microbial growth, BTEX degradation, and

groundwater quality results from both the preliminary and final experiments, along with insights gained throughout the process.

In Chapter 4, toxicology assays were conducted and analyzed to assess the potential effects of toxic intermediates formed from BTEX biodegradation on germinated seeds. Their respective materials, analytical methods and instruments were described in accordance with standard regulatory protocols—this chapter addresses objective 2.

Chapter 5 highlights a summary of the research findings, their significance, and recommendations for future work.

Chapter 2: Literature Review

2.1 From heat to healing: Geothermal energy as a catalyst for environmental remediation

2.1.1 Introduction

Globally, about 50% of the world's population depends on groundwater for domestic use (UNESCO World Water Assessment Programme, 2022). In Canada, almost 9 million people solely depend on groundwater as their main source of water, which equates to 30.3% of Canada's population (Environment and Climate Change Canada (ECCC), 2013). Additionally, 80% of Canada's rural population uses groundwater as their water source, while 52% of Canada's First Nations rely on groundwater (Council of Canadian Academics (CCA), 2009; Indigenous Services Canada, 2013). Access to clean water is vital for survival, yet the quest for potable water is increasingly challenged by pervasive groundwater and soil contamination (Shores et al., 2017; B. Yu et al., 2022). This pressing issue highlights the global urgency to address and mitigate pollution in our essential water resources. Due to accidental leakages and spills of petroleum hydrocarbons, such as crude oil and natural gas, the subsurface can become contaminated, and depending on the conditions of the subsurface, the contaminant can persist for a long duration and cause irreversible damage to aquifer zones (Braddock & McCarthy, 1996; UNESCO World Water Assessment Programme, 2022). These contaminants can infiltrate the unsaturated zone, contaminate groundwater, and disperse further into the environment, which poses a threat to our ecosystems. If our groundwater supply is not safeguarded and is continuously polluted, many Canadians will not have access to clean drinking water. Additionally, if the thousands of contaminated sites across around Canada are not treated, this can further create adverse health effects.

A large quantity of remediation techniques exists to degrade these toxic chemicals, including chemical, physical, and biological remediation techniques. Common remediation techniques such as pump-and-treat, air sparging, and chemical oxidation often have drawbacks including high costs, long timelines, and risks of incomplete contaminant removal (Vidali, 2001; S. Zhao et al., 2023). In contrast, bioremediation offers a scalable, cost-effective, environmentally friendly approach that utilizes natural microbial processes to degrade contaminants without introducing harmful byproducts (Vidali, 2001; S. Zhao et al., 2023). However, natural attenuation—a form of bioremediation relying solely on unassisted microbial activity—can be limited by slow degradation rates in cold subsurface environments, $\leq 8°C$ (Van Stempvoort & Biggar, 2008). By coupling bioremediation with geothermal heating, microbial activity can be enhanced, providing optimal temperatures that accelerate the breakdown of contaminants and improve overall remediation

efficiency. However, the degradation of BTEX in groundwater has not been addressed under cyclic temperatures that range from high temperatures to low temperatures, to mimic geothermal heating and cooling cycles. Previous reviews have focused on the effects of temperature on bioremediation though thermally enhanced bioremediation at constant temperatures or energy storage systems and heating technologies, such as aquifer thermal energy storage systems and electrical resistance heating systems (Bonte et al., 2013; Kaur et al., 2021; Moradi et al., 2018; Q. Wang et al., 2022). As such, this chapter focuses on the assessment of bioremediation with geothermal heating to decontaminate groundwater. This is done by summarizing the thermal effects on the biogeochemical cycle and on bioremediation, providing an overview of BTEX and its impact on the subsurface. The removal of BTEX through remediation techniques and its enhancing parameters, including geothermal heating, and the impact of temperature on groundwater quality, is also discussed. The results of each section should be used to assess and evaluate if geothermal heat pumps can theoretically be utilized to remediate BTEX-contaminated groundwater.

2.1.2 Background on BTEX

The release of aromatic hydrocarbons, specifically petroleum hydrocarbons, into the environment is a serious global issue for our ecosystem. They are environmentally harmful pollutants, that can be released into the environment or found naturally in rock formations or volcanoes (J. W. Moore & Ramamoorthy, 1984; National Research Council (US) Committee on Pyrene and Selected Analogues, 1983). It is estimated that 10% of total global anthropogenic nonmethane organic carbon emissions are from aromatic species, such as benzene and formaldehyde (Barnes, 2015). There are different forms of aromatic hydrocarbons, including monoaromatic, polycyclic, and heterocyclic aromatic compounds. These structure forms have common features in their structure; however, they can behave differently due to differences in chemical properties and reactivity. Some examples of monoaromatic hydrocarbons are benzene, toluene, ethylbenzene, and (o-, m-, p-) xylene isomers (BTEX), the simplest chemical structure being benzene, C_6H_6 (Z. Wang et al., 2016). BTEX shares similar chemical properties and environmental behaviours to other aromatic hydrocarbons like naphthalene and styrene.

BTEX is a ubiquitous mutagenic substance that receives significant attention due to its presence in various products and processes, different media samples (air, water, soil), and the risk it poses to the environment and human health. According to the International Agency for Research on Cancer (IARC), ethylbenzene is a possible human carcinogen, while benzene is a classified carcinogen (Environment Canada, 1984). BTEX or its monomers, can be found and sourced from petroleum and petroleum products, crude oil, hydraulic fracturing, leaks from underground storage tanks and landfills, oil spills, automative products, improper treatment and disposal of wastewater, and consumer and personal care products like household cleaners, detergent agents, sunscreen, and hand sanitizer (Bolden et al., 2015b; Fetter et al., 2017; Hinwood et al.,

2007; Nicole, 2022; Pal et al., 2022, 2023; Shores et al., 2017; Van Stempvoort & Biggar, 2008; B. Yu et al., 2022; Zanello et al., 2021). Additionally, BTEX is widely used as an industrial solvent and additive, potentially allowing these compounds to be unintentionally added to building materials, cosmetics, paints, and carpets, which can aid in the emissions of these volatile organic compounds (VOCs) (Weschler & Shields, 1997). Specifically in the petrochemical industry, BTEX is one of the most common chemicals produced, with benzene accounting for approximately 50 million metric tons globally per year (Somboon et al., 2020). These sources can contaminate indoor and outdoor air, different types of water bodies (surface water, groundwater, aquifers, oceans) and soil media (Bakhtiari et al., 2018; Honda & Suzuki, 2020; Robinson et al., 2009; B. Yu et al., 2022). With the increasing use of BTEX, its contamination is expected to continue to rise (Shores et al., 2017).

The Treasury Board of Canada identified about 24,000 federal contaminated sites, where 3,152 of those sites contained hydrocarbon groundwater contamination. These sites were contaminated with petroleum hydrocarbons, polyaromatic hydrocarbons, or halogenated hydrocarbons (Treasury Board of Canada Secretariat, 2024). Typical field concentrations in groundwater have been reported to be 24-95 mg/L for benzene, and 12-60 mg/L for toluene (EPA, 2004). A 2008 study analyzed 33,189 wells that were primarily used for conventional oil and gas operations from 13 states in the USA. The highest concentrations for benzene, toluene, ethylbenzene, and m-xylene were 27 mg/L, 37 mg/L, 19 mg/L, and 0.611 mg/L. The median concentrations however were lower, 10 mg/L, 9.7 mg/L, 1.8 mg/L, and 0.137 mg/L (Guerra & Drewes, 2008). A 2016 study in Eastern subarctic Canada examined BTEX levels in tree cores located at and near a former landfill site (1990's) where BTEX contamination and BTEX migration were present. A previous study at this site, from 2006 to 2011, indicated that the groundwater samples had concentration ranges of benzene (2.0– 990.0 µg/L), toluene (6.5– 27.9 µg/L), ethylbenzene (0.7– 27.9 µg/L), and xylenes (2.0–17.1 µg/L) (Fonkwe & Trapp, 2016). All the studies above have BTEX concentrations or its compounds higher than the allowable limits set for these compounds, as shown in Table 2.1, according to the Canadian and American allowable groundwater limits of BTEX in groundwater. This indicates that the concentration of BTEX in the environment exceeds the legal limits, which poses a risk to human health and the environment.

2.1.2.1 Mobilization and Persistence of BTEX in the Subsurface

BTEX compounds transport in the subsurface through several mechanisms, including advection, diffusion, sorption/desorption, and volatilization. Understanding the transport mechanisms is crucial for developing effective remediation strategies and protecting groundwater resources from contamination, as these mechanisms can greatly contribute to BTEX migration. Advection and dispersion transport dissolved contaminants by groundwater flow velocities. If the flow rate increases, the mass transfer of BTEX

increases (Savoie et al., 1998). Under diffusion, BTEX molecules move from areas of higher concentration to areas of lower concentration. This process is typically slower than advection but can be significant in low-permeability zones where groundwater movement is limited (Powers et al., 2001). In soil media, the extent of sorption and desorption depends on soil properties, such as organic matter content and soil texture. Sorption can have a positive or negative effect on the environment. If BTEX adheres to soil particles, it can retard the movement of contaminants, which can decrease contaminant transport. In comparison, the negative effect occurs when desorption releases the BTEX compounds into the surrounding groundwater. (Zytner, 1994). (Zytner, 1994) demonstrated that the sorption coefficients (K_d) for BTEX compounds can vary significantly. Higher sorption coefficients result in greater retardation, particularly for compounds like ethylbenzene and xylenes, compared to benzene which would remain mobile and bioavailable over time (B. J. Moore et al., 2002; Zytner, 1994). For volatilization, BTEX can transfer from the liquid phase to the gas phase and migrate through soil air spaces (such as the vadose zone), which is influenced by soil temperature, vapour pressure, and BTEX volatility. (Y. Han et al., 2024) studied the volatilization of BTEX in air-dry soils and observed that BTEX volatilized linearly with time. Typically, higher vapour pressure and Henry's constant indicate a greater possibility for the contaminant to volatilize (Arthurs et al., 1995; W. S. Chen et al., 2010; Seagren & Becker, 2002). Table 2.1 summarizes these parameters at 25 °C, they are expected to increase with temperature.

When BTEX compounds leak into the subsurface, they migrate through the unsaturated zone and disperse as residual saturation. In cases of substantial release, these compounds can reach the capillary fringe and spread laterally above the saturated zone, facilitating further contaminant transport. BTEX compounds can exist in the subsurface as light non-aqueous phase liquids (LNAPLs). These LNAPLs typically have a density lower than water (1 g/mL) and therefore migrate downward through the vadose zone until reaching the water table, where they spread laterally and form a floating layer. Once present, BTEX compounds partition from the LNAPL phase into the aqueous phase, creating a dissolved contaminant plume in groundwater, further contaminating it. LNAPLs can partition into both water and air phases and can be retained in the soil media as immobile components (Zanello et al., 2021). Mobility depends on subsurface parameters like soil type, pH, and temperature. An increase in temperature can increase the volatilization rate of the LNAPL, allowing the contaminant to enter the vapour phase (W. Chen et al., 2010; A. Liu et al., 2018). Immobile hydrocarbons exist within the soil-water phase, while mobile hydrocarbons may undergo partial or full dissolution. BTEX, particularly, is considered a mobile contaminant due to its tendency to partially dissolve in water, allowing dissolved contaminants to be transported over long distances (Chesnaux, 2008; Feenstra et al., 1991; Illangasekare et al., 1995; Zanello et al., 2021). Studies have shown that benzene and toluene have the highest mobility in the dissolved phase, and in sandy loam and clay loam, while xylene and ethylbenzene tend to concentrate near the source zone (Shores et al., 2017; Zanello et al.,

2021). Benzene is notable for its lower sorption potential and higher solubility compared to other BTEX compounds, which enables the formation of extensive and larger dissolving plumes in the subsurface (Ruiz-Aguilar et al., 2003; Zanello et al., 2021).

While the fate and transport of BTEX is a concern, the persistence of this contaminant in the environment is another component that needs to be studied. Unfavourable redox conditions can make BTEX degradation difficult. Factors that influence this are microbial activity, oxygen availability (aerobic or anaerobic), temperature, and soil and groundwater properties. Studies have shown that in field sites, BTEX can be biodegraded by aerobic microorganisms faster than anaerobic ones, due to the availability of oxygen (Barbaro et al., 1992; Lovley, 1997, 2001; National Research Council, 2000; Patterson et al., 1993). Additionally, some studies have reported the half-life of BTEX compounds in aerobic conditions in laboratory microcosms. Studies measuring primary biodegradation reported half-lives of 0.2-679 days with a mean of 1.5 days and initial concentrations of ≤ 5 mg/L for benzene. For toluene, it was reported that half-lives ranged from <1- 495 days with a mean of 0.5 days. For the half-lives of ethylbenzene, o-xylene, and p-xylene, the initial concentrations used for the experiments were ≤ 2 mg/L. From this, the half-lives reported for ethylbenzene were 1- 22 days with a mean of 4.4 days, 1- 41 days with a mean of 4.3 days for o-xylene, and 1- 28 days with a mean of 4.4 days for p-xylene (Aronson et al., 1999). It could be observed that the order of mean half-lives is ethylbenzene > p-xylene > o-xylene > benzene > toluene. These reported half-lives do vary, depending on the redox conditions mentioned above and temperature. Subarctic regions like Northern Canada have lower temperatures, which can decrease the diffusion rate, volatilization, and solubility of organic compounds, thereby reducing microbial activity. Additionally, permafrost can restrict groundwater flow and flux, which impacts contaminant transport (Braddock & McCarthy, 1996; Ulrich et al., 2010). BTEX persistence can also be impacted by the dissolution rate of BTEX. Table 2.1 has the dissolution rate of the BTEX compounds in water, and it has been found that benzene has a dissolution rate of 2.6, which is 20.6 times higher than toluene, and ethylbenzene (Njobuenwu et al., 2005). This does vary depending on factors such as temperature, surface area, and the concentration, the compounds that have a slower rate take a longer time to dissolve in groundwater, which allows it to persist longer in the environment.

Table 2.1. BTEX Properties

Property	Benzene	Toluene	Ethylbenzene	Xylene
Chemical Formula	C_6H_6	$C_6H_5CH_3$	$C_6H_5CH_2CH_3$	$C_6H_4(CH_3)_2$
Density (g/cm^3)[1]	0.87	0.86	0.87	m-xylene: 0.86 p-xylene: 0.10 o-xylene: 0.88

[1] (Fedrizzi et al., 2013)

Property	Benzene	Toluene	Ethylbenzene	Xylene
Water Dissolution rate (cm^2/s)[2]	10.30×10^{-6}	9.10×10^{-6}	8.23×10^{-6}	m-xylene: 8.23×10^{-6} p-xylene: 8.23×10^{-6} o-xylene: 8.23×10^{-6}
Solubility (mg/L) at 20 °C[3]	1780	515	152	m, p-xylene: 198 o-xylene: 175
Diffusion coefficient in liquid (cm^2/d)[4]	0.9504	0.81216	0.7344	0.7344
Mean Aerobic Half-life (days)[5]	1.5	0.5	4.4	p-xylene: 4.4 o-xylene: 4.3
Henry's Constant (dimensionless) [4]	0.19792	0.23391	0.28789	0.260899
Vapour Pressure at 25 °C (atm)[1]	0.125	0.037	0.009	m-xylene: 0.00108 p-xylene: 0.00115 o-xylene: 0.00861
Canadian Maximum Acceptable Concentration (MAC) in Drinking Water (mg/L)[6]	0.005	0.06	0.14	0.09
Ontario Potable Groundwater Maximum Concentrations (mg/L)[7]	0.005	0.0024	0.024	0.3
American Allowable Limit in Groundwater (mg/L)[8]	0.005	1.00	0.7	10
Where can it be found? [3] [9]	Synthetic rubbers, insecticides, cosmetics, cigarette smoke, auto exhaust	Found naturally in petroleum products, paints, gums, stain removers	Gasoline additive, paints, pesticides	Gasoline, solvent in rubber and printing

*Biodegradation half-life is influenced by factors such as pH, temperature, microbes, and nutrients

2.1.2.2 Impacts of BTEX on the Environment and Human Health

As mentioned above, BTEX can contaminate soil, air, and water, allowing it to easily enter human systems through the pathways of ingestion, inhalation, and adsorption through the skin (Honda & Suzuki, 2020; B. Yu et al., 2022). BTEX is known for its potential toxic, mutagenic, and carcinogenic effects (Werder et al., 2019; B. Yu et al., 2022). Due to the severity of the side effects to organisms, there are guidelines for this substance under *the Canadian Environmental Protection Act (CEPA)* 1999 and the U.S. *Environmental*

[2] (Njobuenwu et al., 2005)
[3] (Mitra & Roy, 2011)
[4] (Shores et al., 2017)
[5] (Aronson et al., 1999)
[6] (Government of Canada, 2025)
[7] (Ministry of the Environment, 2011)
[8] (EPA, 2025)
[9] (John V. Headley S. Lee Barbour & Gong, 2000)

Protection Agency (US EPA). However, as mentioned, the concentration of BTEX in the environment often exceeds the stated allowable limits for drinking and potable groundwater.

Subsurface BTEX contamination can also significantly impact wildlife and ecosystems. Habitat degradation is a common side effect of oil spills or leaks, whether on land or in water bodies, as observed in the Deepwater Horizon oil spill in the Gulf of Mexico and in other wetland cases (Barron, 2012; McGenity, 2014). This degradation can have a significant impact on wildlife that depends on these habitats for food and shelter. In aquatic life, when BTEX enter water bodies, they can cause acute toxicity to fish, invertebrates, and plant species (Asejeje et al., 2021; Gissi et al., 2021; Headley et al., 2001). A study on biota BTEX concentrations showed that common species consumed by community members had higher BTEX levels than those in their surface water exposure media (Asejeje et al., 2021). In 2010, grocery stores in Belgium recorded the highest benzene levels in smoked (18.90 µg/kg) and canned (7.40 µg/kg) fish (World Health Organization, 2019). Additionally, epidemiological studies have shown that when wildlife is exposed to or consumes high concentrations of BTEX or its components, there's an impact on their immune system, lungs, kidneys, liver, reproductive organs, neurobehaviors, and can cause dermatological symptoms and mortality (C. J. Davidson et al., 2021; Folkerts et al., 2021; Saunders et al., 2018; L. Wang et al., 2023).

Similarly with humans, BTEX exposure can lead to adverse effects on the nervous system, heart, kidneys, liver, and increase the risk of developing nonlymphocytic leukemia (Wongbunmak et al., 2020; B. Yu et al., 2022). Studies have also shown that BTEX can have endocrine-disrupting properties that can impact reproductive systems (Bolden et al., 2015a; Kassotis et al., 2016). As mentioned, humans can be exposed to BTEX through ingestion of food and groundwater. The United States Food and Drug Administration evaluated 70 "table-ready" foods from 1996 to 2006. In the majority of the 70 foods, benzene was detected, ranging from 1- 190 µg/kg (e.g., fully cooked ground beef), which is above the allowable limits (World Health Organization, 2019). Additionally, a study showed that with high peak concentration spills, benzene and toluene exceeded the EPA drinking water limit in sandy loam, and benzene exceeded the limit in clay loam soil (Shores et al., 2017). According to the regulations, the allowable limits of benzene and toluene in groundwater, 0.005 mg/L and 0.06 mg/L, respectively, are less than those of ethylbenzene and xylenes. This shows that benzene and toluene are riskier and pose a threat to human health and the ecosystem, when their concentrations exceed environmental limits.

2.1.3 BTEX Remediation Techniques

Understanding the fate, transport, and distribution of BTEX and other hydrocarbons within the environment is vital to effectively managing and remediating this abundant chemical. There are multiple in-situ, on-site, and ex-situ remediation technologies that can remove BTEX, including physical, chemical, and biological

methods. In-situ remediation occurs within the subsurface without any excavation, on-site remediation occurs on the site of the contamination with excavation, and the decontaminated media is returned to the original location, while ex-situ remediation occurs off-site for the treatment and processing of the contaminant (Caliman et al., 2011; Ossai et al., 2020).

Physical methods can be in situ, ex situ, or on-site. Some physical remediation techniques include soil washing, soil vapour extraction, pump and treat, and incineration. These methods tend to isolate and separate the contaminants in the media (Lim et al., 2016; Ossai et al., 2020). Chemical treatment methods can be in-situ or ex-situ. Some chemical remediation technologies include chemical oxidation using different types of oxidants like ozone and persulfate, activated carbon treatment, electrochemical systems, and ultraviolet and photocatalytic oxidation (Ambaye et al., 2022; Ossai et al., 2020). The chemicals used can contain, sequester, separate, and remove the contaminants from the polluted media. Biological remediation technologies include phytoremediation (using plants to extract contaminants), and different bioremediation (supplying nutrients and oxygen to the native microbes in the subsurface) techniques, such as bioaugmentation (supplying microbes to the subsurface), biostimulation (supplying nutrients to the subsurface), and bioventilation (supplying oxygen to the subsurface) (Ossai et al., 2020; Van Stempvoort & Biggar, 2008). Most bioremediation methods occur in-situ.

From these different techniques, some methods are favoured over others due to cost-effectiveness and environmental sustainability (Caliman et al., 2011; Chesnaux, 2008; Fleuchaus et al., 2018a; Koshlaf & S Ball, 2017; Yuniati, 2018). Bioremediation is favoured because it is non-invasive, cost-effective, and it has the potential to eliminate waste and contamination permanently (Firmino et al., 2015; Lim et al., 2016; Vidali, 2001). According to the US EPA, bioremediation represents 24% of the remediation technologies chosen for groundwater and soil remediation (Q. Wang et al., 2022).

2.1.3.1 Bioremediation of BTEX

Bioremediation is commonly used for the removal of petroleum hydrocarbons, as it has been in practice since the 1940's (Ossai et al., 2020). Microorganisms, such as bacteria or fungi, are either introduced into the subsurface or natively present and utilized to consume and metabolize BTEX compounds. During the biodegradation process, microorganisms use the carbon in the BTEX compounds as a food source and convert them into less harmful by-products such as carbon dioxide and water (Farhadian et al., 2008; Ossai et al., 2020; Wongbunmak et al., 2020).

The success of bioremediation depends on various factors, including the type and concentration of the pollutant, the availability of appropriate microorganisms, and environmental conditions such as temperature, pH, and oxygen levels (Kaur et al., 2021; Lim et al., 2016). In soils, depending on soil type

and its properties, BTEX behaves differently, as bioremediation in soils is governed by the interactions of biological, chemical, and physical processes that control contaminant degradation, mobility, and persistence. Microbial activity is the primary driver, with hydrocarbon-degrading microorganisms transforming BTEX compounds through enzymatic pathways (e.g., monooxygenases and dioxygenases) into intermediate products that can be further mineralized to CO_2 and H_2O (Vidali, 2001). The efficiency of these processes is strongly influenced by redox conditions and the availability of electron acceptors such as oxygen, nitrate, and sulphate, which determine degradation pathways and rates (Kaur, 2026; Kaur et al., 2023; Miri et al., 2022). Contaminant bioavailability also plays a critical role, as sorption to soil organic matter and mineral surfaces can limit microbial access, particularly in fine-grained soils. Soil physical properties, including texture and porosity, influence oxygen diffusion, water retention, and mass transport, with clay and silt fractions often restricting permeability while increasing sorption capacity. Chemical properties such as pH, nutrient availability, and organic carbon content further regulate microbial activity, with optimal biodegradation typically occurring near neutral pH and sufficient nitrogen and phosphorus levels. Temperature is another key factor, as increased temperatures enhance microbial metabolism and contaminant desorption, although in clay-rich soils these benefits may be offset by diffusion limitations (Kaur et al., 2023, 2025). Additionally, moisture content controls both microbial activity and solute transport, while plant–microbe interactions in the rhizosphere can further stimulate degradation through the release of root exudates (Butcher et al., 2020). Together, these mechanisms highlight the complexity of soil bioremediation and the importance of considering site-specific conditions when evaluating contaminant fate and remediation efficiency.

The subsurface itself is home to many microbes with different metabolic pathways: chemotrophic (aerobic), chemotrophic (anaerobic), and phototrophic (anoxygenic), and some of them have the potential to degrade contaminants. However, the biodegradation process depends on the availability of the microbe and the contaminants' transport mechanisms, such as sorption, dissolution, and volatilization (Kaur et al., 2022). Additionally, microorganisms have optimal temperature ranges where they experience peak growth and degradation rates. In Ontario, Canada, studies have shown that hydrocarbons can be degraded in cold climates below 15 ºC (Van Stempvoort & Biggar, 2008). In comparison, a study reported that the temperature range of 20- 35 ºC increased cell growth by 4-fold; however, when the temperature increased beyond that range to 40 ºC, growth declined rapidly (Deeb & Alvarez-Cohen, 1999). Microorganisms that can grow and adapt to cold environments, below 15 ºC, are considered psychrophiles. Mesophilic microorganisms grow within the temperature range of 15 ºC to 45 ºC, and thermophilic microorganisms' optimal temperature range is 45 ºC to 75 ºC (Kaur et al., 2021). However, in the literature, most identified bacterial strains are mesophiles. Commonly identified genera and species that degrade BTEX include *Pseudomonas*, *Burkholderia*, *Sphingomonas*, *Ralstonia*, *Azoarcus*, *Thauera*, *Dechloromonas*,

and *Rhodococcus* (Chicca et al., 2020; Coppotelli et al., 2010; Kim & Jeon, 2009; Morya et al., 2020). Table 2.2 summarizes hydrocarbon-degrading bacterial strains with respect to their optimal environmental conditions, reflecting the diversity and adaptability of microbial communities in the subsurface. Other parameters, like pH, affect degradation rates. For example, the optimal pH for hydrocarbon degradation is 7.5, with a degradation rate of 78% (Pawar, 2012; Rahman et al., 2002). Extreme pH levels can affect the degradation process as they can create a toxic environment for the microbial community.

Table 2.2. Bacterial Strains that can degrade hydrocarbons in the subsurface

Bacterial Strains	Optimum Growth Conditions	Hydrocarbon Degraded	Bio-degradability	Degradation Efficiency	Study Scale	References
Bacillus infantis	28 ºC	BTEX	Aerobic	B- 65.2% T- 71.02% E- 76.44% X- 74.04%	Lab Scale	(Kaur et al., 2023)
Microbacterium esteraromaticum	28 ºC	Degrades BTEX in liquid medium and soil slurry	Aerobic	B- 67.98% T- 72.8% E- 77.52% X- 74.58%	Lab Scale	(Kaur et al., 2023; Wongbunmak et al., 2017)
Bacillus cereus	30- 40 ºC, can grow at 55 ºC	BTEX	Aerobic or facultative anaerobic	63.9-74.7%	Lab Scale	(Gilbert et al., 1974; Handayani et al., 2019)
Pseudomonas putida	28 ºC	Alkanes, BTEX	Aerobic	89%	Field Study	(Chicca et al., 2020; Fonseca et al., 2011a; I. et al., 2022; Palleroni* et al., 2010; Szulc et al., 2014; X. Xu et al., 2018)
Acinetobacter sp. 16S	30- 37 ºC	Alkanes	Aerobic	69.17%	Lab Scale	(Cai et al., 2021; Metzgar, 2004)
Pseudomonas aeruginosa	25- 39 ºC	Alkanes, BTEX	Aerobic	73- 81.49%	Lab Scale	(Ikram et al., 2022; Kumar et al., 2008; LaBauve & Wargo, 2012; Ravi et al., 2022)

Bacterial Strains	Optimum Growth Conditions	Hydrocarbon Degraded	Bio-degradability	Degradation Efficiency	Study Scale	References
Alcanivorax borkumensis	25– 30 °C	BTEX	Aerobic	64.7%	Lab Scale	(Bookstaver et al., 2015; Kadri et al., 2018)
Mycobacterium austroafricanum	25– 37 °C	Aromatic hydrocarbons (BTEX), methyl tert-butyl ether (MTBE)	Aerobic	MTBE: 99% removal for concentrations up to 15mg/L	Lab Scale	(Maciel et al., 2008)
Sphingomonas paucimobilis	20– 30 °C	Polycyclic aromatic hydrocarbons (PAHs), phenanthrene	Aerobic	52.9%	Lab Scale	(Coppotelli et al., 2010)
Bacillus subtilis	25– 37 °C	Alkanes (C10–C20)	Aerobic	32.61%	Lab Scale	(Parthipan et al., 2017)
Burkholderia sp. K24	25– 37 °C	Aromatic hydrocarbons, BTEX	Aerobic	Not detected	Lab Scale	(Morya et al., 2020)
Janibacter sp. SB2	25– 35 °C	PAHs, BTEX	Aerobic	45.5%	Lab Scale	(H. M. Jin et al., 2013)
Peptococcaceae	20– 30 °C	Alkanes	Anaerobic	Not detected	Lab Scale	(Mohamad Shahimin & Siddique, 2017; Siddique et al., 2015)
Bacillus licheniformis ARMP2	29 °C	Petroleum hydrocarbons	Aerobic	88%	Lab Scale	(Ravi et al., 2022)
Bacteria consortium*: Pseudomonas sp., Yarroia sp., Acinetobacter sp., Corynebacterium sp., Sphingomonas sp.*	28- 30 °C	BTEX	Aerobic	B: 97% T: 93% E: 90% X: 98%	Lab Scale (batch)	(Jo et al., 2008)
Dechloromonas aromatica RCB	30 °C	BTEX	Anaerobic	Mineralized 100% B, T, E to CO_2	Lab Scale	(Chakraborty et al., 2005)

Some successful techniques for enhancing the biodegradation of petroleum hydrocarbons are by oxygenating the groundwater, or amendments with nitrate (Van Stempvoort & Biggar, 2008). Oxygenating groundwater is an effective way to enhance degradation, as it provides oxygen to microbes, increasing the metabolism of organic compounds. In confined aquifers, the groundwater has no air phase, the petroleum hydrocarbon plume is anaerobic, which prolongs the degradation process. However, oxygenating the groundwater is not the most effective in low-permeability or shallow aquifers. Other electron acceptors, like sulphate, nitrate, or ferric iron, can be used to substitute oxygen for anaerobic microorganisms to degrade hydrocarbons (Van Stempvoort & Biggar, 2008). Besides providing electron acceptors to increase metabolism, another way to improve bioremediation is to increase the temperature of the microbe's environment.

2.1.3.2 Thermally Enhanced Bioremediation

Thermally enhanced bioremediation helps to address the challenges of efficiency and duration of the pre-existing bioremediation technique. Microbes grown at their optimum temperature will positively enhance the bioremediation process. There are multiple ways to thermally enhance the bioremediation process, such as electrical resistance, radio frequency techniques, and hot fluid injection (Z. Han et al., 2020; Moradi et al., 2018; Price et al., 1999).

The increment in temperature does depend on the contaminants and the microbes, but studies have shown that a substantial increase in temperature can enhance bioremediation. Temperature is responsible for controlling microbial metabolism, diffusion rates, and solubility (Garnier et al., 2011; Riedel, 2019; Somboon et al., 2020). A study on the bioremediation of toluene showed that every time the soil-water temperature increased by 10 ºC, the degradation increased by 2-folds (Q. Wang et al., 2022; Yadav et al., 2012). Additionally, BTEX is usually found in the LNAPL phase, and the permeability of microbial cell membranes to LNAPL increase with temperature. This further promotes the uptake of organic compounds and nutrients, which are provided to the bacteria (Corseuil & Weber, 1994; Lekmine et al., 2014; Zeman et al., 2014) The higher the rate of contaminant uptake, the higher the rate of contaminant decomposition. Studies have shown that in warm environments, CO_2 concentration increases in the subsurface, which impacts microbial respiration, which further helps to understand the relationship between temperature and microbial activity (Kaur et al., 2021). Temperature also affects contaminant transport properties. An increase in temperature increases contaminant solubility and diffusivity, while decreasing its viscosity (Garnier et al., 2011). Majority of the studies highlight the benefits of increasing temperature for microbial growth.

These thermal methods however can be energy demanding, making it not cost-effective and can increase GHG emissions with the burning of fossil fuels (Hinchee & Smith, 1992; Yadav & Gupta, 2022). A viable

alternative to this problem is using renewable energy, like solar, wind, and geothermal energy, to enhance bioremediation (Kaur et al., 2021; Moradi et al., 2018; Nakamura et al., 2000). While harnessing and storing the heat from solar and wind is complex because it is not possible to control the timing of the supply, underground thermal energy storage (UTES) is often used (Moradi et al., 2018). The most common UTES technologies are borehole thermal energy storage (BTES), and aquifer thermal energy storage (ATES) (Ni et al., 2020; Q. Wang et al., 2022).

BTES uses U-shaped pipes that circulate fluid to transport the heat from the subsurface to buildings. This technology has been coupled with in-situ bioremediation (BTES-ISB), and a study showed that after increasing the BTES-ISB working temperature 40 ºC to 80 ºC, microbial activity increased by 2-folds (Moradi et al., 2018; Roohidehkordi & Krol, 2021). A numerical BTES-ISB study reported 97% degradation of benzene over 1 year (Roohidehkordi & Krol, 2021). However, in the field, BTES is reported to have a low efficiency of 27- 30% (Moradi et al., 2018).

Globally, there are more than 2800 ATES systems in operation, where 85% of all systems are in the Netherlands (Fleuchaus et al., 2018a). ATES uses groundwater as a sink and source to store heat, for the cooling and heating of buildings. ATES systems often have the same depth as groundwater contamination (Zuurbier et al., 2013). Thus, ATES has been coupled with in-situ bioremediation (ATES-ISB) (Kaur et al., 2021). (Zuurbier et al., 2013) presented a numerical study that highlighted a decrease in contaminant mass and plume volume of chlorinated solvents. This coupled system was implemented in 2 field scale tests in the Netherlands, and one of them showed conditions for reductive dichlorination (Sommer et al., 2013). Studies have also shown that this system can degrade chlorinated volatile organic compounds (CVOCs) (Ni et al., 2020). (Ni et al., 2016)) carried out a study that revealed ATES environments provided ideal conditions for bio-reductive dechlorination through introducing *Dehalococcoides* into the ATES warm wells (20–25 ˚C). The limitations of the ATES systems are clogging of wells and heat exchangers, corrosion of wells, and thermal breakthroughs (Fleuchaus et al., 2018a).

2.1.4 Geothermal Heating with GHPs

Electricity generation and space heating account for a large share of global energy consumption, with most of this energy sourced from fossil fuels. Fossil fuels are non-renewable, and their use has significant environmental consequences, including the release of greenhouse gases and other pollutants that drive climate change (Farzanehkhameneh et al., 2020). On average, buildings are responsible for 30% of the world's greenhouse gas (GHG) emissions. In 2011, residential buildings accounted for 6% of direct, and 11% of indirect CO_2 emissions (Soltani et al., 2019). These challenges highlight the need for an alternative sustainable energy solution. A renewable energy source that has been leveraged for space heating in the

past couple of decades is subsurface geothermal heating and cooling (Lee, 2009; Soltani et al., 2019). Geothermal heat pumps (GHPs), also known as ground source heat pumps (GSHPs), can be used to harness the geothermal heat in the subsurface (Kaur et al., 2021). Studies have shown that GHPs can reduce CO_2 emissions by 40- 60% and provide energy savings of 40- 70%, in comparison to traditional energy sources (Fleuchaus et al., 2018b; Kaur et al., 2021). GSHPs initial costs can be high due to borehole drilling, but they are economically feasible with their low maintenance cost over a 25-year lifetime period (Daemi & Krol, 2019).

There has been a positive growing trend in the use of GHPs globally. The number of countries with GHP installations has increased from 26 in 2000, to 43 in 2010, to 54 in 2020. GHPs now have the largest geothermal use worldwide, accounting for 59.2% of the annual energy use (Lund & Toth, 2021). In Europe, about 1.7 million ground source heat pump systems with over 20,000 MWt (megawatt thermal) capacity were operating at the end of 2015 (Soltani et al., 2019; Vienken et al., 2019). In 2020, Canada had more than 700,000 residential GHPs installed with a capacity of 1,822.5 MWt and 14,235 TJ/yr (Lund & Toth, 2021). As the global adoption of geothermal heat pumps continues to grow, it is critical to examine the limitations of this technology and explore its potential applications beyond reducing energy consumption and greenhouse gas emissions.

2.1.4.1 How do GHPs work

GHPs utilizes ground source thermal energy for space heating and cooling. This low-enthalpy heat is derived from the absorption and storage of solar energy within the subsurface, facilitated by the Earth's thermal conductivity and high heat storage capacity (Daemi & Krol, 2019; Kaur et al., 2021). Thus, during the summer months when temperatures are high, the subsurface acts like a sink. Furthermore, the subsurface is separated into two sections: the zone of seasonal fluctuations and below the zone of seasonal fluctuations (D. Banks, 2012). The seasonal fluctuations zone is directly below the ground surface, and this zone is affected by the seasonal temperature changes (D. Banks, 2012; Kaur et al., 2021). In Canada, this zone is reported to be less than 20 m below the ground surface (Kaur et al., 2021).

Studies have shown that, below this zone, the subsurface temperature is reported to be constant and equal to the mean annual surface temperature, equal to around 10- 15 °C (D. Banks, 2012; Wolf, 1980). Thus, during the cold seasons, the ground temperature is higher than the atmospheric temperature, so the ground temperature can be used for heating. In contrast, during the hot seasons, the atmospheric temperature is greater than the ground temperature, so the ground temperature can be used for cooling (Kaur et al., 2021; Mustafa Omer, 2008; Roohidehkordi & Krol, 2021; Soltani et al., 2019). The GHPs extract the thermal energy from the shallow subsurface through ground loops, which either have groundwater or an antifreeze solution, and the heat is then transferred to a building using a heat exchanger. During the cooling mode, the

warm indoor air from the building is injected into the subsurface for it to "cool down," and the cool air produced is injected into the building as air conditioning, as shown in Figure 2.1.

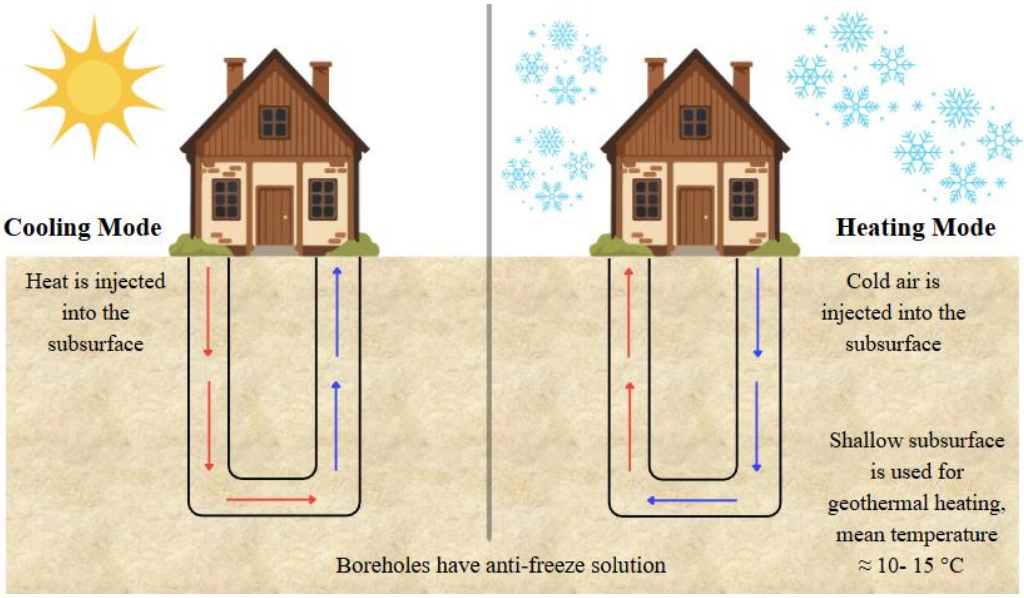


Figure 2.1. Schematic illustration of a shallow geothermal ground-source heat pump system operating in cooling and heating modes

GHP loops can either be vertical or horizontal in the subsurface, vertical GHPs require less space and, as such, tend to be preferred. These are typically installed at with a depth of 50- 200 m below the surface, as the geothermal gradient in the subsurface increases with depth. In southern Canada, the gradient is 0.01 °C m^{-1} at 50–100 m depth, 0.012 °C m^{-1} at 100–150 m, 0.014 °C m^{-1} at 150–200 m and 0.016 °C m^{-1} at 200–250 m (Kaur et al., 2021). Depending on the latitude, at a depth of 50-200 m, the ground temperature ranges from 7°C to 21 °C. In comparison, the temperature ranges for GHP loops usually cycle between 15 ºC to 37 ºC during the seasons, with the minimum temperature at -5 ºC or the highest temperature at 80 ºC (Lee, 2009; L. Zhao et al., 2003). While research on the interaction between temperature gradients generated by geothermal heat pumps and subsurface temperatures remains limited, this topic will be further explored in Section 2.1.5.

2.1.4.2 Geothermal Remediation

A way to thermally enhance bioremediation with geothermal heating is through the usage of GHPs. GHPs can be utilized in the process of bioremediation to enhance the effectiveness and efficiency of remediation efforts by providing a renewable heat source to enhance microbial activity, and increase contaminant solubility, which will cumulatively accelerate the biodegradation of the contaminants (Garnier et al., 2011; Kaur et al., 2021; Moradi et al., 2018; Roohidehkordi & Krol, 2021). It has been proven through temperature-based studies that increasing temperature has a positive effect on the performance of certain

microbes (Kaur et al., 2022; Roohidehkordi & Krol, 2021; Van Stempvoort & Biggar, 2008; Vidali, 2001; Q. Wang et al., 2022). However, there are very few studies, both lab-based and site-based, that analyze the effects of geothermal heating on subsurface bioremediation.

(Roohidehkordi & Krol, 2021) conducted a numerical study, using FEFLOW, of a GHP system remediating a well-defined mass of monoaromatic hydrocarbons for 1 year. The model had a ground temperature baseline of 10 °C, and a temperature profile derived from a heat transport simulation. The model switched between the heating and cooling mode, and the GSHP resulted in more sorption during the heating season (lower subsurface temperatures in November–May). However, during cooling mode, the subsurface temperatures rose to 18- 25 °C and the biodegradation rate drastically increased. The study highlighted a substantial decrease of the total contaminants mass; 97% for benzene and 93% for toluene (Roohidehkordi & Krol, 2021).

(Garnier et al., 2011) explored the concept of "biothermoremediation". The article suggested that bioremediation with geothermal heating can degrade 90% of organic pollutants. In the study, a field site was contaminated with polyaromatic hydrocarbons (PAH), volatile aromatic compounds (VAC), volatile organic hydrocarbons (VOHC) and hydrocarbon oil. The site remediation was enhanced with an ATES system, and after 5 years, 80% of the organic compounds degraded. The microbial activity was enhanced by the temperature increase from the ATES system, and to examine this temperature effect on the metabolic activity, 2 methods were used. The first method used the *Dehydrogenase* activity (respiratory activity) approach, and the second approach used the carbon source utilization profile. In the study it was observed that increased temperatures up to 36 °C promoted higher bacterial respiratory activity, enhancing the breakdown of carbon substrates. However, beyond this point, a decline in metabolic activities was observed due to the denaturation of enzymes. Additionally, higher temperatures reduced the viscosity, increased the mobility of contaminants which improved the breakdown efficiency by the bacteria, and enhanced the solubility and volatilization of organic pollutants (Garnier et al., 2011). In contrast, increased contaminant transport can be a concern if groundwater velocities are high (Kaur et al., 2021).

(Yadav & Gupta, 2022) investigated the impact of temperature variations on biodegradation rates of LNAPLs, specifically toluene, in soil-water environments by using a one-dimensional column setup. The biodegradation rates of toluene at the certain temperatures were 0.002 mg/L/h at 4 °C, 0.008 mg/L/h at 20 °C, 0.012 mg/L/h at 28 °C, and 0.015 mg/L/h at 36 °C (Yadav & Gupta, 2022). The elevated temperatures, particularly 28– 36 °C, promoted microbial growth, as the microbial count at 36 °C increased from 28×10^4 CFU/mL to 120×10^4 CFU/mL. Additionally, the dissolution rates of NAPLs increased with temperature, resulting in higher concentrations of dissolved toluene in the soil water (Yadav & Gupta,

2022). This is good for the microbes because it enhances the bioavailability of toluene as a carbon source for the microbes, which accelerates its biodegradation.

(Ni et al., 2020) conducted a comparative life cycle assessment (LCA) between the integration of ATES with in-situ bioremediation (ISB), and conventional heating and cooling with ISB. The study focused on remediating chlorinated volatile organic compounds (CVOCs). The results demonstrated that the ATES-ISB system enhanced the biodegradation of CVOCs 13-folds compared to natural conditions. As well, the LCA estimated that it would take 30 years to remediate the contamination, whereas the conventional method would require 390 years to achieve the same level of remediation (Kaur et al., 2021; Ni et al., 2020).

Based on the reviewed studies, geothermal heating can effectively enhance the bioremediation process. The findings demonstrated that the temperature generated by GHPs impacts the existing subsurface thermal conditions and microbial ecosystem. Microorganisms have temperature requirements for optimal growth and activity, and geothermal systems can help maintain the desired temperature range, providing favorable conditions for the microbial populations involved in bioremediation processes (Jesußek et al., 2013; Kaur et al., 2021). While these 2 technologies do form a more efficient technology, the feasibility of GHPS with bioremediation relies on the lasting impacts on the environment.

2.1.4.3 Environmental Implications of GHPs

While geothermal heat pumps are celebrated for their sustainable energy potential, their environmental impacts, ranging from subsurface temperature changes to effects on groundwater quality and ecosystems, reveal a complex balance between benefits and unintended consequences. The policies and regulatory guidelines are currently evolving for geothermal heating and ground source heat pumps. The guidelines already established vary across the globe, where some just focus on water supply issues, and product and installation guidelines, instead of thermal impacts. In Canada, there is an Ontario Regulation in the Environmental Protection Act about ground source heat pumps, and it only mentions the restrictions on the heat transfer fluid (Environmental Protection Act, 2012). The National Ground Water Association in the U.S. performed a study, and it stated that heat transfer calculations are approximately only required in 4% of the state regulatory agencies (Freedman et al., 2012). In comparison, some European countries have regulations specifying the minimum distance between nearby pumps, the size of the thermal plumes, and the operating temperature (Casasso & Sethi, 2015, 2019). With the popularity of this technology and the influx of research studied in this area, policies need to be formed to protect the future of the subsurface as aquifers have long term memory, so the long-term effects of GHP systems can be mitigated.

GHPs and geothermal heating offer several significant advantages. Firstly, GHPs are highly energy efficient. This efficiency translates into reduced energy consumption and associated cost savings.

Additionally, as a renewable energy source, geothermal heating contributes to a reduction in greenhouse gas emissions, further enhancing its environmental benefits. For example, in northern China, a life-cycle assessment was calculated for the energy and emissions of GHP utilization, considering the extraction, manufacturing, transportation, construction, and operation. The life-cycle energy consumption was 192TJ, with the building heating and cooling allocating 56% and 36%. The SO_2, NOx, and GHG emissions were estimated to have a footprint of 35 MT (metric tons), 45 MT, and 19130 MT CO2e (Chang et al., 2017). Moreover, compared to municipal heating and cooling, there was an energy use reduction of 84% and 83%, and the facility is projected to achieve economic cost-effectiveness in 7.4 years, which is similar to other studies (Allouhi et al., 2016; Farzanehkhameneh et al., 2020; Mustafa Omer, 2008; X. Zhao et al., 2015).

With these benefits of GHPs, some of the unintended environmental consequences can be derived from issues with the system itself, or from thermal effects. The temperature effects on the subsurface ecology and composition will be explored in Section 2.1.5. There have been reports of pipe leakages of anti-freeze solutions from BHEs into the subsurface, as well as defective borehole filling. Both incidents can lead to subsurface contamination and the distribution of contaminants between aquifers (Casasso & Sethi, 2019). With open-loop GHP systems (groundwater is used as the fluid in the underground loops), the re-injection of groundwater into the same aquifer can create a shift in the flow pattern up to several kilometres. This can mix different groundwater types which can impact the groundwater chemistry and cause mineral precipitation, resulting in possible clogging at the wells (Bonte et al., 2013). Additionally, a study from Washington State conducted a heat transport analysis study to compare the impacts of flow rate and temperature on the subsurface. The study compared different wells for a 20-year simulation period for peak and average load scenarios. In one scenario, the pumping rates were the same, and the subsurface injection temperatures were 18.1 °C and 23.1 °C. The results showed a minimal temperature drift of <0.3 °C. In comparison to increasing the pumping rate to 0.14 m^3/s with an injection temperature of 18.1 °C, the temperature drift at the extraction wells increased to 0.7 °C (Freedman et al., 2012). However, it is important to note that the temperatures in this study are relatively close in comparison to the temperature range GHPs operate within (15 °C and 40 °C) as there are studies that indicate that temperature changes the subsurface.

GHP systems can also alter subsurface temperatures in both heating and cooling modes. This has been demonstrated in numerous studies that discuss the impact of the alterations of subsurface temperatures (Barla et al., 2020; Casasso & Sethi, 2015; Herbert et al., 2013; Moradi et al., 2018; Piga et al., 2017; Roohidehkordi & Krol, 2021). Heating-dominating systems extract more heat in winter compared to the amount injected in summer, leading to a gradual subsurface temperature decline, while cooling-dominating systems increase ground temperatures (Marazuela & García-Gil, 2022; Roohidehkordi & Krol, 2021). In urban areas like Lyon, France, high GHP density resulted in permanent subsurface temperature increases,

sometimes exceeding 10 °C, due to the imbalance in heating and cooling demands (Garnier et al., 2011). Additionally, due to long-term or unbalanced operations, the propagation of thermal plumes can lead to a possible increase in temperature up to 70 °C (Jesußek et al., 2013). Thermal plumes can affect the efficiency of GSHPs, which rely on stable subsurface temperature dissipation (Casasso & Sethi, 2015). Overuse of GHPs can reduce system sustainability, potentially causing ground freezing, which damages structures, or ground warming, which decreases efficiency of the overall system and the geothermal resources (Daemi & Krol, 2019; UNESCO World Water Assessment Programme, 2022).

2.1.5 Implications for Groundwater Quality and Geothermal Bioremediation

Groundwater is a hidden resource that is sensitive to temperature and climate change effects. Increased groundwater and surface water temperatures, precipitation intensity, and land use changes are anticipated to amplify water pollution, which can negatively impact natural environments and human health (Bates et al., 2013). This situation is concerning because groundwater is being prioritized as a solution to alleviate poverty, improve access to food and water, and promoting socio-economic development (Egidio et al., 2024). Thus, understanding and quantifying the availability of good-quality groundwater is critical. Quantitative projection studies have been developed for predicting the future availability of groundwater through quantifying groundwater recharge rates, predicting precipitation intensity, and groundwater vulnerability (Amanambu et al., 2020; Atawneh et al., 2021; Riedel, 2019; Stevenazzi et al., 2017; Taylor et al., 2013). However, there is a lack of projection tools and studies to predict groundwater quality.

Groundwater quality considers the physical, chemical, and biological properties of this resource. Additionally, the biogeochemical cycle in groundwater and soils above aquifers involves the transformation and movement of chemical elements through biological, geological, and chemical processes. Groundwater is a key part of the hydrological cycle and plays a significant role in the cycling of carbon, nitrogen, sulphur, iron, and other elements (Ciais et al., 2014; Kappler et al., 2021; Pathak et al., 2022; Watanabe & Ortega, 2011). Microbial activity, chemical reactions, and interactions between dissolved substances and minerals in the subsurface environment mediate this cycle. Microorganisms facilitate critical processes such as carbon respiration, nitrification, denitrification, sulphate reduction, and iron cycling, utilizing available electron donors (e.g., dissolved organic carbon) and electron acceptors (e.g., oxygen, nitrate, sulphate, Fe^{2+}). These microbial processes regulate the mobility of nutrients and contaminants, influencing the chemical composition of groundwater. For example, sulphate-reducing bacteria transform sulphate into sulphide, immobilizing metals, while iron-reducing bacteria convert Fe^{3+} to Fe^{2+}, affecting the fate of contaminants like arsenic by absorbing and immobilizing them (Bonte et al., 2013; Kappler et al., 2021; Pathak et al., 2022). Phosphorus cycling, controlled by sorption to iron oxides and microbial uptake, can limit microbial growth, affecting organic contaminant degradation. In the biogeochemical cycle of soil, climate change can

alter soil moisture and atmospheric CO_2 levels, thereby affecting soil microbial communities and hydrological cycles (Riedel, 2019). Furthermore, studies have shown that warming can affect microbial respiration, thereby altering the cycling of soil carbon (E. A. Davidson & Janssens, 2006; Moradi et al., 2018). Overall, environmental contaminants and temperature changes not only affect groundwater quality but also alter various biogeochemical cycling processes by altering native microbial communities (Borch et al., 2010; Hemme et al., 2015; Madsen, 2011), and this will be investigated in the following subsections.

2.1.5.1 Microbial Activity and Ion Interactions Influencing Groundwater

The intersection of geothermal heating, microbial activity, ion interactions, and biodegradation in groundwater is a critical aspect of the biogeochemical cycling of elements, particularly in subsurface environments. As aforementioned, geothermal heating can enhance microbial activity, thereby accelerating the biodegradation of organic pollutants under favourable conditions (Bonte et al., 2013). However, the influence of geothermal systems on microbial communities, ion mobility, and contaminant degradation is complex.

Groundwater ecosystems host diverse microbial communities that drive biodegradation by metabolizing organic pollutants, such as hydrocarbons, into less toxic forms. Microbial degradation processes depend heavily on subsurface conditions, including the availability of electron acceptors (oxygen, nitrate, sulphate, Fe3+) and the presence of dissolved organic carbon (DOC), which serves as an energy source for microbes (Kaplan & Newbold, 2000). Ions such as sulphate, nitrate, iron, and manganese serve as electron acceptors and donors in redox reactions that are vital to microbial respiration in groundwater. Studies have shown that sulphate-reducing bacteria (SRB) reduce sulphate to hydrogen sulphide during the degradation of organic matter at 30 °C (Deng et al., 2018, 2023). Similarly, iron-reducing bacteria utilize Fe^{3+} as an electron acceptor reducing it to Fe^{2+}, which may influence the mobility of other contaminants, such as arsenic, through sorption or precipitation processes (Xue et al., 2020). These ion-driven processes regulate the supply of essential elements, such as nitrogen, sulphur, and carbon in groundwater, and directly modify the efficiency of contaminant degradation (Flynn et al., 2021). Redox conditions in the subsurface control microbial respiration rates and dictate whether biodegradation occurs via aerobic or anaerobic pathways. Under anoxic conditions, iron and sulphate reduction are dominant processes that can enhance the breakdown of certain organic compounds while simultaneously immobilizing metals through sulphide or iron precipitation (Weber et al., 2006).

2.1.5.2 Temperature Effects on Groundwater Quality

The influence of temperature on groundwater quality is an essential factor in understanding the complex interactions between chemical, biological, and physical processes within subsurface environments.

Elevated temperatures, whether from climate change, natural geothermal sources, or anthropogenic activities such as geothermal energy systems, can notably alter groundwater chemistry, microbial activity, and the mobility of contaminants, thereby altering overall water quality and ecosystem health. Most of the available knowledge on temperature-associated changes in groundwater quality is derived from studies on geothermal energy use (Bonte et al., 2013; Griebler et al., 2016; Vienken et al., 2019).

2.1.5.3 Temperature Effects on Groundwater Ecology

With respect to groundwater ecology, an increase in subsurface temperature can alter recharge rates, the volume of groundwater discharge, and flow rates, and can affect living organisms. With climate change increasing air temperatures and precipitation, studies have projected a 34% increase in groundwater discharge volume and a maximum temperature increase of 3.6 °C in adjacent rivers during summer months (Kurylyk et al., 2014). Laboratory studies suggest that a few-degree increase can alter microbial community structure and fauna. Groundwater invertebrates are sensitive to heat discharge; for example, stygobites survive in low-temperature groundwater, less than 16 °C, which is in line with the average subsurface temperature (Brielmann et al., 2009; Kaur et al., 2021).

High temperatures and thermal plumes can affect groundwater flow and increase contaminant mobilization. Thermal plumes with high temperatures can alter groundwater density, changing the direction of groundwater flow. For example, groundwater can become denser due to GHP cooling or increased salinity, causing denser groundwater to sink while less dense groundwater rises. Differences in density can create a convective flow pattern, thereby impacting the general (Bonte et al., 2011, 2014; Daemi & Krol, 2019). Additionally, ATES operational studies have demonstrated that changes in subsurface temperatures can affect mineral desorption, microbial communities, and groundwater quality. A study in the Netherlands with an ATES system reported that a well 570 m away contained fecal microbes and that groundwater quality affected by the ATES system (Bonte et al., 2011; Jesußek et al., 2013). Furthermore, a laboratory study observed an increase in temperature from 5 °C to 60 °C, which enhanced the mobilization of heavy metals such as arsenic and trace elements such as potassium and phosphorus (Bonte et al., 2013).

2.1.5.4 Temperature Effects on Groundwater Chemical and Physical Properties

The temperature of groundwater plays an important role in modifying its chemical and physical properties, including solubility, ion exchange, redox reactions, and mineral dissolution. Large temperature variations can significantly alter groundwater chemistry and the behaviour of dissolved substances, thereby affecting processes such as contaminant transport, nutrient availability, and overall water quality. Studies have shown that naturally occurring temperature changes also affect groundwater quality. A time series analysis observed that climate change has been increasing soil and groundwater temperature by 0.3 °C per decade

(Riedel, 2019). In Italy, a 16-year study (2004-2020) examined the relationship between climate change and dissolved ions in groundwater. The study indicated that water hardness was significantly affected, aquifer discharge was reduced, and sulphate increased by about 5 mg/L at all field locations (Barbieri et al., 2023). This may have occurred because temperature can increase the dissolution rate of minerals, thereby increasing the concentrations of ions such as sulphate and iron. A large-field study found that when the temperature is naturally in the range of 5- 20 °C, a difference of +1K can decrease oxygen saturation by 4%, and pH by 0.02 (Riedel, 2019). Similarly, in an in-situ subsurface heating experiment, temperature reductions below 25 °C were associated with changes in the concentrations of Mg, Si, Li, and ammonium (Riedel, 2019). In a laboratory batch and column experiment, the experimental temperatures were in the range of 0- 100 °C, and it was observed that significant changes in the groundwater composition occurred in temperatures less than 40 °C (Bonte et al., 2013; Riedel, 2019). This indicates that minimal changes in the subsurface temperature relative to the average can impact overall groundwater quality and ecology.

2.1.5.5 Groundwater Quality Impacts on Bioremediation

Impact of Ions on Bacterial Metabolic Rates and Bioremediation

Ions and chemical compounds in groundwater play a vital role in bacterial metabolism, serving as nutrients, electron donors, and acceptors, and directly influencing microbial processes such as bioremediation. pH affects enzyme activity and microbial cellular function, with neutral pH levels (6.5- 7.5) being optimal for growth, while extreme pH may impair metabolism and enzyme functions (Srivastava et al., 2014; Yates et al., 1988). CO_2 supports microbial respiration, serving as a carbon source for autotrophic bacteria, improving the breakdown of organic pollutants. High levels of CO_2 can enhance the microbial breakdown of organic pollutants by providing an additional carbon source, thereby stimulating microbial growth and activity (Q. Jin & Kirk, 2016). Dissolved organic carbon (DOC) fuels heterotrophic bacteria growth and pollutant degradation, though excessive DOC can lead to oxygen depletion, hindering aerobic processes (F. Liu & Wang, 2022). Dissolved oxygen (DO) is necessary for aerobic biodegradation. Aerobic microbes use oxygen as the final electron acceptor in the breakdown of organic compounds, making DO a limiting factor in bioremediation. Low DO levels slow down aerobic degradation, but in anaerobic conditions, alternative electron acceptors (such as nitrate or sulphate) can be used (Wilson & Bouwer, 1997). Chemical oxygen demand (COD) reflects organic compound levels and promotes microbial activity, but excessive COD can deplete oxygen and create anoxic conditions (Kuo et al., 2012).

Iron, in both Fe^{3+} and Fe^{2+} forms, participates in redox reactions and anaerobic respiration, aiding in the degradation of pollutants (Gadd, 2004; Rivett et al., 2008; Weber et al., 2006). Sulphate and nitrate serve as electron acceptors in anaerobic conditions, supporting sulphate-reducing and nitrate-reducing bacteria in the breakdown of organic pollutants (Z. Zhang et al., 2022). Sulphate is an important electron acceptor in

anaerobic conditions. SRB can use sulphate to degrade organic pollutants, producing hydrogen sulphide as a by-product. Sulphate availability is important for supporting anaerobic degradation pathways. Like sulphate, nitrate acts as an electron acceptor in the absence of oxygen. Nitrate-reducing bacteria facilitate denitrification, a process where nitrate is reduced to nitrogen gas while degrading organic contaminants (Rivett et al., 2008).

Impact of Ions and Chemical Compounds on Temperature in Bioremediation

The interactions among groundwater temperature (GWT), microbial activity, and chemical compounds are crucial to bioremediation. Increased GWT has been shown to enhance bacterial proliferation, as temperature is a key factor in promoting microbial diversity and activity. However, studies reveal that rapid temperature shifts, such as those near geothermal heat exchangers, may reduce bacterial populations due to thermal shock (Egidio et al., 2024). These temperature variations can also affect water quality parameters such as pH, dissolved oxygen levels, and manganese levels, leading to higher water treatment costs. More significantly, temperature increases may destabilize ecosystems, making them less habitable for certain organisms, thus leading to imbalances that are difficult to resolve.

Temperature directly influences the natural attenuation of contaminants, including chlorinated solvents, by enhancing microbial activity. Studies have demonstrated that technologies such as ATES can maintain optimal subsurface temperatures, thereby considerably improving the efficiency of bioremediation processes (Ni et al., 2020). Research consistently shows that elevated temperatures increase biodegradation rates across soil, marine, and freshwater ecosystems (Roohidehkordi & Krol, 2021). In the subsurface, “augmented bioremediation” techniques, including the use of residual heat from thermal technologies, have been employed to boost the breakdown of contaminants. Seasonal temperature changes, particularly in colder climates, also affect biodegradation efficiency. For instance, in Nunavut, Canada, cyclic temperature shifts significantly improved the biodegradation rates due to the growth of psychrophilic microorganisms (Roohidehkordi & Krol, 2021). These data emphasize that even minor temperature fluctuations can remarkably enhance biodegradation, a promising concept for future bioremediation strategies.

Temperature changes also impact groundwater quality. For example, increases in groundwater temperature during GSHP operations may trigger the mobilization of harmful elements, such as heavy metals, and alter microbial communities. Some laboratory studies found that increasing groundwater temperature enhanced the mobility of elements such as arsenic, iron, and phosphorus, as well as elevating DOC concentrations (Bonte et al., 2013). Additionally, an increase in GWT tends to decrease DO levels, limiting aerobic microbial activity, which is more exothermic than anaerobic processes. The reduction in DO levels can slow

down biodegradation, leading to higher chemical oxygen demand (COD), as more oxygen is required to break down organic compounds (Kuo et al., 2012).

Moreover, ions and compounds such as CO_2, iron, sulphate, and nitrate interact with temperature to affect microbial metabolism and, consequently, bioremediation. For example, SRB and methanogens display greater activity at elevated temperatures, thereby accelerating the degradation of contaminants such as hydrocarbons (Bethke et al., 2011). Higher temperatures can similarly enhance redox reactions, influencing the concentration of these compounds in groundwater. For instance, warmer temperatures accelerate the reduction of iron and sulphate, promoting biodegradation but also increasing the risk of heavy metal contamination in aquifers.

Chemical compounds such as pH and CO_2 further represent the link between temperature and microbial function. Higher temperatures can lead to a drop in pH, as water becomes slightly more acidic due to increased hydrogen ion dissociation (Yates et al., 1988). This pH change can affect microbial growth, as most microbes thrive within a narrow pH range. Similarly, elevated temperatures enhance microbial respiration, which increases CO_2 production. This rise in CO_2 can decrease pH levels by forming carbonic acid in water, additionally influencing microbial communities (Q. Jin & Kirk, 2016). DOC levels are also influenced by temperature, with elevated temperatures encouraging more rapid microbial degradation of organic matter and increasing DOC concentrations (Rajendiran et al., 2023).

In conclusion, ions and chemical compounds, including pH, CO_2, DOC, DO, and various redox-sensitive elements, strongly influence temperature changes during bioremediation. Their interactions with microbial processes influence the overall rate and capability of contaminant degradation, making them important factors in the success of bioremediation strategies. Understanding these complex relationships is key to optimizing bioremediation in various environmental contexts, particularly in response to climate-induced changes in groundwater temperatures.

2.1.6 Conclusion

The combination of geothermal heat pumps with bioremediation is a promising, energy-efficient solution for degrading BTEX in groundwater. Through leveraging controlled temperature increases, GHPs enhance microbial activity and significantly improve the degradation rates for contaminants. The review identifies a range of operational parameters, including the optimal temperature range (20–35 °C), that maximize microbial degradation while sustaining system sustainability, and how these temperature changes impact the groundwater ecosystem. However, the study also reveals multiple challenges, including thermal plume management and potential adverse effects on groundwater chemistry, such as the release of heavy metals due to elevated temperatures.

To enhance Geothermal Remediation, subsequent research will need to investigate field experiments and numerical models and conduct environmental risk and sustainability assessments. Operational parameters of GHPs, including flow rates, temperature cycles, and microbial consortium selection, need to be optimized to assess the long-term impacts of sustained temperature increases on microbial diversity and groundwater quality. Numerical models need to simulate the interactions among geothermal heat, microbial kinetics, and contaminant transport to improve efficiency. Lastly, geothermal heating of groundwater chemistry, particularly the mobilization of ions (e.g., Fe, Mn, As) and organic matter, warrants further investigation to better understand the subsurface biogeochemical limits.

2.2 Ecotoxicological Assessment of Bioremediated BTEX-Contaminated Soils Using Seed Germination Bioassays

2.2.1 Introduction

BTEX compounds are characterized by relatively high volatility, moderate water solubility, and low to moderate sorption to soil organic matter (W. Chen et al., 2010; John V. Headley S. Lee Barbour & Gong, 2000). These properties allow partitioning among soil, groundwater, and the atmosphere. Particularly, benzene exhibits high mobility in groundwater and soil due to its solubility and high soil organic carbon sorption coefficient (K_{oc} = 60- 83), increasing the risk of aquifer contamination (Agency for Toxic Substances and Disease Registry (US), 2007; Braddock & McCarthy, 1996). Toluene and xylenes can similarly migrate through porous media, depending on soil composition and hydraulic gradients (Lueders, 2017; Njobuenwu et al., 2005). The environmental persistence of BTEX varies with oxygen availability, temperature, nutrient status, and microbial community composition (Morgan et al., 1993). Under aerobic conditions, BTEX compounds can biodegrade relatively quickly; however, in oxygen-limited or heterogeneous subsurface environments, degradation may be slower and incomplete. Sorption to soil particles and diffusion into low-permeability zones may further prolong their environmental residence time (Zytner, 1994). Therefore, BTEX contamination can persist for extended periods, notably in complex subsurface systems.

Due to their hazardous and mutagenic properties, BTEX compounds represent risks to both environmental and human health. Benzene is classified as a known human carcinogen and is linked to hematological disorders and leukemia (Bolden et al., 2015a). Ecologically, BTEX compounds can cause acute and chronic toxicity to aquatic organisms, soil invertebrates, and plants. Exposure may impair fish survival, alter microbial communities, and inhibit plant germination and growth (Gissi et al., 2021; Headley et al., 2001). Plants are particularly important indicators of soil ecosystem health, as contaminated soils can reduce soil stabilization and delay ecological recovery. Given the widespread occurrence and environmental importance of BTEX contamination, remediation strategies are commonly implemented to reduce environmental risk.

2.2.2 Need for Ecotoxicological Assessment in Remediated Soils

Bioremediation is widely applied to treat BTEX-contaminated soils by stimulating microbial degradation of hydrocarbons. The success of remediation is typically evaluated using chemical analyses that measure decreases in contaminant concentrations. However, chemical measurements alone may not accurately represent ecological recovery (Alexander, 2000; National Research Council, 2003). Aging processes,

sorption to soil organic matter, and sequestration within soil micropores may alter bioavailability independently of measured concentration (Alexander, 2000; Chung & Alexander, 1998; Xiao & Zytner, 2019). In addition, microbial degradation of hydrocarbons can produce intermediate metabolites such as phenols, aldehydes, and organic acids that may retain toxicological properties (El-Naas et al., 2014; Hernández-Ospina et al., 2024; Ladino-Orjuela et al., 2016; Weelink et al., 2010). Consequently, soils that meet regulatory concentration thresholds may still exhibit residual toxicity due to transformation products or complex mixture effects.

Ecotoxicological assessments address this limitation by measuring the biological responses of organisms exposed to contaminated soils. Instead of evaluating individual chemicals in isolation, these assays assess endpoints such as survival, growth, and reproduction (Gong et al., 2001; Valerio et al., 2007). In the context of remediated BTEX soils, biological testing provides insight into whether remediation has restored ecological functionality rather than reduced measurable contaminant concentrations (National Research Council, 2013; United States Environmental Protection Agency, 1998). Biological endpoints reflect organism-level responses to complex environmental contaminants. Integrating bioassays with chemical analyses is essential for realistic environmental risk assessments, since the bioavailable fraction of contaminants often drives ecological effects more than total concentrations (Q. Li et al., 2023). Evidence from studies of hydrocarbon-contaminated soils demonstrates that decreases in contaminant concentrations do not always correspond to reduced toxicity. A study on microbial degradation of polycyclic aromatic hydrocarbons (PAHs) reduced concentrations of benzo[a]anthracene and benzo[a]pyrene by 70.4% and 49.9%, respectively, yet soil toxicity temporarily increased due to the formation of toxic intermediate metabolites. These intermediates inhibited wheat seed germination and growth, induced oxidative stress, and reduced soil enzyme activity, demonstrating that microbial degradation of pollutants can generate transient toxic by-products that affect ecological health. Some of the metabolites, intermediates, and derivatives identified were cis-1,2-diacetoxy-1,2-dihydrochrysene, 2,4-di-tert-butylphenol, phthalic acid, bis(7-methyloctyl) ester (Du et al., 2025).

2.2.3 BTEX Bioremediation and Formation of Toxic Intermediates

Bioremediation relies on microbial metabolic pathways to transform organic contaminants into less complex compounds and ultimately mineralize them into carbon dioxide and water. Currently, the literature contains an abundance of toxicology research on phytoremediation, which uses plants to remediate land (L. Li et al., 2020; Steliga & Kluk, 2020, 2021; J. Tang et al., 2010; Xi et al., 2018). However, there is a lack of research on toxicology studies completed on remediated lands (Hernández-Ospina et al., 2024; Kaur et al., 2025).

During microbial metabolism, aromatic hydrocarbons undergo enzymatic transformations that yield intermediate metabolites before complete mineralization. Under aerobic conditions, BTEX degradation is initiated by monooxygenase or dioxygenase enzymes that introduce oxygen into the aromatic ring structure (El-Naas et al., 2014). These reactions produce intermediate compounds such as benzyl alcohol, benzaldehyde, benzoic acid, catechol, benzoic acid, and propionic acid (Hernández-Ospina et al., 2024; Kaur et al., 2023, 2025; Sathesh-Prabu et al., 2023; Wu et al., 2023; H. Yu et al., 2001; Yuxuan et al., 2023). Different BTEX compounds produce distinct intermediates. Benzene's main intermediate product is catechol, while toluene and ethylbenzene produce substituted catechols such as 3-methylcatechol and 3-ethylcatechol. Additionally, xylene isomers are metabolized to mono-methylated catechols. M-xylene degrades to 3-methylcatechol, and some studies show that p-xylene leads to 3,6-dimethylcatechol (El-Naas et al., 2014).

Anaerobic BTEX degradation occurs through different biochemical pathways. For example, toluene may undergo fumarate addition to form benzyl succinate intermediates, which are subsequently converted to benzoyl-CoA (Beller & Spormann, 1998; Leuthner et al., 1998). These processes ultimately contribute to hydrocarbon mineralization, although intermediate metabolites may temporarily accumulate and influence soil toxicity. Depending on the bacterial strain or degradation pathway, ethylbenzene can degrade into carbon dioxide. Xylene isomers exhibit different degradation pathways under different redox conditions. For example, under nitrate-reducing conditions, p- and m- xylene degraded only (Weelink et al., 2010). Benzene is considered recalcitrant under anaerobic conditions unless the degradation is under different redox conditions, such as nitrate (Weelink et al., 2010). Several studies have identified phenolic intermediates, such as phenol, cresols, and catechols, as transient products of BTEX biodegradation (El-Naas et al., 2014; Hernández-Ospina et al., 2024). Not all metabolic intermediates are harmful, and aerobic biodegradation is less likely to produce harmful intermediates (Hernández-Ospina et al., 2024).

2.2.4 Seed Germination Bioassays in Soil Ecotoxicology

Seed germination bioassays are widely used to evaluate the phytotoxic effects of contaminated soils. These assays assess the ability of seeds to germinate and develop into early seedlings when exposed to contaminated substrates. Standardized methodologies such as *ISO 11269-2* and *OECD guidelines* ensure consistency and reproducibility in phytotoxicity testing (International Organization for Standardization (ISO), 1993). Multiple protocols have been developed by individual researchers, the Organization Cooperation and Development (OECD), and the U.S. Environmental Protection Agency (EPA) (OECD, 2006; US EPA, 1996).

Seed germination assays typically evaluate several endpoints, including germination percentage, root elongation, shoot length, and seedling vigour index. Among these endpoints, root elongation is often the most sensitive indicator of toxicity because roots are in direct contact with the soil matrix and respond rapidly to bioavailable contaminants (OECD, 2006). Inhibition of root growth may occur even when germination rates remain unaffected, making it a valuable indicator of sublethal toxicity (Adam & Duncan, 2002; M. K. Banks & Schultz, 2005; Wei et al., 2013).

A wide range of plant species has been employed in soil ecotoxicological testing. Commonly used species include lettuce (*Lactuca sativa*), garden cress (*Lepidium sativum*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), and onion (*Allium cepa*). Table 2.3 compares the different plant species used in ecotoxicology assays on remediated soil. These plants are selected based on several criteria, including rapid germination, uniform seed size, high sensitivity to contaminants, and reproducibility under controlled laboratory conditions (M. K. Banks & Schultz, 2005).

Table 2.3. Plant germination responses to hydrocarbon-contaminated soils following remediation treatments

Common name	Scientific name	Key reference	Reported sensitivity to contamination	Crop relevance (Canada)	Residual contaminant levels reported
Lettuce	*Lactuca sativa*	(Baud-Grasset et al., 1993)	37/40 seeds germinated (94%), indicating low residual phytotoxicity after remediation	Yes — greenhouse leafy crop	Soil remediated ~49%; total PAHs remaining ≈ 2997.78 mg/kg
Garden cress	*Lepidium sativum*	(Marzi et al., 2025)	Slight inhibition, less than 20%. Germinated seeds after 3 days C- 9.5/10, S- 5.25/10, SB- 1.5/10, SBB- 1/10, SBP- 7/10, SBBP- 1/10	Primarily laboratory bioassay species	The soil was treated with these methods with a final hydrocarbon concentration of i) natural attenuation (S) – 5500 ±607 mg/kg, ii) treatment with biochar (SB) – 3363 ±292, iii) treatment with microorganism-enriched biochar (SBB) – 969 ±28.3, iv) treatment with biochar and phytoremediation (SBP) – 1178 ±40.3, v) treatment with microorganism-enriched biochar and phytoremediation (SBBP) 1199 ±99.3
Tall fescue	*Festuca arundinacea*		Slight inhibition, less than 20%. Germinated seeds after 3 days C- 5.5/10, S- 2.25/10, SB- 2.5/10, SBB- 4.75/10, SBP- 6/10, SBBP- 4.75/10		
Oat	*Avena sativa*	(Baud-Grasset et al., 1993)	21/22 seeds germinated (95%), indicating relatively low residual toxicity after remediation	Yes — cereal crop grown in Canada	Soil remediated ~49%; total PAHs remaining ≈ 2997.78 mg/kg
Wheat	*Triticum aestivum*	(Du et al., 2025)	Reduced germination, seedling growth and chlorophyll due to toxic intermediates; oxidative stress observed. Root length inhibition rates 26.24% with a germination potential of 60% at 20 days	Yes — major crop	TPH concentration was 134 mg/kg Benzo[a]anthracene was reduced by 70.4% and benzo[a]pyrene by 49.9% during microbial degradation, with a final concentration of 2.66 mg/kg BaA, 3.5 mg/kg BaP
Wheat	*Triticum aestivum*	(Miri et al., 2022)	p-xylene inhibits seed germination and root elongation.	Yes — major crop	Mean p-xylene removal for sample X (94.1%) and X/5 (92.9%)

Common name	Scientific name	Key reference	Reported sensitivity to contamination	Crop relevance (Canada)	Residual contaminant levels reported
			Sample X had 18/20 seeds germinated, X/5 had 15/20 seeds germinate		
White mustard	*Sinapis alba*	(M. Wang et al., 2025)	10 seeds, triplicates, germination rate after 4 months BT1: 90% BT2: 100% BT3: 90%	Minor crop/ cover crop	After 4 months, these are the degradation rates of BT1 (bacterial treatment 1), 63.42% BT2 (bacterial treatment 2), 73.08% BT3 (bacterial treatment 3), 78.34%
Sorghum	*Sorghum saccharatum*		10 seeds, triplicates, germination rate after 4 months BT1: 86.7% ± 9.4% BT2: 83.3% ± 12.5% BT3: 83.3% ± 4.7%	Regional forage crop	TPH concentrations at 3 sampling points: BT1- 11036 ± 225 mg/kg, 5736 ± 324 mg/kg, 4038 ± 71 mg/kg BT2- 11318 ± 103 mg/kg, 4731 ± 99 mg/kg, 3046 ± 60 mg/kg BT3- 10941 ± 145 mg/kg, 2433 ± 76 mg/kg, 2370 ± 34 mg/kg
Garden cress	*Lepidium sativum*		10 seeds, triplicates, germination rate after 4 months BT1: 90% BT2: 100% BT3: 100%	Primarily laboratory bioassay species	

The selection of plant species is an important consideration in seed germination bioassays because different species exhibit varying sensitivities to environmental contaminants. Studies have shown that lettuce and garden cress are among the most sensitive species for detecting hydrocarbon toxicity, while cereal crops such as wheat and barley may demonstrate greater tolerance to certain petroleum hydrocarbons (M. K. Banks & Schultz, 2005). Smaller seeds may also demonstrate increased sensitivity due to limited nutrient reserves during early development (Robson et al., 2004). In addition to sensitivity considerations, the ecological relevance of selected species should be considered. For studies conducted in Canada, crop species such as wheat, barley, and lettuce are particularly relevant because they are widely cultivated and represent economically important agricultural plants. Evaluating the effects of contaminants on these species can therefore provide insight into the potential impacts of soil contamination on agricultural productivity and ecosystem health.

Studies have demonstrated variable plant responses to hydrocarbon-contaminated soils. *Lactuca sativa* and *Avena sativa* showed high germination success following remediation, with germination rates of 94% and 95%, respectively, suggesting relatively low residual phytotoxicity despite total PAH concentrations remaining at approximately 2997.78 mg/kg (Baud-Grasset et al., 1993). Similarly, *Lepidium sativum* exhibited only slight inhibition (<20%) across several remediation treatments, with germination occurring within three days; however, treatments involving microorganism-enriched biochar substantially reduced hydrocarbon concentrations to as low as 969 ± 28.3 mg/kg (Marzi et al., 2025).

In contrast, *Triticum aestivum* demonstrated greater sensitivity to contamination. (Du et al., 2025) reported a germination potential of approximately 60% after 20 days, with root length inhibition of 26.24%, indicating significant phytotoxic effects likely associated with toxic intermediate metabolites formed during PAH degradation. Other species, such as *Sinapis alba* and *Sorghum saccharatum* demonstrated relatively high germination rates after microbial treatment, ranging from 83.3% to 100%, suggesting improved soil conditions following biodegradation (M. Wang et al., 2025).

2.2.5 Advantages and Limitations of Seed Germination Bioassays

Seed germination bioassays offer several advantages for evaluating soil toxicity. One of the primary strengths of these assays is their simplicity and rapid turnaround time. Germination tests can typically be completed within a few days to two weeks, allowing researchers to screen multiple soil samples or remediation treatments rapidly (Adam & Duncan, 2002; Masakorala et al., 2013). Additionally, the assays require relatively inexpensive materials and minimal laboratory equipment, making them accessible for routine environmental monitoring. Another advantage is the ecological relevance of plant endpoints. Plants play a fundamental role in terrestrial ecosystems by stabilizing soil, cycling nutrients, and forming habitats.

Consequently, inhibition of plant germination or early growth may signal broader ecological impairment, because seed germination assays measure biological responses to the bioavailable fraction of contaminants, they can detect toxicity even when chemical analyses suggest that contaminant concentrations are below regulatory thresholds (Zaman et al., 2025).

However, seed germination bioassays have limitations. Laboratory test conditions may not fully replicate field environments, where factors such as soil heterogeneity, microbial interactions, and climate influence plant growth. In addition, germination assays focus primarily on early developmental stages and may not capture long-term ecological effects on plant communities. For this reason, seed germination assays are often used in combination with other ecotoxicological tests, including microbial respiration assays, earthworm survival tests, and enzymatic activity measurements (M. K. Banks & Schultz, 2005; Knoke et al., 1999; Roques et al., 2023). Integrating multiple biological endpoints with chemical analyses provides a more comprehensive evaluation of soil toxicity and remediation effectiveness.

2.2.6 Knowledge Gaps and Future Directions

Despite extensive application of seed germination bioassays in contaminated soil studies, several knowledge gaps persist. First, there is no universally accepted species selection framework specific to BTEX-impacted soils, and sensitivity data for many crops remain inconsistent across studies (M. K. Banks & Schultz, 2005). Standardization of test species, exposure durations, and endpoint metrics is needed to enable direct comparisons among remediation approaches and contaminated sites. Second, while germination endpoints are useful for early toxicity screening, there is limited understanding of the long-term ecological relevance of early plant responses, particularly for plant establishment and community dynamics in situ. Integrating seed assays with longer-term growth experiments or field trials could help bridge this gap.

Furthermore, most bioassays focus on parent compounds, yet toxic intermediates produced during biodegradation may exert distinct or delayed effects on plant physiology (M. Wang et al., 2025; W. Wang, 1991). Future research should therefore expand to include targeted analysis of known transformation products alongside biological endpoints and biomarkers. Finally, incorporating seed germination assays into regulatory risk assessment frameworks for contaminated land could improve decision-making by emphasizing functional recovery alongside chemical thresholds.

2.2.7 Conclusion

Seed germination bioassays represent a valuable ecotoxicological tool for assessing the environmental impacts of BTEX-contaminated soils and evaluating the effectiveness of remediation strategies. While

chemical analyses provide essential information regarding contaminant concentrations, they do not necessarily reflect the biological availability or ecological effects of pollutants and their transformation products. Biological assays, therefore, play a crucial complementary role in environmental risk assessment.

During the bioremediation of petroleum hydrocarbons, microbial metabolic processes transform BTEX compounds via a series of intermediate metabolites before complete mineralization. Some of these intermediates may temporarily increase soil toxicity and affect plant development.

Seed germination bioassays are particularly useful for this purpose because early plant development is highly sensitive to environmental stressors. Measurements of germination rate, root elongation, and seedling vigour provide rapid, cost-effective indicators of soil phytotoxicity. When applied alongside chemical analyses and other biological assays, seed germination tests can provide a comprehensive evaluation of soil quality and ecological recovery following remediation. Overall, integrating ecotoxicological bioassays into remediation assessment frameworks enhances the understanding of contaminant bioavailability, transformation processes, and ecological risk.

Chapter 3: Temperature Impacts on Groundwater Properties and BTEX Biodegradation

3.1 Introduction

Groundwater is a critical source of drinking water in many regions worldwide, including Ontario, Canada, where a significant portion of the municipal water supply relies on subsurface aquifers. However, groundwater systems are vulnerable to contamination from petroleum hydrocarbons, particularly monoaromatic compounds such as benzene, toluene, ethylbenzene, and xylene (BTEX). These compounds are commonly released into the subsurface through fuel leaks, industrial activities, and improper waste disposal. Due to their relatively high solubility and mobility in water, BTEX compounds can migrate through aquifers, posing significant risks to human health and aquatic ecosystems. Among these compounds, benzene is particularly concerning due to its persistence and known carcinogenic properties.

Biodegradation is widely recognized as one of the most effective and sustainable mechanisms for removing petroleum hydrocarbons from contaminated groundwater. Indigenous and introduced microorganisms can metabolize hydrocarbons as carbon and energy sources through aerobic or anaerobic pathways. Numerous bacterial genera have demonstrated the ability to degrade BTEX compounds, including *Bacillus*, *Microbacterium*, and various mixed microbial consortium. Compared to monocultures, microbial consortium often exhibits enhanced degradation efficiency due to synergistic metabolic interactions, in which one organism further transforms intermediate products generated by another.

Environmental factors play a critical role in controlling microbial activity and biodegradation rates in groundwater systems. Among these factors, temperature is particularly important because it directly influences microbial metabolism, enzyme activity, and substrate utilization rates. Temperature variations can alter microbial growth kinetics, affect the stability of hydrocarbon-degrading enzymes, and influence the physical properties of groundwater such as dissolved oxygen availability. In subsurface environments influenced by geothermal heat pump (GHP) systems or seasonal temperature fluctuations, groundwater temperatures may vary substantially, potentially impacting the natural attenuation of hydrocarbon contaminants.

Despite the importance of temperature in microbial degradation processes, there is limited experimental data examining how temperature variations influence microbial growth and BTEX biodegradation in groundwater systems representative of Ontario aquifers. Understanding these interactions is important for

predicting biodegradation potential in subsurface environments and for designing effective bioremediation strategies.

Therefore, the objective of this chapter was to investigate the influence of temperature on microbial growth dynamics and BTEX biodegradation in groundwater. Groundwater samples collected from the Waterloo region were first characterized to determine their physicochemical and biological properties. Two bacterial strains (*Microbacterium esteraromaticum* and *Bacillus infantis*) and a laboratory-prepared microbial consortium were then evaluated for their growth performance and BTEX degradation ability in groundwater microcosms. Experiments were conducted at three temperatures (15 °C, 28 °C, and 40 °C) to represent potential environmental and geothermal conditions. 15 °C mimics the average temperature of the Canadian shallow subsurface, 28 °C is the optimum temperature for the mesophilic bacteria used in the experiments, and 40 °C was the upper bound to pose stress on the microbes. Microbial growth, BTEX degradation kinetics, and changes in groundwater properties were analyzed to assess how temperature influences microbial activity and contaminant degradation in groundwater systems.

3.2 Materials, Analytical Methods and Instruments

3.2.1 Groundwater

3.2.1.1 Groundwater Collection

Waterloo is the largest urban municipality in Ontario that relies heavily on groundwater for drinking water. In this region, the Waterloo Moraine comprises several aquifers and aquitard units, and the permitted location for water collection is at the Wilmot Centre Well Field. Groundwater samples were collected from an overburden well on November 28th, 2022, and on May 9th, 2024, in a dark opaque 25 L container. The well was screened from 45.4 to 48.5 mBGS (below ground surface), and the borehole profile is available in Appendix A. The well was situated near agricultural farmland, and the water quality reports from the Waterloo region are in Appendix A. The groundwater was stored at 4 °C in a cold room until it was used in experiments.

3.2.1.2 Groundwater Characterization and Analytical Methods

The water quality reports from the Waterloo region served as a baseline for understanding the characterization and types of organics, salts, and metals present, as detailed in Appendix A. Once obtained, the groundwater samples underwent various water quality tests to assess its physicochemical and biological properties which are described below. Biological tests included analyzing the groundwater for microbial communities through colony-forming unit (CFU) plating and a DNA extraction kit. Physicochemical tests

involved measuring pH, dissolved oxygen (DO), total solids (TS), volatile solids (VS), chemical oxygen demand (COD), and analyzing anions using an ion chromatography system.

In the growth kinetic experiments to observe the effects of temperature on groundwater properties, data for the following 3 analytical methods were collected: pH, DO, and ion chromatography for anion analysis.

CFU Plating

The groundwater sample contained some sediments, thus it was mixed to a homogeneous mixture. 50 µL of the groundwater sample was spread onto a prepared agar Petri plate and incubated at 30 ± 1 °C for 48 h. This was completed for both groundwater samples (2022 and 2024).

DNA Extraction Kit

A Zymo Research Quick-DNA Fecal/Soil Microbe Micro-Prep DNA Extraction Kit was used, and the procedure was completed. The remaining sample was analyzed with the Thermo Scientific™ Nanodrop Lite Spectrophotometer UV/V is reader.

pH and DO

The pH and DO meters were calibrated before use, and readings were obtained immediately upon sample preparation.

Total Solids

Total solids were measured according to *US EPA Method 1684*. Samples were taken in triplicate. First, the empty aluminum trays were weighed (W_{dish}). 30 g of the sample was placed on the tray and covered with a watch glass. The samples were then dried at 105 ± 2 °C for 12 h in the VWR Gravity Convection Oven. Trays were cooled in a desiccator containing fresh desiccant and weighed using a bench scale. The trays were placed in the oven again for 1 h, cooled in the desiccator to balance the temperature, and weighed again. The heating, cooling, desiccating, and weighing procedure was repeated until the weight change was less than 4% or 50 mg, whichever was lower. The final weight was recorded (W_{total}) (US EPA Office of Water, 2001).

Volatile Solids

The volatile solids procedure was conducted in conjunction with the total solids procedure, following the *US EPA Method 1684*. The evaporating dishes containing the dried residues were transferred to a cool muffle furnace. The furnace was heated to 550 ± 1 °C, and trays were placed in for 2 hours. The trays were then cooled in a desiccator to balance the temperature. Residues were weighed. The procedure of igniting

(30 min), cooling, desiccating, and weighing was repeated until the weight change was less than 4% or 50 mg, whichever was lower. The final weight was recorded ($W_{volatile}$) (US EPA Office of Water, 2001).

Chemical Oxygen Demand (COD)

The COD method followed the US EPA Reactor Digestion Method 8000. According to WHO Guidelines, groundwater is expected to have a COD less than 10 mg/L because groundwater has low organic matter, thus the ultra-low range was selected: 0.7 to 40 mg/L COD (Huo et al., 2021). The DRB200 Reactor was preheated to 150 ± 1 °C. COD hatch vials were used. 2 mL of a homogeneous groundwater mixture was added to each vial. A blank vial with deionized water was also prepared. Vials were tightly closed and gently inverted several times. The vials were placed in the digester for 2h at 150 ± 1 °C. Afterwards, the digester was turned off, and the vials were cooled to 120 ± 1 °C or lower for 20 minutes. Vials were inverted, and the COD was measured with the DR3900 HACH Spectrophotometer (US EPA, 2021).

Ion Analysis

Ion concentrations were detected using a Thermo Scientific™ Dionex Ion Chromatography (IC) system (Dionex AS158 ion chromatograph) integrated with an autosampler at York University. The anion separator column (Dionex IonPac AS15, 4x250 mm) was integrated into the IC system, with an AG15 guard column and a suppressed conductivity detector. The system temperature was maintained at 30 °C. Sample vials were 1.5 mL, and the injection volume was 25 µL. The system had a flow rate of 1.2 mL/min, with a duration of 20 minutes/ injection. The analysis was performed with 38 mH potassium hydroxide (KOH) as the carrier eluent. The software connected to the system was the data collection software Chromeleon.

The anions phosphate (PO_4), nitrite (NO_2), chloride (Cl), sulphate (SO_4), and nitrate (NO_3) were selected for analysis based on the Region of Waterloo groundwater characteristics and the anions that facilitate microbial growth. Standard samples with the concentration of 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, and 1 mg/L were made with the following salts: $(NH_4)_2SO_4$, $NaNO_3$, KH_2PO_4, NaCl, and $NaNO_2$. $(NH_4)_2SO_4$, $NaNO_3$, KH_2PO_4, NaCl all had purities > 99%, and $NaNO_2$ had a purity of > 97%. The standards and blanks (DI water) were placed in 1.5 mL Dionex AS-AP Autosampler vials with split caps. Samples and blanks were tested on the instrument.

3.2.1.3 Statistical Treatment and Uncertainty

All experiments were completed in duplicates (n=2). Calibration curves were created for each anion phosphate (PO_4), nitrite (NO_2), chloride (Cl), sulphate (SO_4), and nitrate (NO_3), and graphs are in Appendix D, Figure D.1-D.5. The limit of detection (LOD) and limit of quantification (LOQ) of the system for each compound was determined before experimental use to ensure proper use of the chromatography unit. The

LOD was 1 mg/L, and the LOQ was 50 mg/L. 100 mg/L stock solutions were prepared for each ion and diluted to concentrations of 1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L for calibration curves. Duplicate vials and duplicate injections per vial were completed. If sediment or microbes were present in the samples, the samples were filtered using a 0.2 µm syringe filter. All curves had an $R^2 > 0.95$. Data was processed using Excel.

3.2.1.4 Groundwater Sterilization

The groundwater was sterilized to remove native microbial communities noticed from the CFU count. Different sterilization methods were applied to the groundwater, including autoclave steam sterilization at 121 ± 1 °C, dry heat oven sterilization at 160 ± 1 °C for 2 h, and pasteurization at 70 ± 1 °C for 30 seconds. Pasteurization sterilized the groundwater, leaving it free of any precipitate. The final pasteurization method included filtration with a 0.22 µm sterilized syringe filter in an autoclaved flask, which was then placed on a hot plate, and the groundwater temperature was measured repeatedly until it reached 70 ± 1 °C. The groundwater was heated to 70 ± 1 °C for 30 seconds, then cooled to below 15 ± 1 °C.

3.2.2 Microorganisms and Growth Media

3.2.2.1 Microorganism Selection

Microbacterium esteraromaticum, *Bacillus infantis*, and a laboratory-prepared microbial consortium were used in the experimental trials. These microorganisms were isolated from soil samples collected from boreholes located in Toronto, Ontario. The sampling site was non-contaminated, and the boreholes were originally constructed for a planned geothermal heat pump installation. *Microbacterium esteraromaticum*, *Bacillus infantis*, and the strains incorporated into the consortium were selected based on their demonstrated growth performance and BTEX degradation potential, as reported in the literature and supported by preliminary experiments conducted by a laboratory colleague (Kaur et al., 2023). The consortium was created with 12 isolates with higher growth and degradation ability.

Table 3.1. 12 isolates in the consortium with their optimum growth temperature (Kaur et al., 2023; Schober et al., 2025)

	Soil-isolated Strain	**Accession Number by GenBank**	**Optimum Growth Temperature**
1	*Microbacterium paraoxydans*	OQ582075	37 °C
2	*Microbacterium paraoxydans*	OQ582076	37 °C
3	*Bacillus subtilis*	OQ582077	30 – 35 °C

	Soil-isolated Strain	Accession Number by GenBank	Optimum Growth Temperature
4	*Microbacterium esteraromaticum*	OQ582078	30 °C
5	*Microbacterium paraoxydans*	OQ582079	37 °C
6	*Microbacterium lacusdiani*	OQ582080	28 °C
7	*Nocardia asteroides*	OQ582088	30 – 37 °C
8	*Peribacillus simplex*	OQ582088	30 °C
9	*Microbacterium maritypicum*	OQ582090	28 – 30 °C
10	*Microbacterium paraoxydans*	OQ582091	37 °C
11	*Bacillus infantis*	OQ582092	37 – 40 °C
12	*Exiguobacterium mexicanum*	OQ582093	30 °C

3.2.2.2 Microorganism Growth and Preparation Methods

Tryptic soy agar (TSA) was prepared by dissolving 30 g of dehydrated tryptic soy broth (TSB) in 1 L of distilled water, with 1.5% agar added as a solidifying agent. The TSA was poured into sterile Petri plates and allowed to solidify before use for plating and streaking microorganisms. All individual strains and the consortium culture were stored at −80 ± 1 °C before use and defrosted for streaking plates. The streaked plates were incubated in a static incubator for 48h at 28 ± 1 °C. For inoculum preparation, single colonies were grown in flasks containing TSB media for 24 h in a shaker incubator (INFORS HT) at 28 ± 1 °C and 200 RPM. TSB was used as it is a nutrient-rich medium that provides essential compounds for microbial growth. Each 20 mL microcosm was inoculated with a starting OD_{600} = 0.04 (equivalent to 2% of an OD_{600} 2.0 culture).

3.2.2.3 Growth Media Selection

To determine the nutrient media that enhance microbial growth in monocultures, preliminary experiments were conducted. *Microbacterium esteraromaticum* and *Bacillus infantis* were attempted to grow in unsterilized groundwater (no nutrients), 2% Lysogeny broth (LB), mineral salt media (MSM), 3% yeast extract, 3% dextrose, and a combination of 3% yeast extract and 3% dextrose. LB media was curated by

mixing 2.0 g of LB in 1 L of distilled water. MSM media was made with the composition of 1.8 g K_2HPO_4, 4.0 g NH_4Cl, and 0.01 g $FeSO_4$. $7H_2O$, 0.1 g NaCl, and 0.2 g $MgSO_4.7H_2O$ per 1 L of distilled water. The iron sulphate salt was prepared separately in distilled water, sterilized by a 0.5µm syringe filter, and added to the salt media before the experiment. Yeast extract and dextrose were both prepared at 3.0 g/L. All media were autoclave sterilized at 121 ± 1 °C before use in experiments. The selected media for the final bacterial growth experiments vary. *Microbacterium esteraromaticum* and the consortium were grown with 3% yeast extract, and *Bacillus infantis* was grown with 3% yeast extract and 3% dextrose. Yeast extract contains amino acids, peptides and vitamins, while dextrose and BTEX are carbon sources, both essential for microbial growth.

3.2.2.4 Microorganism Analytical Methods

To record the growth rate, microbial samples were collected every 4 h for the first 16 h, then every 8 h until the strain began to decline. A micro-syringe was used to collect the sample, and the cell count was obtained using a spectrophotometer (96-well plate reader, Gen5, Epoch (BioTek)) at 600 nm.

3.2.3 BTEX and Degradation

BTEX, the contaminant of concern, was used as a carbon source for the selective isolation of BTEX-degrading bacteria and for growth experiments. B, T, E, X compounds were acquired from Fisher Scientific at a >97% purity grade. Experiments contained a total of 200 mg/L BTEX at a 1:1:1:1 ratio (therefore 50 mg/L of each compound).

3.2.3.1 BTEX Degradation Analytical Method

BTEX concentrations were detected and analyzed using a Gas Chromatography- Mass Spectrometry (GC-MS) system (Agilent 7820A, Agilent Technologies) connected to a Solid Phase Micro-Extraction (SPME) headspace sampler (Clarus 500, PerkinElmer, USA) at York University, Ontario. The column integrated into the GC system was an Agilent J&W CP-Sil 5 CB GC column (30 m × 0.25 mm × 1.00 µm). The headspace system was maintained at 90 ± 1 °C and 120 ± 1 °C for the needle and the transfer line, respectively. Sample vials were heated and agitated for 10 min at 80 ± 1 °C before injection. The vials were auto-sampled with a needle and transferred to the column. The extraction occurred at 40°C for 24 minutes. The analysis was performed with helium as the carrier gas.

3.2.3.2 GC Sample Preparation

Samples were prepared in 20 mL GC headspace vials. Each vial consisted of 20 µL of the test solution, 5 mL of distilled water, and 30 µL of fluorobenzene-D5 (4 mg/L, diluted in methanol) as the internal standard. Using the dilution formula, 20 µL of the test solution equated to a concentration of 200 µg/L for each BTEX

compound, to remain within the LOQ of the GC system. Vials were sealed with crimp caps to prevent volatilization.

3.2.3.3 Statistical Treatment and Uncertainty

All experiments were completed in duplicates (n=2). Calibration curves were created for each BTEX compound, graphs are in Appendix C, Figure C.1- C.5. Internal standards were used to assess reproducibility. The LOD for all compounds was 2 µg/L. The LOQ for benzene was 200 µg/L, as concentrations past that would plateau and not provide accurate concentration readings. For toluene, ethylbenzene, and m-, p-, o- xylene, the LOQ was 1045 µg/L, but to be consistent, the calibration curves were generated over the range of 2-209 µg/L. All curves had a $R^2 > 0.95$. Data was processed using Excel.

3.2.3.4 BTEX degradation half-life

BTEX half-life was determined by estimating compound half-lives based on measured concentrations through the degradation experiments. To calculate the half-life, first-order degradation kinetics were used. The rate constant k represents the rate of decay over time, and the contaminant half-life $t_{1/2}$ represents the time required for the concentration to decrease by 50%.

The model assumes that contaminant concentration decreases exponentially with time.

Rate constant:

$$k = \frac{\ln (C_0/C_t)}{t}$$

Half-life:

$$t_{1/2} = \frac{\ln (2)}{k}$$

Where,

- C_0= initial contaminant concentration
- C_t= concentration at time t , at the end of the experiment or when the compound was completely degraded
- t= time (h)
- k = first-order degradation rate constant (h^{-1})
- $t_{1/2}$= contaminant half-life (h)

3.2.3.5 Detection of Biodegradation Metabolites Using LC–MS

The following treatment, as shown in Table 3.2, was submitted for chemical characterization by liquid chromatography-mass spectrometry (LC-MS). Similar to the preparation of the serum bottles for the BTEX degradation and growth analysis, the serum bottles with the treatments listed below were incubated in the shaker incubator (INFORS HT) at 28 °C and 180 RPM for 48 h. BTEX control and BTEX treatment samples were submitted at 0 h, while all 3 treatments were submitted for 48 h. Collected samples were filtered using a 0.22 µm syringe filter to remove possible suspended matter. Filtered samples were transferred into 1.5 mL glass vials and stored at 4 °C before analysis to minimize chemical transformation and microbial activity. The three treatment sets were submitted for untargeted and targeted analyses to detect a wide range of organic compounds and to identify compounds reported as intermediates in BTEX biodegradation pathways. LC-MS analysis was performed at the YSCi Core Mass Spectrometry Facility at York University by Maxime Rossato.

Table 3.2. Treatment groups submitted for LC-MS analysis.

	Untargeted		Targeted	
Treatment set	T0	T48	T0	T48
1. Groundwater		x		
2. Groundwater + yeast extract + 200 mg/L BTEX	x	x		
3. Groundwater + yeast extract + 200 mg/L BTEX + consortium	x	x		x

3.2.4 Experimental Set-up

To study the growth effects and biodegradation abilities of the previously mentioned bacterial strains, batch treatments were used. 100 mL serum bottles with septum crimp caps were used, containing 20 mL of groundwater supplemented with/without nutrients, depending on the experiment, and spiked with 200 mg/L of BTEX. Serum bottles were inoculated with the bacteria at 2% of 2 OD. Bottles were incubated in a shaker incubator (INFORS HT, MaxQ 6000 Stackable Incubated and Refrigerated Shakers, and/or MaxQ 4450 Benchtop Orbital Shaker) at 15 ± 1 °C, 28 ± 2 °C, and 40 ± 1 °C. The temperature range was selected based on the GHP working conditions (Roohidehkordi & Krol, 2021). Groundwater characteristics, microbial growth and BTEX degradation were analyzed for each temperature-dependent experiment. Table 3.3 describes the samples in the batch experiment and their purpose. The analysis parameter dictates the negative control in the treatment set. For analyzing bacterial growth, the positive control is a sample containing only bacteria. For analyzing BTEX degradation, the positive control in the sample contains only BTEX. All experiments were completed in duplicates.

Table 3.3. Experimental samples used in batch experiments

Sample	Description	Purpose
1	Groundwater	Abiotic control
2	Groundwater + *bacteria*	Bacterial growth control
3	Groundwater + 200 mg/L BTEX	BTEX volatilization control
4	Groundwater + *bacteria* + 200 mg/L BTEX	Biodegradation experiment

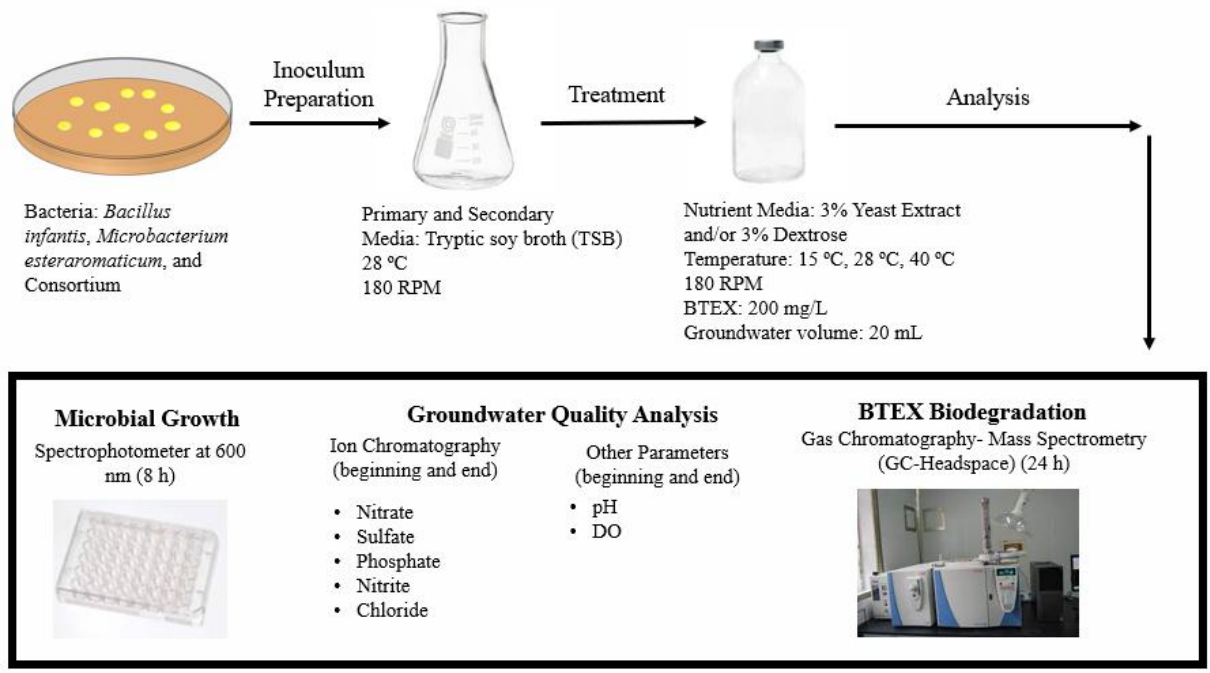


Figure 3.1. Schematic representation of bacterial preparation and BTEX degradation

3.2.4.1 Personal Protective Equipment (PPE)

Due to the nature of the experiments involving bacteria and volatile compounds (BTEX), lab coats, gloves, and masks were always worn. Safety glasses were sometimes worn during the experiment setup. All BTEX sampling was done in the fume hood.

3.3 Results and Discussion

3.3.1 Initial Characteristics and Properties of Groundwater

The presence of microbial communities in the groundwater samples was assessed using the plate count method. Samples were directly inoculated on nutrient agar plates, and the total bacterial count was

expressed as colony-forming units per millilitre (CFU/mL). The 2022 groundwater sample was plated twice, yielding colony counts of 40 CFU/mL and 100 CFU/mL. For the 2024 groundwater sample, the CFU count was 20 CFU/mL of groundwater on 2 different plates, as shown in Figure A.3 in Appendix A. This low microbial count indicates a limited presence of culturable microorganisms within the sampled groundwater. Despite the low CFU count, the presence of even a small number of viable microorganisms suggests that some indigenous bacteria may persist under certain conditions, which requires that the groundwater be sterilized.

The DNA extraction kit showed low 260/280 readings and low concentration readings on the Nanodrop spectrophotometer. The 260/280 reading was 1.18, yielding a concentration of 6.5 ng/µL. This is lower than the ideal ratio of 1.80, with a concentration of 15 ng/µL; thus, the DNA and/or RNA are below the detection limit, indicating the groundwater sample has low DNA/RNA.

pH readings were taken in triplicate, resulting in an average pH of 7.7 ± 0.02. This indicates a neutral pH, typical for water. The pH was expected to be in the range of 6.5-8.8, as the well site was near an agricultural area and nitrate fertilizers could acidify the groundwater. The DO had a concentration of 7.05 mg/L

Groundwater typically has low organic matter content; the VS and TS were expected to be low. The mean total solids were 3 ± 0.003 mg, and the volatile solids mean was 0.8 ± 0.001 mg. Similarly, the COD did result in the ultra-low range value, 26 ± 1.4 mg/L, which was higher than expected, but still in the ultra-low range, 40 mg/L (Huo et al., 2021).

The groundwater had concentrations of chloride (Cl^-), nitrate (NO_3^-), sulphate (SO_4^{2-}), and phosphate (PO_4) of 35 mg/L, 2 mg/L, 71 mg/L, and 8.3 mg/L, respectively. Nitrite (NO_2^-) was not detected with the IC. These results correlate with the ion concentrations reported by the region of Waterloo (Figure A.1 and A.2 in Appendix A), except for Cl, for which the IC detected Cl levels three times higher than what was reported by the region of Waterloo.

3.3.2 Temperature Influence on Microbial Growth in BTEX-Contaminated Groundwater

Microbial growth experiments with *Bacillus infantis, Microbacterium esteraromaticum,* and a mixed microbial consortium were conducted to evaluate bacterial tolerance and growth dynamics in groundwater spiked with BTEX. Cultures were incubated at 15 °C, 28 °C, and 40 °C to examine the influence of temperature on microbial activity. *Microbacterium esteraromaticum* and the consortium were grown in 3 % yeast extract, while *Bacillus infantis* cultures were grown in groundwater supplemented with 3 % yeast

extract and 3 % dextrose. *Bacillus infantis* required an additional carbon source (dextrose) with BTEX, as it exhibited growth issues in preliminary experiments.

The microbial growth experiments with *Bacillus infantis*, *Microbacterium esteraromaticum*, and a consortium demonstrated microbial growth at all temperatures. The results, shown in the growth curves in Figure 3.2, demonstrate the influence of temperature on growth. Abiotic controls with groundwater (not shown on graphs) and groundwater and BTEX showed negligible OD changes. This confirms observed turbidity increases were associated with microbial biomass accumulation rather than abiotic precipitation. Similar observations were reported in groundwater microcosm studies, where BTEX compounds were substrates for microbial metabolism under aerobic conditions (Das & Chandran, 2011; Lovley, 2003).

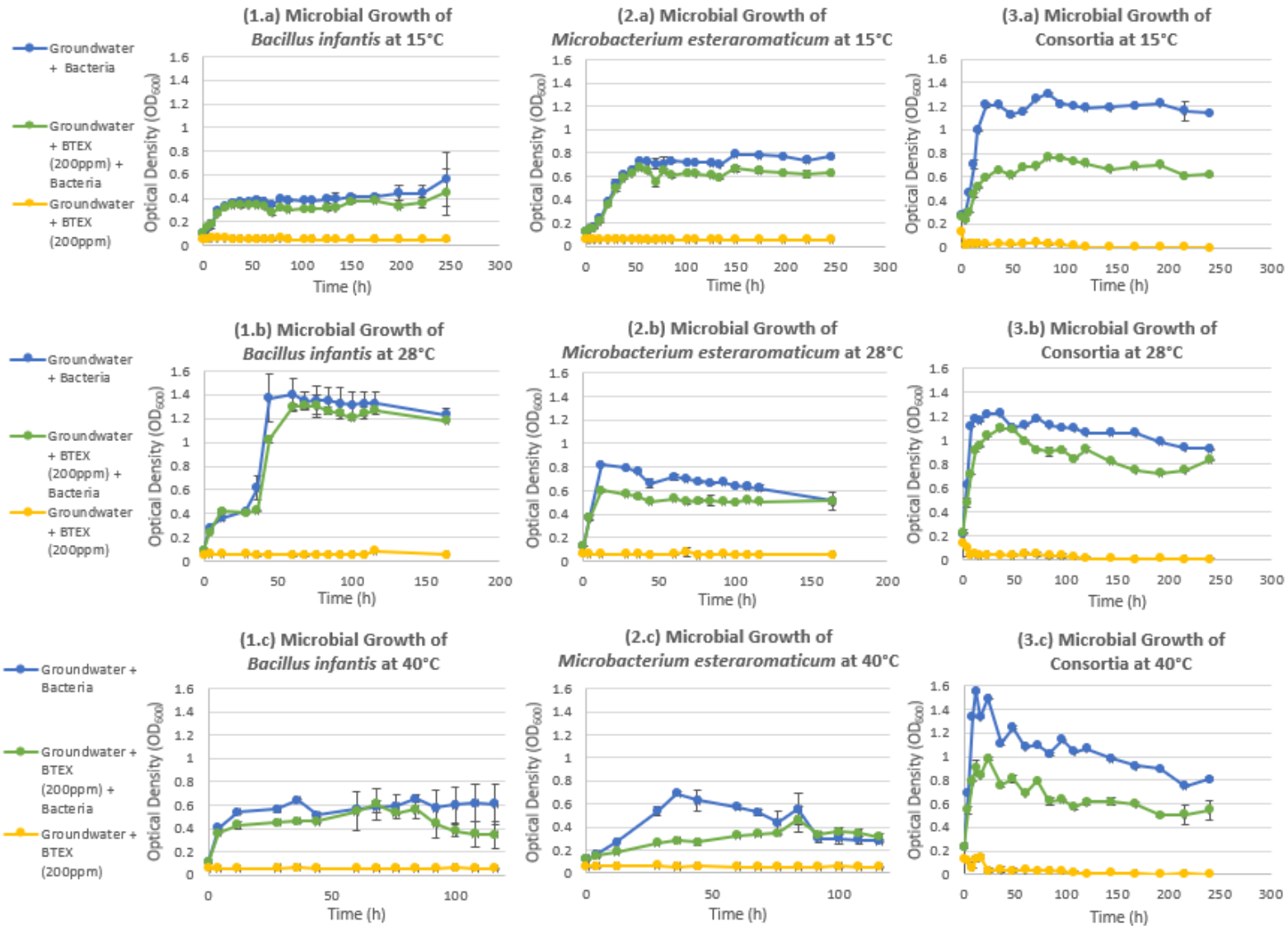


Figure 3.2. Growth curves of (1) *Bacillus infantis*, (2) *Microbacterium esteraromaticum*, and (3) Consortium in the presence and absence of BTEX. Absorbance (OD_{600}) of bacteria at different temperatures (a)15 °C (b)28 °C (c)40 °C

At 15 °C, all microbial systems demonstrated slower growth rates compared to higher temperatures, indicating that cold conditions limit metabolic activity in mesophilic microbial strains. Although 15 °C may not be the optimum environment for mesophilic microbes, they can survive and adapt to low temperatures, like *Pseudomonas putida*, a well studied mesophilic BTEX-degrading strain (Fonseca et al., 2011b; Kaur et al., 2023; Leahy & Colwell, 1990). *Bacillus infantis* (1.a) exhibited gradual growth, with an OD of ≈ 0.55 after 250 hours in groundwater without BTEX, while cultures exposed to BTEX reached ≈ 0.45 OD. This low and slow growth suggests that the low temperature decreased enzyme activity, which slowed the substrate intake and metabolic reaction, as the strain started to decline at 250 h (Margesin & Schinner, 2001). *Microbacterium esteraromaticum* demonstrated slightly greater tolerance to cold conditions, reaching ≈ 0.75 OD within the first 60 hours before stabilizing (2.a). Although BTEX exposure caused a minor reduction in biomass, growth remained relatively stable throughout the incubation period. This observation is consistent with previous reports identifying *Microbacterium esteraromaticum* as a BTEX-degrading bacterium capable of metabolizing BTEX through enzymatic pathways (Hernández-Ospina et al., 2024; Kaur et al., 2025). These enzymes catalyze the initial oxidation of aromatic hydrocarbons, converting them into intermediates such as phenol or catechol that can subsequently enter central metabolic pathways. Similarly, this reasoning can be applied to 3.a, the growth with the consortium. The microbial consortium resulted in the highest biomass accumulation at 15 °C, reaching ≈ 1.2 OD in groundwater without BTEX and ≈ 0.75 OD in microcosms with BTEX. Although growth in the presence of BTEX was reduced, the consortium maintained moderate metabolic activity throughout the incubation period. The enhanced performance likely reflects collaborative interactions among the 12 bacterial species, in which metabolic intermediates produced by one organism can be further degraded by another. Such cooperative interactions are common in hydrocarbon-degrading microbial communities and often lead to higher degradation efficiency than single strains alone (Leahy & Colwell, 1990).

At 28 °C, microbial growth was substantially faster, reaching the exponential phase more quickly and more stably across all treatments, indicating that this temperature provided conditions closer to the optimal metabolic range for the bacteria. *Bacillus infantis* exhibited rapid exponential growth, reaching an OD of approximately 1.3–1.4 within 60–80 h in both groundwater and BTEX-containing microcosms (1.b). The similar growth patterns observed across these treatments suggest that *Bacillus infantis* can tolerate and potentially metabolize BTEX compounds under favourable environmental conditions. Members of the genus *Bacillus* are known to degrade aromatic hydrocarbons through oxygenase-mediated pathways that convert benzene and toluene into catechol intermediates, which are subsequently mineralized via ortho- or meta-cleavage pathways (Das & Chandran, 2011; Hernández-Ospina et al., 2024). Growth of *Microbacterium esteraromaticum* at 28 °C was moderate, reaching ≈ 0.7 OD in groundwater microcosms and slightly lower values when BTEX was present (2.b). Although hydrocarbon exposure reduced

metabolism, the bacteria maintained consistent growth. Thus, suggesting the presence of enzymatic pathways transformed the BTEX compounds into less toxic metabolites, like the results for this strain at 15 °C. The consortium again produced the highest growth with an OD of ≈ 1.25 in groundwater, and a slightly lower growth in the presence of BTEX, OD of ≈ 1.0 (3.b). Rapid growth occurred during the first 20 h and remained relatively stable throughout the incubation period, before gradually declining after 120 h.

At 40 °C, microbial growth patterns differed from those observed at lower temperatures. All microbial systems showed rapid initial growth, but over time, growth declined gradually. *Bacillus infantis* reached ≈ 0.6 OD at 36 h in groundwater and ≈ 0.6 OD at 68 h in BTEX-containing treatments (1.c), suggesting that higher temperatures increased metabolic stress and intensified hydrocarbon toxicity. High temperatures with a 180 RPM can increase membrane permeability, allowing hydrophobic hydrocarbons to diffuse more readily into bacterial cells and disrupt intracellular processes, which can lead to cell death (Heipieper et al., 2007). *Microbacterium esteraromaticum* also exhibited reduced growth at 40 °C (2.c), reaching an OD near 0.8 at 36 h before declining over time. The BTEX sample reached its exponential phase at 84 h, with an OD of 0.46. The optimal growth for this strain is 30– 33 °C, thus metabolic efficiency may be reduced at higher temperatures (Wongbunmak et al., 2017). The decline in biomass observed at 40 °C therefore likely reflects thermal stress and reduced stability of hydrocarbon-degrading enzymes. The microbial consortium showed the highest initial growth among all conditions, reaching approximately 1.5 OD at 12 h (3.c). However, the growth gradually declined after this peak, indicating that sustained exposure to elevated temperature reduced microbial stability over time. In the BTEX-spiked sample, the consortium reached a lower peak of approximately 0.98 OD at 24 h, then gradually decreased. These results suggest that although elevated temperature initially stimulated microbial metabolism and biomass production, prolonged exposure likely induced physiological stress, limiting long-term growth. Similar patterns have been reported in hydrocarbon-degrading microbial communities, where temperatures above 35 °C reduce biodegradation efficiency due to decreased enzyme stability and reduced dissolved oxygen availability. (Das & Chandran, 2011).

3.3.2.1 Overall Temperature Effects

Collectively, the results demonstrate that temperature strongly influences microbial growth kinetics and BTEX tolerance in groundwater systems. Growth was slow but sustained at 15 °C, rapid and stable at 28 °C, and rapid but less stable at 40 °C. Among the three microbial systems tested, the consortium consistently produced the highest biomass accumulation, indicating that cooperative metabolic interactions enhanced microbial resilience and substrate utilization.

These findings are consistent with previous hydrocarbon bioremediation studies demonstrating that most BTEX-degrading bacteria are mesophilic and exhibit optimal biodegradation activity between 25 °C and

30 °C (Leahy & Colwell, 1990). Additionally, previous research examining BTEX-degrading bacteria isolated from subsurface environments reported that higher temperatures significantly enhanced both microbial growth and biodegradation efficiency, with increases of 35–49 % in hydrocarbon removal with consortium compared to colder conditions. (Kaur et al., 2023). The results, therefore, suggest that the microbial consortium used in this study is well-suited for biodegradation.

In all three microbial systems, the groundwater + bacteria treatments consistently showed higher growth than the groundwater + BTEX + bacteria treatments, suggesting that BTEX had some inhibitory effects on microbial proliferation. Aromatic hydrocarbons are hydrophobic molecules that can partition into microbial cell membranes, disrupting membrane structure and impairing cellular respiration. (Heipieper et al., 2007) all stress, limiting long-term growth. Similar patterns have been reported in hydrocarbon-degrading microbial communities, where temperatures above 35 °C reduce biodegradation efficiency due to decreased enzyme stability and reduced dissolved oxygen availability (Das & Chandran, 2011). Despite this inhibition, measurable growth was observed in the BTEX-containing microcosms, suggesting that the microorganisms were able to tolerate and potentially utilize these compounds as carbon sources through aerobic degradation pathways.

3.3.3 BTEX degradation ability of bacterial strains

BTEX degradation experiments were conducted to evaluate the ability of *Bacillus infantis*, *Microbacterium esteraromaticum*, and a microbial consortium to degrade benzene, toluene, ethylbenzene, and xylene in groundwater at 15 °C, 28 °C, and 40 °C. Concentration changes were monitored over time and expressed as normalized concentration ratios (C/C_0), where C_0 is the initial concentration, and C is the final concentration. The results demonstrate that BTEX degradation occurred primarily in the consortium cultures, whereas monocultures of *Bacillus infantis* and *Microbacterium esteraromaticum* showed BTEX loss. However, there were instrumental issues with the monoculture degradation, which did not produce promising results. Additionally, the absence of degradation by the monocultures indicates that the microbial strain can grow in the presence of BTEX but may not have the metabolic ability to degrade it in the presence of nutrients and groundwater. This suggests that other nutrients should be supplemented to assist in the degradation pathways of BTEX.

The consortium cultures in this study demonstrated measurable degradation of several BTEX compounds, as shown in Figure 3.3. The degradation results at 40 °C and the BTEX control graph are included in Appendix C, Figure C.8 and C.9. At 15 °C, degradation of ethylbenzene and xylene was limited but detectable, with concentrations declining gradually over the 96-hour incubation period. The slow degradation at 15 °C is consistent with previous findings of decreased hydrocarbon metabolism due to

reduced enzyme activity and slower substrate uptake (Margesin & Schinner, 2001). Cold groundwater environments are therefore known to limit the rate of BTEX biodegradation even when degradative microorganisms are present. The consortium exhibited high degradation activity at 28 °C. Toluene concentrations declined rapidly within the first 24 hours and was almost 100% degraded at 96 h. Ethylbenzene and xylene also showed progressive declines, reaching approximately 20–50 % of their initial concentrations by 96 h. These results indicate that mesophilic conditions promoted optimal metabolic activity for the microbial community, allowing the consortium to metabolize aromatic hydrocarbons more efficiently. Numerous studies have reported that the optimal temperature range for aerobic hydrocarbon-degrading bacteria lies between approximately 25 °C and 30 °C, where enzymatic activity and microbial growth rates are highest (Das & Chandran, 2011; Kaur et al., 2023; Wu et al., 2023).

In contrast, at 40 °C, none of the BTEX compounds degraded. Based on this observation, an instrumental error was assumed, as no volatilization occurred. The graph is in Appendix C, C.8. Concentrations remained close to their initial values throughout the incubation period (no data reported at 24 h), suggesting that the elevated temperature inhibited biodegradation. High temperatures can destabilize key enzymes involved in aromatic hydrocarbon metabolism and may also increase the toxicity of hydrophobic compounds by enhancing their diffusion across microbial membranes (Heipieper et al., 2007).

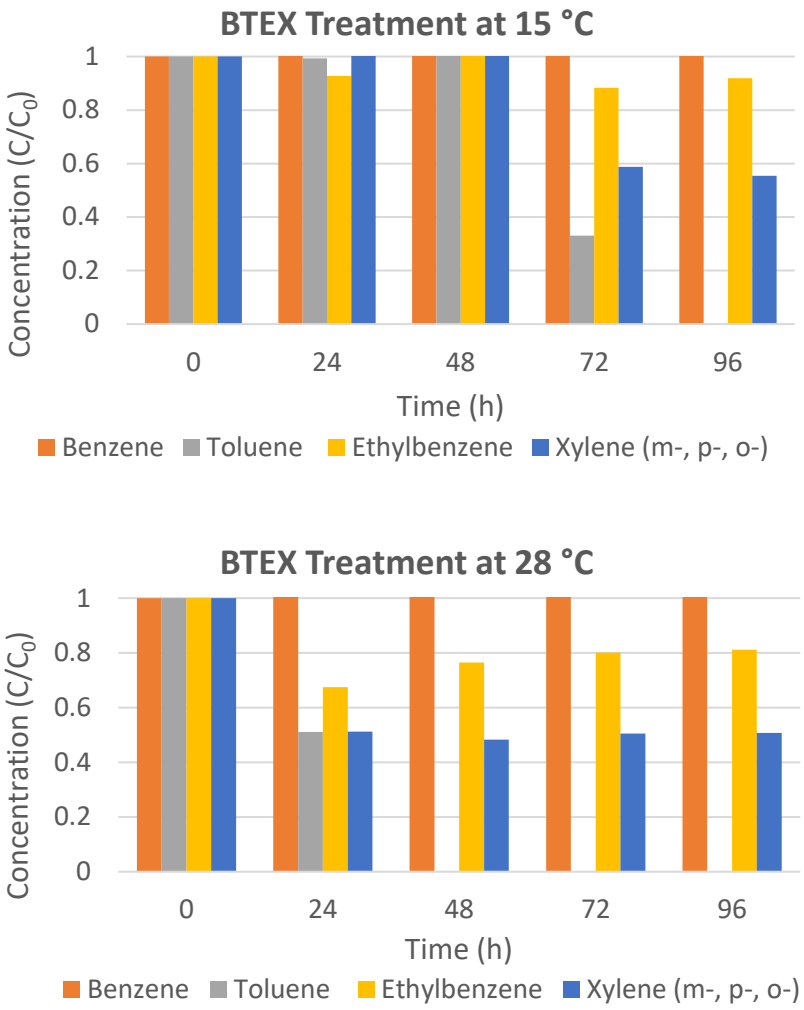


Figure 3.3. BTEX degradation graphs with consortium at 15 °C, and 28 °C for 96 hours

3.3.4 Kinetic Analysis of BTEX Degradation by Consortium

The degradation kinetics of benzene, toluene, ethylbenzene, and xylenes by the microbial consortium were evaluated using first-order kinetic modelling. The results (Table 3.4) indicated that degradation rates varied among compounds and were strongly influenced by temperature. The calculated degradation rate constants (k) and half-life values ($t_{½}$) varied between compounds and temperatures, indicating strong compound-specific and temperature-dependent biodegradation behaviour. Overall, measurable degradation was observed for toluene, ethylbenzene, and xylenes at 15 °C and 28 °C, while benzene showed no degradation (ND = not detected), and none occurred at 40 °C.

Table 3.4. First-order degradation kinetics and half-life values for BTEX compounds degraded by the microbial consortium at different temperatures

	15 °C					
Compound	C_0	C_t	t (h)	k (h^{-1})	$t_{1/2}$ (h)	$t_{1/2}$ (day)
Benzene	1.00	1.00	96.00	≈ 0.00	ND	ND
Toluene	1.00	0.00	96.00	-	<48.00	<2.00
Ethylbenzene	1.00	0.91	96.00	9.82×10^{-4}	705.56	29.40
Xylene (m-, p-, o-)	1.00	0.55	96.00	6.23×10^{-3}	111.31	4.64
	28 °C					
Compound	C_0	C_t	t (h)	k (h^{-1})	$t_{1/2}$ (h)	$t_{1/2}$ (day)
Benzene	1.00	1.00	96.00	≈ 0.00	ND	ND
Toluene	1.00	0.00	48.00	-	<24.00	<1.00
Ethylbenzene	1.00	0.80	96.00	2.32×10^{-3}	298.20	12.43
Xylene (m-, p-, o-)	1.00	0.50	96.00	7.22×10^{-3}	96.00	4.00

Overall, only the consortium shows measurable degradation, especially toluene, nearly 100% in 48- 96 h at 28 °C; ethylbenzene and xylene were slower; benzene was persistent within 96 h. At 15 °C, toluene degraded the fastest, being completely removed within 96 h, corresponding to an apparent half-life based on the last non-zero point of 48 h (2 days). Xylene had a half-life of 111.31 h (4.64 days), followed by ethylbenzene with a half-life of 705.56 h (29.4 days). At 28 °C, degradation rates increased substantially for several compounds. Toluene was completely removed within 48 h, corresponding to an apparent half-life based on the last non-zero point of 24 h (1 day). Xylene had a half-life of 96 h (4 days), while ethylbenzene had a half-life of 298.2 h (12.43 days). These results correspond to the aerobic biodegradation half-lives in groundwater, with toluene ranging from 2- 92 days, ethylbenzene ranging from 0.1- 231 days, and xylene ranging from 3.1 h to 4.1 days (Health Canada, 2015). Benzene has a reported half-life of 28 days, which can be applied to these results, as there was no degradation of benzene in 96 h, which emphasizes how harmful benzene can be to ecosystems as it persists longer (Agency for Toxic Substances and Disease Registry (US), 2007).

3.3.5 Identification of Biodegradation Metabolites Associated with BTEX Degradation

The objective of this analysis was to identify intermediate metabolites produced during microbial degradation and determine whether these compounds could contribute to the phytotoxic effects observed in seed germination bioassays. Targeted and untargeted analyses were completed for the three treatment sets at time = 0 (T0) and time = 48 h (T48). In the untargeted analysis, comparisons were made between (1) microbial treatments at T0 and T48 to identify new metabolites produced through degradation, and (2) microbial treatment to BTEX control to determine if the metabolites were microbially produced. The complete list of chemicals resulting from comparing the data sets can be found in Appendix C.

From the targeted analysis, Benzyl alcohol and Diethyl succinate were detected. The untargeted LC-MS analysis revealed numerous metabolites detected in the bacterial treatment but absent in the BTEX control. Several of the identified compounds belong to chemical classes commonly associated with aromatic hydrocarbon degradation pathways, including phenolic compounds, aromatic acids, and catechol derivatives (Hernández-Ospina et al., 2024; Ladino-Orjuela et al., 2016). These metabolites are known intermediates produced during the microbial transformation of aromatic hydrocarbons. Several compounds identified in the treatment samples, including 2-Methylhippuric acid, 3,4-Dihydroxybenzeneacetic acid, 3,4-Dihydroxyacetophenone, and 2-Aminobenzoic acid, are structurally related to known intermediates in degradation pathways. The formation of catechol-like metabolites is significant because catechol derivatives are central intermediates that facilitate the oxidative cleavage of aromatic rings and subsequent mineralization of hydrocarbons. However, several of these intermediate metabolites have been reported to exhibit greater toxicity than the parent hydrocarbons, particularly toward plants and microorganisms. Table 3.5 has compounds that are very consistent with BTEX transformation pathways. Several aromatic metabolites were detected at T48 that were absent at T0, suggesting the formation of intermediate degradation products during microbial transformation of BTEX compounds.

Table 3.5. Compounds detected by the LC-MS that are consistent with BTEX transformation pathways

Detected compound	**Role in degradation**	**Literature relevance**
2-Methylhippuric acid	xylene oxidation metabolite	reported metabolite of xylene transformation
3,4-Dihydroxybenzeneacetic acid	catechol derivative	catechol pathway intermediate
3,4-Dihydroxyacetophenone	phenolic oxidation product	aromatic ring hydroxylation product

Detected compound	Role in degradation	Literature relevance
2-Aminobenzoic acid (anthranilic acid)	aromatic degradation intermediate	detected in aromatic compound biodegradation
4-Hydroxy-3-methylbenzoic acid	methylbenzene oxidation product	toluene/xylene oxidation
Quinolin-6-ol / methylquinolinol	heterocyclic aromatic products	microbial transformation of aromatic compounds
3-Coumaric acid	phenolic aromatic compound	secondary aromatic metabolite

In Section 3.3.3, toluene was degraded in less than 48 h, indicating that there was early-stage oxidation of toluene. The proposed pathway of toluene to catechol is in Figure 3.4 (a) (Basu et al., 2003). In Figure 3.4 (b), ethylbenzene had a proposed degradation pathway to 3,4-Dihydroxyacetophenone (Gao et al., 2023). For Figure 3.4 (c), the proposed toluene degradation pathway led to 3,4-Dihydroxybenzeneacetic acid through the microbial metabolism of p-hydroxyphenylacetic acid (Sparnins et al., 1974; Y. Tang et al., 2016a). The following proposed pathways were created from using Kegg Pathway and from research articles. Figures C.9, C.10, and C.11 in Appendix C have the correlating putative ID level, m/z, and retention times.

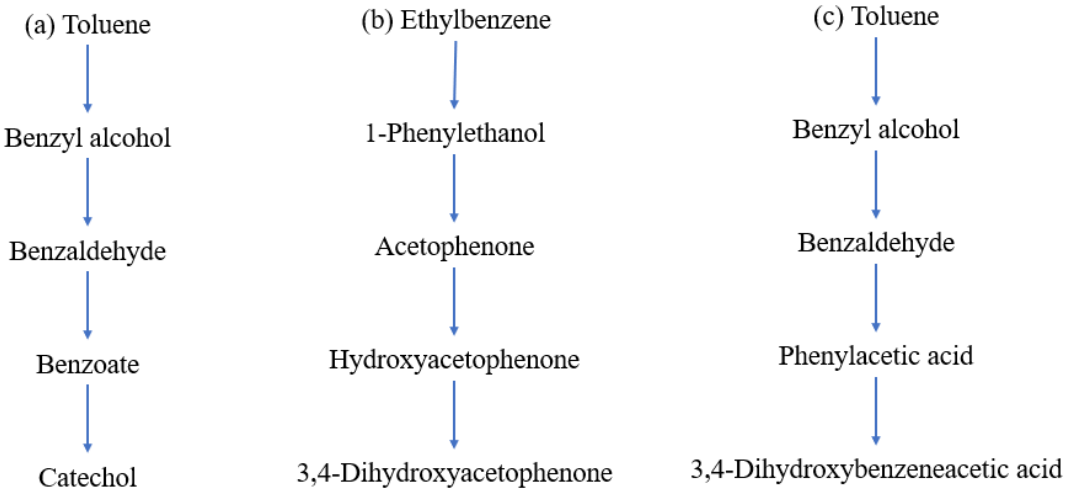


Figure 3.4. Comparison of three proposed microbial degradation pathways for aromatic compounds: (a) Toluene degradation to Catechol, (b) Ethylbenzene degradation to 3,4-Dihydroxyacetophenone, and (c) an alternative Toluene pathway leading to 3,4-Dihydroxybenzeneacetic acid

These types of phenolic and catechol-derived intermediates are widely reported during aerobic BTEX degradation. Phenolic compounds and catechol derivatives, such as 3,4-Dihydroxybenzeneacetic acid, are known to induce oxidative stress, disrupt cellular membranes (Y. Tang et al., 2016b). Additional aromatic compounds identified in the treatment samples included 2-aminobenzoic acid, 4-hydroxy-3-methylbenzoic acid, and 3-coumaric acid, consistent with the oxidative transformation of substituted benzene rings. These metabolites suggest that microbial oxidation reactions, including hydroxylation and side-chain modification, occurred during the degradation process. Similar transformation products have been reported during microbial metabolism of toluene and xylene in contaminated soils and groundwater systems.

The LC-MS analysis also revealed the presence of several dicarboxylic acids, including pimelic acid, suberic acid, azelaic acid, and 3,3-dimethylglutaric acid. These compounds are commonly formed during the oxidative degradation of hydrocarbons and may represent downstream products resulting from aromatic ring cleavage. The formation of these organic acids suggests that partial mineralization of BTEX compounds occurred, resulting in smaller carbon intermediates that can enter central microbial metabolic pathways such as the tricarboxylic acid (TCA) cycle (Y. Tang et al., 2016b).

In addition to degradation intermediates, numerous microbial biomass metabolites and cellular metabolism were detected in the treatment samples. These included nucleobase derivatives such as uracil, dihydrouracil, and hypoxanthine, as well as amino-acid-related metabolites including N-acetyl-L-phenylalanine and guanidinopropionic acid. The presence of these compounds likely reflects increased microbial activity and biomass production stimulated by the addition of yeast extract and BTEX substrates.

Overall, the LC-MS metabolomic profile indicates that the microbial consortium actively transformed BTEX compounds, producing a diverse suite of secondary metabolites. The detection of aromatic oxidation products, catechol derivatives, and organic acids suggests that microbial biodegradation pathways were actively operating during the incubation period. Importantly, several of the detected compounds, particularly phenolic and quinone-like metabolites, have been reported to exhibit phytotoxic properties, which may contribute to the inhibitory effects (Hernández-Ospina et al., 2024; Kaur et al., 2025; Ladino-Orjuela et al., 2016). The formation of such intermediates cannot be fully evaluated through chemical analysis alone; complementary ecotoxicological assays are necessary to assess their potential biological impacts. Seed germination bioassays are widely used as sensitive indicators of soil phytotoxicity and have been applied extensively in studies evaluating the ecological safety of bioremediated soils (Du et al., 2025; Gong et al., 2001).

3.3.6 Effects of Temperature on Groundwater Properties

Groundwater properties were observed for the BTEX degradation experiments with the consortium and not observed for *Microbacterium esteraromaticum* and *Bacillus infantis* experiments, as no degradation occurred.

3.3.6.1 pH and Dissolved Oxygen Influence

Table 3.6 shows the pH and DO data collected from the degradation experiment. Across all experimental treatments and temperatures (15 °C, 28 °C, and 40 °C), pH values remained relatively stable and within a narrow range of 7.0–8.5. Groundwater control consistently exhibited slightly alkaline conditions (~8.4), whereas treatments containing microbial consortium ranged from 7.0 to 7.8. During aerobic BTEX biodegradation, microorganisms oxidize hydrocarbons to produce carbon dioxide and water. Dissolved CO_2 can form carbonic acid, which may slightly lower the pH. However, carbonate buffering generally prevents significant pH shifts in groundwater systems. Similar observations have been reported in microcosm studies examining petroleum hydrocarbon biodegradation, where pH remained relatively constant despite active microbial degradation due to the buffering capacity of groundwater matrices (Chapelle, 2000; Riedel, 2019). Groundwater studies investigating BTEX degradation have reported that microbial biodegradation occurs primarily within a near-neutral pH range, as extreme pH values inhibit microbial activity (Z. Liu et al., 2024).

Table 3.6. Observed pH and DO concentrations (mg/L) in the BTEX degradation consortium experiment

			GW	GW + Consortium	GW + BTEX	GW + BTEX + Consortium
15	pH	Day 1	8.40	7.06	7.89	7.12
		Day 10	8.35	7.52	7.93	7.55
	DO	Day 1	7.15	1.62	7.04	1.07
		Day 10	7.38	2.53	12.33	0.44
28	pH	Day 1	8.42	7.21	8.03	7.07
		Day 10	8.39	7.58	7.99	7.64
	DO	Day 1	7.20	2.66	6.80	1.74
		Day 10	6.67	1.91	8.90	3.04
40	pH	Day 1	8.48	7.30	8.07	7.34
		Day 10	8.47	7.75	8.02	7.78
	DO	Day 1	7.22	2.16	7.00	2.04
		Day 10	7.03	2.52	12.16	3.03

Dissolved oxygen concentrations showed distinct trends between treatments with and without microbial addition. Groundwater control treatments maintained relatively high DO concentrations (~7 mg/L), whereas treatments containing the microbial consortium exhibited substantially lower DO values. The lower DO concentrations in treatments with microorganisms are attributed to aerobic respiration during BTEX biodegradation. During this process, bacteria oxidize hydrocarbons and use oxygen as the terminal electron acceptor, producing CO_2 and water (Kaur et al., 2025). Aerobic degradation of aromatic hydrocarbons such as BTEX is known to require significant oxygen consumption. The relatively consistent DO levels observed in the groundwater controls indicate that abiotic oxygen consumption was minimal, confirming that the DO decreases observed in consortium treatments were primarily due to microbial respiration. Interestingly, DO values remained relatively similar across temperature treatments but consistently decreased when microbial consortium was present. This pattern suggests that oxygen consumption was primarily driven by microbial metabolism rather than by temperature alone. Although microbial activity typically increases with temperature within the mesophilic range, the availability of oxygen and substrate can limit respiration rates. Therefore, similar DO trends across temperatures likely reflect comparable metabolic oxygen demand across treatments rather than temperature-dependent effects on oxygen solubility.

3.3.6.2 Anion Trends during BTEX degradation experiments

The concentrations of the anions chloride (Cl^-), nitrate/nitrite (NO_3^-/NO_2^-), sulphate (SO_4^{2-}), and phosphate (PO_4^{3-}) were monitored throughout the consortium experiments to assess potential geochemical changes associated with microbial metabolism and BTEX degradation. Table D.1 and Figure 3.5 shows that anion concentrations remained relatively stable across the different temperature treatments (15 °C, 28 °C, and 40 °C), suggesting that temperature did not exert a direct effect on anion concentrations within the time scale of the experiment. Instead, variations in anion concentrations are more likely associated with microbial metabolic processes and groundwater geochemistry than with thermal effects alone, as evidenced by changes from day 1 to day 10.

Nitrate and nitrite had moderate fluctuations between treatments with and without consortium. Such variability is consistent with microbial utilization of nitrate as an alternative electron acceptor during hydrocarbon degradation. In oxygen-limited environments, microorganisms capable of degrading aromatic hydrocarbons may shift from aerobic respiration to denitrification, reducing nitrate to nitrite and ultimately to nitrogen gas. This metabolic shift is commonly observed in petroleum-contaminated aquifers once dissolved oxygen becomes depleted. Studies investigating natural attenuation of petroleum hydrocarbons have reported that nitrate reduction often accompanies microbial degradation of BTEX compounds, particularly in groundwater systems where oxygen concentrations decline due to microbial respiration (Barbaro et al., 1992; Chapelle, 2000; McDonough et al., 2020). The small changes in nitrate observed in

the present study, therefore, likely reflect localized microbial activity rather than temperature-driven geochemical processes.

Sulphate concentrations also remained relatively consistent across the temperature treatments, but they did increase from day 1 to day 10. This suggests that the increase in sulphate concentrations results from the oxidation of reduced sulphur compounds present in the groundwater matrix or in microbial biomass. When dissolved oxygen is available, sulphur-oxidizing microorganisms can convert reduced sulphur species such as sulphide (H_2S) or elemental sulphur into sulphate. This oxidation process can lead to gradual increases in sulphate concentrations in aerobic groundwater systems (Chapelle, 2000). With DO remaining detectable in several of the microcosm treatments, conditions may not have progressed sufficiently toward the strongly reducing environments required to stimulate significant sulphate reduction.

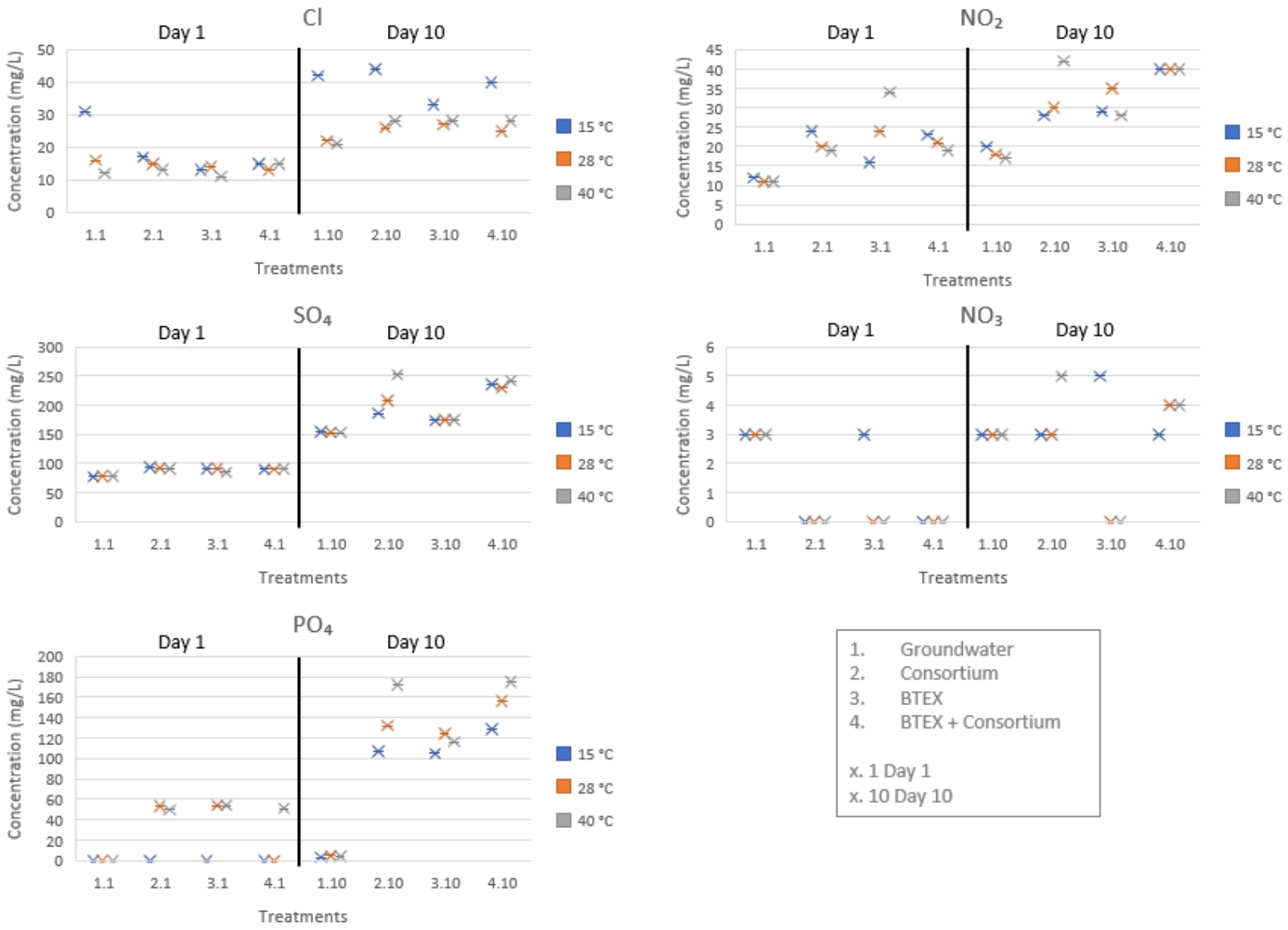


Figure 3.5. Anion plots by concentration vs temperature and time

Chloride concentrations had minimal variation across treatments and temperatures. This observation is expected because BTEX compounds do not contain chlorine, and thus their degradation does not produce chloride as a metabolic by-product. In groundwater, chloride concentration is largely unaffected by biological or chemical reactions (Nagpal, 2003). Phosphate concentrations had minor changes across treatments, which may be attributed to microbial nutrient uptake and release during biomass growth. Phosphorus is an essential nutrient required for microbial metabolism, cellular energy transfer, and nucleic acid synthesis (Nicholls et al., 2023). During hydrocarbon biodegradation, microorganisms often digest available phosphate to support cell growth and enzymatic activity. On the contrary, microbial life-death cycles and mineralization processes can release phosphate back into solution, resulting in small variations in measured concentrations (Richardson & Simpson, 2011).

Overall, the anion data suggest that temperature did not directly affect anion concentrations in groundwater. Instead, the differences appeared to be primarily controlled by microbial metabolic processes and the geochemical buffering capacity of the groundwater system. Temperature can influence microbial activity and degradation kinetics; however, it does not typically alter the concentrations of conservative ions or electron acceptors unless microbial metabolism drives redox transformations. The experiment was conducted over a relatively short duration, and oxygen remained present in several treatments; major shifts toward strongly reducing conditions were not observed. Consequently, the modest anion fluctuations recorded in this study are consistent with early-stage biodegradation processes occurring in a buffered groundwater environment.

3.4 Conclusions

This chapter investigated the influence of temperature on microbial growth and BTEX biodegradation in groundwater systems using two bacterial monocultures (*Bacillus infantis* and *Microbacterium esteraromaticum*) and a mixed microbial consortium. Groundwater collected from the Waterloo region was first characterized to establish baseline physicochemical and biological conditions prior to conducting biodegradation experiments. Groundwater characterization results indicated low microbial abundance, low organic matter content, and near-neutral pH, typical of natural groundwater environments. The low abundance of indigenous microorganisms confirmed the need to sterilize the groundwater before conducting controlled microbial growth and degradation experiments. Physicochemical parameters, including dissolved oxygen, total solids, volatile solids, and chemical oxygen demand, were consistent with values expected for relatively clean groundwater systems.

Microbial growth experiments demonstrated that temperature significantly influenced bacterial growth dynamics. At 15 °C, all microorganisms exhibited slower metabolic activity and reduced growth rates,

reflecting the limitations of mesophilic bacteria under colder conditions. At 28 °C, microbial growth was substantially enhanced, with all cultures entering exponential growth phases more rapidly and achieving higher biomass concentrations. In contrast, experiments conducted at 40 °C showed rapid initial growth followed by a gradual decline in biomass, suggesting that prolonged exposure to elevated temperatures induced physiological stress and reduced microbial stability. Among the tested microbial systems, the consortium consistently exhibited the highest biomass accumulation across all temperatures. This observation highlights the advantages of mixed microbial communities, where cooperative metabolic interactions allow microorganisms to collectively utilize substrates and tolerate environmental stressors more effectively than individual strains.

BTEX degradation experiments revealed that significant biodegradation occurred primarily in the microbial consortium cultures. Monocultures of *Bacillus infantis* and *Microbacterium esteraromaticum* showed little to no degradation beyond the losses observed in abiotic controls, indicating that volatilization was the dominant mechanism for BTEX removal in these treatments. In contrast, the microbial consortium demonstrated measurable degradation of toluene, ethylbenzene, and xylene, particularly at 28 °C, where toluene was completely degraded within 48 hours. Degradation rates were slower at 15 °C and negligible at 40 °C, indicating that mesophilic temperature conditions are optimal for BTEX biodegradation in the studied groundwater system. Benzene degradation was not observed within the experimental time frame, reflecting its known persistence and resistance to microbial breakdown. LC-MS analysis identified common metabolic intermediates generated during the degradation process, including phenolic compounds, aromatic acids, and catechol derivatives.

Temperature also influenced groundwater properties during the biodegradation experiments. Dissolved oxygen concentrations decreased significantly in treatments containing microbial consortium, confirming oxygen consumption during aerobic microbial metabolism. However, pH values remained relatively stable due to the groundwater's buffering capacity. Anion concentrations showed only minor fluctuations throughout the experiments, suggesting that short-term temperature variations had minimal direct influence on groundwater geochemistry.

Overall, the results demonstrate that temperature is a key environmental factor influencing microbial growth and BTEX biodegradation in groundwater systems. Mesophilic conditions near 28 °C supported the highest microbial activity and degradation efficiency, while colder temperatures slowed metabolic processes and higher temperatures induced microbial stress. Additionally, the superior performance of the microbial consortium highlights the importance of microbial community interactions in hydrocarbon biodegradation. These findings contribute to a better understanding of temperature-dependent biodegradation processes in groundwater environments and provide valuable insight for predicting natural

attenuation and designing bioremediation strategies for BTEX-contaminated aquifers, particularly in systems influenced by geothermal heating or subsurface temperature fluctuations.

Chapter 4: Bioremediation assessment of BTEX-contaminated soil using toxicology assay

4.1 Introduction

Industrial exploration, fuel storage, transportation, and accidental spills are all ways in which petroleum hydrocarbons contribute to global environmental pollution. Many countries, including Canada, are facing hydrocarbon contamination on land, surface water, air, and the subsurface (Lins, 2019; Mohanta et al., 2024; Treasury Board of Canada Secretariat, 2024; Wanjala et al., 2025; C. Xu et al., 2019). Bioremediation has emerged as an effective and environmentally sustainable strategy for the treatment of petroleum-contaminated soils, as microorganisms can metabolize aromatic hydrocarbons under both aerobic and anaerobic conditions. During microbial degradation, hydrocarbons are utilized as carbon and energy sources, initiating enzymatic oxidation reactions that ultimately convert these compounds into carbon dioxide, water, and biomass (Hernández-Ospina et al., 2024; Pandolfo et al., 2023). Aerobic degradation of BTEX typically begins with oxygenase-mediated hydroxylation reactions that produce common intermediate compounds such as benzyl alcohol, benzaldehyde, benzoic acid, cresols, and catechol derivatives. These intermediates are subsequently metabolized through central aromatic degradation pathways, which generate metabolites such as cis,cis-muconate and 3-oxoadipate before entering central carbon metabolism (Wu et al., 2023). Under anaerobic conditions, BTEX degradation may proceed through alternative activation mechanisms such as fumarate addition reactions, producing intermediates like benzyl succinate and phenylitaconate before further transformation (Hernández-Ospina et al., 2024).

Although bioremediation can substantially reduce concentrations of parent BTEX compounds, incomplete degradation may lead to the accumulation of toxic intermediate metabolites. Aromatic compounds such as phenol, catechol, cresols, and benzaldehyde have been identified as common intermediates during BTEX biodegradation and may exhibit toxic effects on microorganisms and plants at elevated concentrations (Hernández-Ospina et al., 2024; Wu et al., 2023; H. Yu et al., 2001). Catechol is a common microbial metabolite formed during benzene and toluene degradation, and it has been shown to exhibit greater microbial toxicity than the parent compound benzene, with reported EC_{50} values of approximately 0.77 mg/L compared with 39.9 mg/L for benzene in bacterial bioassays and in vitro studies (Boyd et al., 1997; Wan & Winn, 2004). Consequently, removal or reduction of the original hydrocarbon concentrations does not necessarily guarantee that remediated soils are free from toxic effects.

For effective bioremediation assessment, biological toxicity assays are widely employed to evaluate the ecological safety of soils following remediation treatments. Seed germination and early seedling growth assays represent a widely accepted ecotoxicological approach because early stages of plant development are highly sensitive to environmental contaminants (Cruz et al., 2013; Gong et al., 2001; OECD, 2006). Germination tests provide rapid indicators of phytotoxicity by measuring parameters such as germination percentage, root elongation, and shoot growth. These bioassays are valuable because they assess the combined biological effects of complex mixtures of residual contaminants and transformation products that may be present after remediation.

Wheat (*Triticum aestivum*) is frequently used in phytotoxicity testing due to its rapid germination, well-characterized physiology, and sensitivity to environmental stressors, and environmental regulatory organizations recommend it for evaluating the chronic effects of soil contaminants (Du et al., 2025; Khaeim et al., 2022; Khan et al., 2018; Salisbury et al., 1987). Wheat seedlings respond rapidly to chemical stress through measurable changes in germination, root elongation, and shoot development, making them useful indicators of soil quality and contaminant toxicity. In addition, wheat produces sufficient biomass within short experimental periods, allowing for both morphological measurements and chemical analysis of contaminant uptake (Du et al., 2025). Plants growing in contaminated soils may absorb organic pollutants through their root systems, providing insight into the potential transfer of contaminants or degradation products into biological systems and the broader food chain (Du et al., 2025; Khan et al., 2018).

This study aimed to evaluate the bioremediation efficiency of BTEX-contaminated soil using a seed germination bioassay. Wheat seeds were grown in remediated soil samples containing varying residual concentrations of BTEX and potential biodegradation byproducts. Germination success, root and shoot growth, and early seedling development were monitored over 14 days to assess potential phytotoxic effects. In addition, BTEX concentrations in both soil and plant tissues were quantified using gas chromatography–mass spectrometry (GC-MS) to determine the persistence of aromatic hydrocarbons and evaluate potential contaminant uptake by plants. By integrating chemical analysis with biological toxicity testing, this study provides a comprehensive assessment of the ecological safety of soils following BTEX bioremediation.

4.2 Materials and Methods

4.2.1 Soil Samples

The soil samples used for the germination study were collected after a bioremediation study performed by the Inzymes group at York University using the same microbial consortium described and used in Chapter 3 (Kaur et al., 2023). The thermally enhanced bioremediation process was conducted in stainless steel

columns (15 cm x 3.5 cm) filled with silty loam soil. The columns were monitored to assess the effects of cyclic temperatures (5 °C to 40 °C) on bacterial growth and BTEX degradation over 18 days. The treatment columns were initially supplemented with 200 mg/L of a BTEX solution and 2% bacterial cells, at a flow rate of 1 mL/min. Each temperature point (10 °C intervals) was maintained for 48 h, and injections of bacteria and the contaminant were administered every 48 h (Kaur, 2026). The control columns were kept at 12 °C, which is the average annual temperature of the shallow subsurface. Table 4.1 presents the physicochemical properties, determined by another member of the group, and the remaining fractions of BTEX detected in the treated soil. The soil samples were labelled as SS1 (soil sample 1), SS2, and SS3. SS3 was virgin, non-contaminated soil and was used as a control sample to validate seedling growth relative to seeds grown in BTEX-contaminated soil. SS1 had lower BTEX concentrations than SS2.

Table 4.1. Soil characterization and BTEX concentration in soil samples after remediation (Kaur, 2026)

Physicochemical property			
Soil type		Silty loam	
pH		7.6	
Clay content		13%	
Silt content		56%	
Sand content		29%	
Moisture content		13.80%	
Plasticity index		10.1	
Nitrate		76 mg/L	
Sulphate		184 mg/L	
Ferric		1.20%	
Fraction of organic carbon (foc)		0.021	
Cation exchange capacity (CEC)		2.15 mg/kg	
Heavy metals		Not detected	
BTEX concentration in soil samples			
	SS1	SS2	SS3
Benzene	14.5 mg/kg	67.5 mg/kg	0 mg/kg
Toluene	26.5 mg/kg	72.4 mg/kg	
Ethylbenzene	30.1 mg/kg	52.8 mg/kg	
m-, p-, o- Xylene	30.7 mg/kg	42.3 mg/kg	

Soil type played a significant role in controlling bioremediation processes in this study. The silty loam soil, characterized by moderate clay content (13%) and a high silt fraction (56%), provided a balance between contaminant retention and microbial accessibility, retaining high moisture due to its fine-grained, small-pore structure. Clay minerals contributed to the sorption of contaminants and nutrients, reducing bioavailability, explaining the low foc value, while also influencing microbial activity and redox conditions. However, the fine-grained nature of the soil limited oxygen and nutrient transport, constraining degradation of more recalcitrant compounds such as benzene. Under thermally enhanced conditions, elevated temperature stimulated microbial metabolism and contaminant desorption, but the influence of clay and silt continued to restrict mass transport. These findings highlight that soil texture, particularly the presence of clay, plays a critical role in governing the efficiency of thermally enhanced bioremediation.

4.2.2 Seed Germination

4.2.2.1 Seed selection

Several plant species have been recommended in protocols and literature for phytotoxicity testing due to their sensitivity to environmental contaminants and rapid germination rates. Among these species, lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum*) are frequently used as model organisms in soil toxicity assays (M. K. Banks & Schultz, 2005; Miri et al., 2022; OECD, 2006; Valerio et al., 2007). Lettuce is commonly used in standardized soil toxicity protocols because it is highly sensitive to soil contaminants and produces measurable responses in germination and root elongation assays. For example, lettuce germination tests have been widely applied to evaluate soil phytotoxicity associated with heavy metals and other pollutants (M. K. Banks & Schultz, 2005). Additionally, studies have demonstrated that soil contaminants can significantly influence wheat germination and early seedling growth, making it a useful indicator species for evaluating soil toxicity.

The agricultural relevance of wheat and lettuce in Canada also informed the selection of plants for this study. Wheat is one of the most widely cultivated cereal crops in Canada and is commonly grown across a variety of soil types. Lettuce is also an economically important crop grown in Canadian agricultural systems. These commonly cultivated crops provide environmentally relevant information regarding the potential impact of soil contamination on agricultural productivity in Canada.

Before the main experiment, preliminary germination trials were conducted to evaluate the germination of wheat, lettuce, barley, and oats. The seed supplier, C&M Seeds, an agricultural seed supplier located in southwestern Ontario that provides certified crop seeds for research and commercial agricultural use, provided samples for barley, oats, and wheat. These preliminary tests included sterilizing the seeds and growing them on Whatman filter paper in Petri dishes. The filter paper was saturated with deionized water

to provide moisture for seed germination. Wheat showed 100% germination rate, while lettuce showed the fastest germination rate within 2 days. Wheat was selected for further experiments because it produced more biomass, was easier to work with, and had the highest germination rate. Table E.1 in Appendix E has the results from this preliminary trial.

4.2.2.2 Seed sterilization

Seeds were surface sterilized using a modified standard sterilization protocol. Similar sterilization procedures using ethanol, followed by sodium hypochlorite and sterile water rinses, have been used in seed germination to eliminate surface microorganisms before experimentation (Dos Santos et al., 2020; Sauer & Burroughs, 1986; Varasteh et al., 2015). 70% ethanol and 1.2% sodium hypochlorite were prepared in separate flasks. The sterilization was performed in a UV-sterilized laminar air flow cabinet. Seeds were wrapped in cheesecloth and immersed in 70% ethanol in a 50 mL beaker for 1 min. Afterwards, the excess ethanol was squeezed from the cheesecloth and immersed in 1.2% sodium hypochlorite for 12 min. The seeds were then rinsed three times with sterile deionized water to remove residual disinfectant.

4.2.2.3 Petri dish plating

In the laminar airflow cabinet, 100 g of soil was weighed and plated into a 100 mm by 17 mm Pyrex petri dish. Pyrex dishes were selected for the study to prevent BTEX from adsorbing to the plates. After sterilization, plump, uniform, undamaged seeds were selected for plating in soil. Each dish had 12 seeds, and all seeds were spaced about 10 mm apart, to provide space for germination (Miri et al., 2022). The dishes were immediately covered with the corresponding Pyrex cover and placed in the enclosed environment described in Section 4.2.2.4. All treatments were plated in Pyrex dishes with glass lids; the control set used a glass vessel of equivalent volume and lid to maintain an identical headspace. The glass lids minimized volatilization loss.

4.2.2.4 Germination set-up/conditions

The Petri dishes containing the seeded soil samples were placed inside a controlled growth chamber to maintain stable environmental conditions for seed germination and early seedling development. The chamber dimensions were 24 in × 21 in × 12 in (L × W × H), and the interior was lined with aluminum foil to help stabilize internal temperature and distribute light more uniformly. An image of the chamber setup is provided in Figure 4.1. The chamber was located inside a laboratory fume hood with proper ventilation during the experimental period to prevent exposure to BTEX. The temperature within the chamber was maintained at 25 ± 1 °C with a relative humidity of 20% to 30%, which falls within the optimal range for wheat (*Triticum aestivum*) germination and early seedling growth. Previous studies have shown that *Triticum aestivum* seeds typically germinate most effectively at temperatures between 20–30 °C, with

approximately 25 °C commonly used in laboratory germination experiments (Khaeim et al., 2022; Salisbury et al., 1987; Siddiqui, 2008).

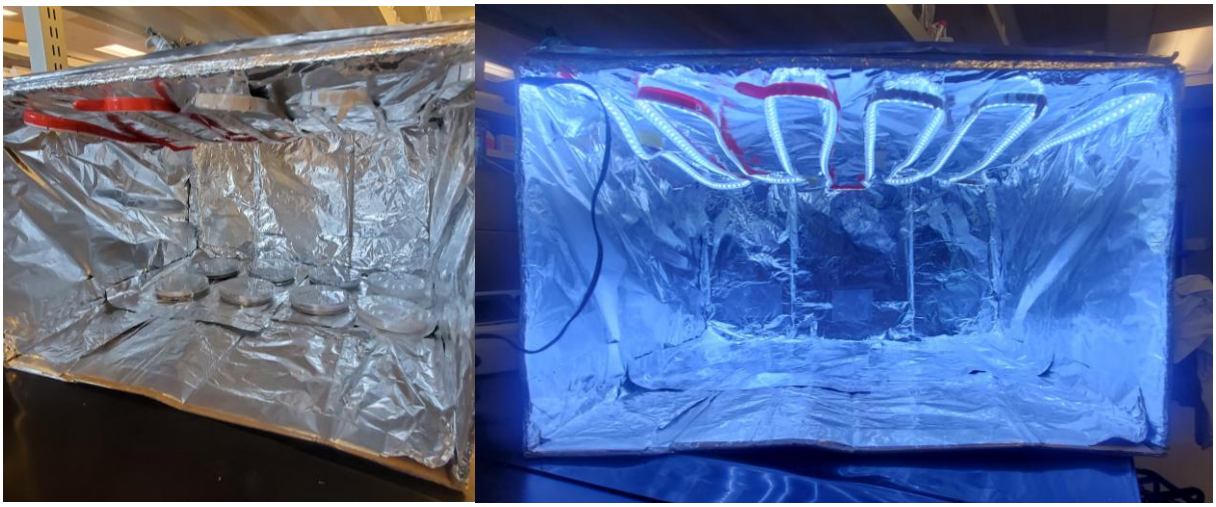


Figure 4.1. Growing chamber used for seed germination study, unlit and lit

Illumination was provided using white LED lights installed within the chamber. The photoperiod was maintained at 16 hours of light and 8 hours of darkness, with the dark period occurring between 9:00 AM and 5:00 PM to simulate typical plant growth conditions used in controlled laboratory experiments (OECD, 2006). The LED lights had a wavelength of 451 nm (blue light). Light intensity was measured with a light meter and recorded as 33 ± 3 μmol m^{-2} s^{-1}, corresponding to photosynthetic photon flux density (PPFD). Additional spectral measurements were obtained using a FLAME miniature spectrometer. The mean radiant intensity within the chamber was measured as 7.06 × 10^2 ± 15 μW cm^{-2}. Blue wavelengths within the 450 nm range are known to influence plant photoreceptors, which regulate early seedling development and photomorphogenic responses (Kang et al., 2008; P. Xu et al., 2009). Additionally, Petri dishes were randomly arranged within the chamber and periodically rotated to minimize potential variability in light exposure.

Soil moisture within the Petri dishes was monitored periodically throughout the experiment to ensure adequate hydration for seed germination. When the soil surface appeared dry, 1 mL of deionized (DI) water was added to maintain sufficient moisture conditions. Maintaining adequate moisture is critical for seed germination, as water uptake initiates metabolic activity and radicle emergence. All environmental conditions, including temperature, photoperiod, and light intensity, were kept consistent across treatments to ensure that any observed differences in germination or seedling growth could be attributed to BTEX exposure rather than variations in growth conditions.

4.2.3 Evaluation of Germination, Growth, and BTEX Concentrations

4.2.3.1 Germination and Growth Assessment

Seed germination and seedling growth were evaluated throughout the experimental period using both qualitative observations and quantitative measurements. Observations were conducted at three time points: the day of sowing (Day 0), midway through the study (Day 8), and at the end of the experiment (Day 14). At each time point, seeds and developing seedlings were visually inspected to document morphological characteristics associated with germination and early plant development. Qualitative observations included the emergence of the radicle, development of root hairs, plumule sprouting, and the formation of shoots and leaves. In addition, the overall condition of the seedlings was assessed, including signs of discoloration, abnormal growth, or other morphological changes that may indicate stress caused by BTEX exposure. Photographs were taken during each observation period to document seed and seedling development over the 14-day study period and to facilitate comparisons of morphological responses among treatments.

Quantitative analysis included measurements of germination percentage, germination rate, and seedling growth parameters. Germination percentage was determined on Days 8 and 14 by counting the number of seeds that had successfully germinated relative to the total number of seeds planted (n = 12). A seed was considered germinated when the radicle visibly emerged from the seed coat. The rate of germination was calculated based on the number of seeds germinated within the observation period (8 or 14 days), allowing for comparison of germination timing among treatments. At the conclusion of the experiment (Day 14), root and shoot lengths were measured for each germinated seedling. Figure 4.2 demonstrates the typical plant structure of wheat. Root and shoot length were measured separately from the base of the seed to the tip of the primary root and from the seed to the tip of the shoot, respectively, using a measuring tape. These measurements were used to evaluate the effects of BTEX exposure on early seedling growth and development.

Figure 4.2. Plant structure of *Triticum aestivum* (wheat)

4.2.3.2 BTEX Concentration Analysis

BTEX concentrations in both soil and plant tissues were analyzed at the conclusion of the 14-day experimental period to evaluate contaminant persistence in the soil and potential uptake by germinated seedlings. Before sampling, the soil in each Petri plate was visually inspected to assess contamination and overall soil condition. At Day 14, representative seedlings were collected from each experimental plate for further analysis. Depending on the growth per plate, 1-2 seedlings were selected from each Petri plate. The seedlings were carefully removed from the soil, placed on tissue paper, photographed, and measured for root and shoot length. They were then gently rinsed with deionized (DI) water to remove any adhering soil particles.

Following the initial measurements, the plant tissues were mechanically homogenized to prepare samples for BTEX analysis. The roots, shoots, and leaves were crushed and weighed before being transferred to headspace vials for gas chromatography (GC). The purpose of analyzing the root and shoot tissues was to determine whether uptake or accumulation of BTEX compounds had occurred within the plant tissues during the experimental period.

We normalized BTEX concentrations to sample mass (mg/kg). Plant tissues (0.05- 0.20 g) and soil (0.01-0.20 g) were weighed into 20 mL headspace vials with 5 mL DI and fluorobenzene-d_5 internal standard. An internal standard was used to assess the reproducibility. Samples exceeding the calibration range were diluted prior to analysis. Concentrations were back-calculated to mg/kg using the recorded mass. The vials were immediately sealed using crimp caps to prevent volatilization of the target compounds prior to analysis.

Gas Chromatography Analysis

BTEX concentrations were analyzed using a Gas Chromatography–Flame Ionization Detector (GC–FID) system (Agilent 7820A, Agilent Technologies) equipped with a Solid Phase Micro-Extraction (SPME) headspace sampler (Clarus 500, PerkinElmer, USA) at York University, Ontario. Separation of the BTEX compounds was achieved using an Agilent J&W CP-Sil 5 CB GC column (30 m × 0.25 mm × 1.00 µm). Prior to injection, sample vials were heated and agitated for 10 minutes at 80 ± 1 °C to promote volatilization of BTEX compounds into the headspace. The headspace system was maintained at 90 ± 1 °C for the needle and 120 ± 1 °C for the transfer line. The samples were automatically injected onto the GC column using the autosampler. Solid-phase microextraction was performed at 40 °C for 24 minutes before analysis. Helium was used as the carrier gas throughout the chromatographic separation. The resulting chromatograms were analyzed to quantify BTEX concentrations in both soil and plant tissue samples.

4.2.4 Data Processing and Concentration Calculations

Quantification of BTEX compounds was performed using a calibration curve generated from known standard concentrations, in Appendix E, Figure E.1- E.5. A series of BTEX standards with known concentrations was analyzed using the same GC–FID conditions to establish a calibration curve relating peak area and retention time to compound concentration. The retention times and peak areas of the soil and plant tissue samples were then compared with the calibration curve to determine the corresponding BTEX concentrations in each sample.

To account for variations in sample mass, dilution factors were applied to calculate the final contaminant concentrations. The measured GC concentration values were converted to soil or plant tissue concentrations using the following equation:

$$C_{final} = \frac{C_{GC} \times V_{water}}{M_{sample}}$$

where:

- C_{final}= final BTEX concentration in the sample (mg/kg soil or mg/kg plant tissue)

- C_{GC}= concentration obtained from the GC calibration curve (µg/L)
- V_{water}= volume of distilled water used in the GC vial (L)
- M_{sample}= mass of the soil or plant tissue sample placed in the vial (g)

This calculation accounted for dilution during sample preparation and allowed BTEX concentrations to be expressed in standardized units of mg/kg soil or mg/kg plant tissue.

4.3 Results and Discussion

4.3.1 Soil Characteristics

The soil, Table 4.1 was classified as silty loam, with silt having the dominant fraction of 56% (Kaur, 2026). The composition included other fine particles, sand and clay, which influence contaminant behaviour in soil through sorption capacity. Soils with a low clay content exhibit a lower adsorption capacity, and greater contaminant mobility (H. Yu et al., 2014). Moreover, the relatively low clay content (13%) and low cation exchange capacity (2.15 mg/kg) indicated a limited ability for the soil to retain positively charged ions and contaminants which reduces the sorption potential and allows BTEX compounds to remain more mobile within the soil matrix (Nguyen & Marschner, 2016; Schönsee et al., 2021). The pH (7.6) and the soil moisture content of 13.8% supports the conditions for microbial growth and hydrocarbon biodegradation (Chandra et al., 2013). Additionally, the presence of the electron acceptors such as nitrate (76 mg/L), sulphate (184 mg/L), and ferric iron (1.20%) suggested that multiple microbial respiration pathways can occur when oxygen becomes limited. Microorganisms can utilize nitrate, sulphate, and ferric iron as alternative electron acceptors during the degradation of hydrocarbons, which can explain the difference in the BTEX concentrations in the soil, SS1 and SS2 (K. Zhang et al., 2019; Zhu et al., 2020).

4.3.2 Seed Germination and Growth

Seed germination and early seedling development were monitored over a 14-day period to evaluate the potential phytotoxic effects of residual BTEX compounds in the remediated soil samples. Germination observations were recorded at Day 0, Day 8, and Day 14, and are summarized in Table 4.2 and Table 4.3. Wheat seeds demonstrated germination in all soil samples, differences in germination rate and seedling growth were observed between contaminated soils and the uncontaminated control soil (SS3).

In the control soil, germination occurred rapidly, with 91.7% germination observed in 8 days, producing healthy seedlings with well-developed root systems and shoots. By Day 14, germination reached 100% and the seedlings revealed a root length of 5.6 cm, and a shoot length of 21.6 cm. 8 out of 12 germinated seeds

developed shoots with 2 leaves, indicating that the seeds entered the seedling growth phase, but had not reached the tillering phase (> 3 leaves) (Grains Research & Development Corporation, 2016)(Grains Research & Development Corporation, 2016).

Seedlings grown in soils containing residual BTEX compounds exhibited reduced germination and variability in seedling morphology compared with the uncontaminated control soil. SS1, which contained the lower initial BTEX concentration, resulted in a higher germination rate, 50% by Day 8 and increased to 66.7% by Day 14, than SS2. Seedlings grown in SS1 exhibited a root length of 5.0 cm and a shoot length of 14.5 cm. Although shoot growth was somewhat reduced compared with the control soil, root development remained relatively similar. In contrast, SS2 contained higher initial BTEX concentrations and had the lowest germination rate. Germination reached only 16.7% by Day 8 and increased to 33.3% by Day 14. Seedlings grown in SS2 also displayed reduced root development, with an average root length of 1.8 ± 0.4 cm, while shoot length reached 18.2 cm. These results indicate that elevated BTEX concentrations may have inhibited early plant development, particularly root elongation.

Root growth is often the most sensitive indicator of phytotoxic stress because the root system is the first plant structure to interact directly with soil contaminants (Ranucci et al., 2024). Organic pollutants are toxic, and exposure can cause oxidative stress in seedlings that can inhibit cell division and elongation in root tissues. This leads to reduced root length and impaired nutrient uptake, which can explain the short root length in SS2 (Wei et al., 2013). Similar inhibitory effects on root growth have been reported in previous studies examining plant responses to polycyclic aromatic hydrocarbons (PAHs), where exposure to compounds such as phenanthrene significantly reduced root length in wheat seedlings (Wei et al., 2013; Zeng et al., 2025).

Previous studies examining hydrocarbon-contaminated soils have reported similar inhibitory effects on seed germination and root development. For example, petroleum hydrocarbons and aromatic compounds present in contaminated soils have been shown to significantly reduce germination rates and seedling growth in various plant species, including cereals and grasses (Adam & Duncan, 2002; Haider et al., 2021). Experiments investigating the phytotoxicity of benzene and toluene in contaminated soils have also demonstrated reductions in seed germination and early plant survival, confirming the sensitivity of seedlings to volatile aromatic hydrocarbons (Adam & Duncan, 2002; M. K. Banks & Schultz, 2005; Masakorala et al., 2013; Wei et al., 2013)

Although germination still occurred in SS2, the reduced germination percentage and shorter root length observed in this treatment suggest that higher BTEX concentrations exerted measurable phytotoxic effects on early plant development. Nevertheless, wheat seeds germinated across all soil treatments, indicating that

the residual contaminant concentrations following bioremediation were not sufficiently high to completely inhibit germination.

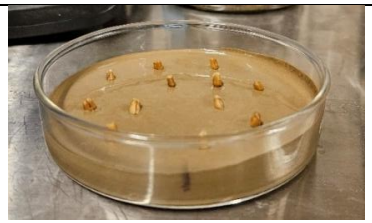
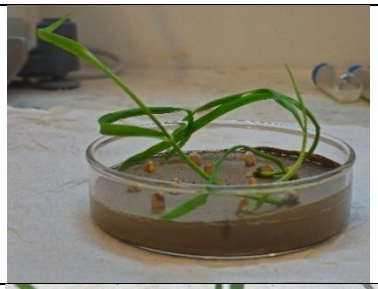
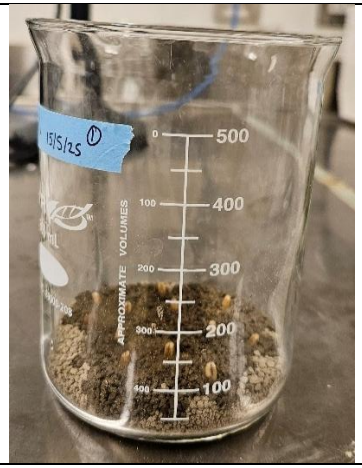
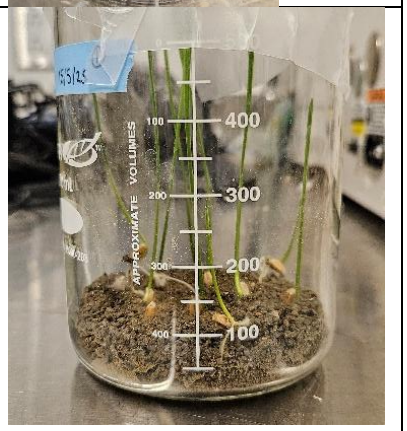
Table 4.2. Summary table of the seed germination results

	Soil initial concentration (mg/kg)		Soil final concentration (mg/kg)		Seed final concentration (mg/kg)	
	SS1	SS2	SS1	SS2	SS1	SS2
Benzene	14.5	67.5	0.0	0.0	0.0	0.0
Toluene	26.5	72.4	0.0	0.0	0.0	0.0
Ethylbenzene	30.1	52.8	3.9	5.3	1.2	2.3
m-, p-, o- Xylene	30.7	42.3	2.8	2.8	1.3	1.2
Wheat germination rate, root and shoot length						
	SS1		SS2		SS3	
Day 8 Germination Rate	50%		16.7%		91.7%	
Day 14 Germination Rate	66.7%		33.3%		100%	
Root and Shoot length of sample in GC vial	Root: 5.0 ± 0.3 cm* Shoot: 18.2 cm		Root: 1.8 ± 0.4 cm Shoot: 14.5 cm		Root: 5.6 ± 0.3 cm Shoot: 21.6 cm	

*Not all germinated seeds had multiple root hairs available to determine a standard deviation, as some hairs broke off during the extraction from the silty loam

Table 4.3. *Triticum aestivum* seed germination and early seedling development in remediated soil samples and control soil over a 14-day period. Representative photographs and germination percentages are shown for Day 0, Day 7, and Day 14

		May 15th: Day 0	May 23rd: Day 8		May 29th: Day 14	
		Photos	Photos	Germination Rate	Photos	Germination Rate
SS1	Top view			50%		66.7% Final day notes - 8/12 germinated seeds had 2 leaves - average shoot lengths >15 cm
	Side view					
SS2	Top view			16.7%		33.3% Final day notes - 3/12 germinated seeds had 2 leaves - average shoot

		May 15th: Day 0	May 23rd: Day 8		May 29th: Day 14	
		Photos	Photos	Germination Rate	Photos	Germination Rate
	Side view					lengths >15 cm
SS3	Top view			91.7%		100% Final day notes - 8/12 germinated seeds had 2 leaves - average shoot lengths >20 cm
	Side view					

4.3.3 BTEX Concentrations in Soil and Plant Tissues

Table 4.2 includes the BTEX concentrations measured in both soil and plant tissues after the 14-day growth period to evaluate contaminant persistence and potential uptake by the wheat seedlings. The analytical results indicated that benzene and toluene were not detected in the soil or plant tissue samples at the conclusion of the experiment, despite initial soil concentrations ranging from 14.5–67.5 mg/kg for benzene and 26.5–72.4 mg/kg for toluene. In contrast, ethylbenzene and xylenes remained detectable in soil samples, with final concentrations of 3.9– 5.3 mg/kg for ethylbenzene and approximately 2.8 mg/kg for total xylenes. This suggests that benzene and toluene were removed more rapidly than ethylbenzene or xylene during the experiment.

The absence of benzene and toluene in the final soil samples is consistent with the well-documented environmental behaviour of these compounds. Benzene and toluene are generally less persistent in soils than ethylbenzene and xylene due to their higher volatility and greater susceptibility to microbial degradation under aerobic conditions (Braddock & McCarthy, 1996). As a result, these compounds are often depleted earlier during remediation processes and may be present at lower residual concentrations following treatment. Previous studies examining BTEX-contaminated soils have reported that benzene and toluene typically exhibit faster degradation rates and shorter environmental half-lives than ethylbenzene and xylenes, which tend to persist longer due to slightly greater hydrophobicity and stronger sorption to soil organic matter (Environment and Climate Change Canada (ECCC), 2024; Hernández-Ospina et al., 2024). Consequently, their absence in the post-treatment soil samples used in the germination assay likely reflects differences in compound persistence rather than processes occurring during the germination experiment itself.

4.3.4 Plant Uptake of BTEX Compounds

Trace concentrations of BTEX compounds detected in plant tissues indicate that wheat seedlings could absorb hydrocarbons from the contaminated soil. In the assay, ethylbenzene concentrations of 1.2–2.3 mg/kg and xylenes concentrations of 1.2–1.3 mg/kg were detected in plant tissues, while benzene and toluene were not detected. This suggests that some BTEX compounds were taken up by the plants, but that uptake and accumulation were relatively limited. Plants can absorb organic contaminants through their root systems when compounds are sufficiently small and moderately hydrophobic. BTEX compounds have log $K_o w$ values between 1.56 and 3.53, a range that exhibits root uptake and partial translocation through plant tissues (Namiki, 2022). Plant uptake of BTEX was minor relative to microbial removal and volatilization, and the root suppression observed likely reflects residual toxicity from possible intermediates highlighted in Section 3.3.5.

Previous studies have demonstrated that aromatic hydrocarbons can enter plant tissues via both root uptake and atmospheric exposure. Experimental research using benzene and toluene showed that these compounds can penetrate plant leaves and be metabolized within plant tissues, where they are transformed into organic acids and incorporated into plant metabolites (Ugrekhelidze et al., 1997). These findings indicate that plants can absorb and metabolize monocyclic aromatic hydrocarbons once they enter plant tissues. Field and laboratory studies examining phytoremediation systems have also reported that BTEX compounds can be detected in plant transpiration streams when plants grow in contaminated environments. For example, research investigating hybrid poplar trees at hydrocarbon-contaminated sites detected toluene within plant tissues and demonstrated that plants can contribute to the transport of BTEX compounds within vegetated systems (BenIsrael et al., 2025). However, direct plant uptake generally represents only a minor pathway for BTEX removal compared with microbial degradation occurring in the rhizosphere. Investigations of vegetated remediation systems have shown that plant roots primarily enhance contaminant removal by stimulating microbial communities that degrade petroleum hydrocarbons in the surrounding soil. Root exudates and oxygen release from plant roots can increase microbial activity and support the degradation of aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (Simmer & Schnoor, 2022).

Consequently, the trace BTEX concentrations detected in wheat tissues in the present study suggest that, while some uptake occurred, plant accumulation likely accounted for only a small fraction of the total contaminant reduction observed during the earlier soil treatment process. Instead, the primary mechanism responsible for the reduction of BTEX concentrations in the soil was likely microbial degradation stimulated by the soil microbial community during remediation, and the root suppression observed was possibly from toxic intermediates produced from the catechol-pathway products.

4.4 Conclusions

This study evaluated the phytotoxicity of soils following BTEX bioremediation using a wheat seed germination assay. The results demonstrate that remediated soils supported successful seed germination and early seedling development, indicating that the bioremediation process substantially reduced contaminant toxicity. This study applies only to early stage phytotoxicity. Soil texture and type strongly impacted the outcome of the bioassay. Soil characterization showed that the silty loam texture and relatively low clay content contributed to increased contaminant mobility and bioavailability within the soil matrix. Environmental conditions maintained during the experiment, including temperature, light intensity, and soil moisture, supported optimal germination conditions for wheat seedlings.

Although germination rates remained moderate across all treatments, differences in seedling growth parameters suggested that residual contaminants may still exert minor phytotoxic effects on early plant

development. Root elongation appeared to be the most sensitive growth parameter, which is consistent with previous studies evaluating plant responses to hydrocarbon-contaminated soils. Chemical analysis of soil and plant tissues revealed that benzene and toluene were not detected after the 14-day experimental period, likely due to volatilization and microbial degradation. However, the detection of BTEX compounds in plant tissues suggests that residual contaminants remained bioavailable and capable of being absorbed by plant roots.

Overall, the results indicate that while bioremediation significantly reduced BTEX contamination in silty loam soil, trace levels of hydrocarbons may persist and remain biologically accessible. Seed germination assays, therefore, provide a valuable complementary approach for evaluating the ecological safety of remediated soils. Additionally, common crop plants can be grown on sites with very low levels of trace contaminants.

Chapter 5: Conclusions and Recommendations for Future Work

This study investigated the effects of temperature on BTEX biodegradation in groundwater and evaluated the ecological safety of remediated soils using a wheat seed germination bioassay. The research addressed an important knowledge gap by examining an integrated assessment of BTEX biodegradation with geothermal heating and post-bioremediation impacts. The novelty of this thesis resides in the investigation of these two technologies with monocultures, a consortium, and observing the effects on groundwater chemistry, as there are limited studies.

Toluene was completely removed within 48 h at 28 °C; xylene $t_{½} \approx 96$ h; ethylbenzene $t_{½} \approx 298$ h in the consortium. The results showed that temperature plays an important role in influencing microbial activity and the degradation behaviour of BTEX compounds. Changes in groundwater physicochemical parameters, including pH, dissolved oxygen, and anion concentrations, were investigated under different thermal conditions, and the results showed that temperature can alter microbial metabolic functions and contaminant transportation pathways. This evidence suggests that thermal inputs from geothermal systems may affect both contaminant degradation rates and groundwater chemistry within subsurface environments.

The phytotoxicity study further demonstrated that soils subjected to bioremediation could support wheat seed germination and early seedling growth, indicating that the remediation process reduced contaminant toxicity. Post-remediation, SS1 had a lower BTEX concentration than SS2, and the germination rates were 66.7% and 33.3%. Differences in root elongation and seedling growth in the soil treatments suggested that trace levels of residual contaminants may still have minor phytotoxic effects, as SS1 had a longer root length than SS2, by 3.2 cm. Root growth was identified as the most sensitive indicator of residual toxicity, which was consistent with previous studies evaluating plant responses to hydrocarbon-contaminated soils. The chemical analysis showed that some BTEX compounds were undetectable at the end of the experimental period, likely due to volatilization and microbial degradation. Nevertheless, the detection of trace hydrocarbons in plant tissues indicates that residual contaminants may remain bioavailable and capable of being absorbed by plant roots. These results emphasize the importance of integrating biological toxicity assessments with chemical measurements when evaluating remediation success.

Overall, this research indicates that bioremediation can significantly reduce BTEX contamination and restore soil conditions appropriate for plant growth. At the same time, the study highlights the importance of evaluating both contaminant transformation processes and ecological responses when assessing remediation technologies. By combining temperature-controlled biodegradation experiments with

ecotoxicological bioassays, this research offers a more comprehensive approach for evaluating the environmental impacts of subsurface remediation strategies.

Despite the promising outcomes of this research, certain limitations warrant consideration. Batch experiments were conducted in a controlled environment, which does not fully reflect the spatial and temporal variability of natural subsurface environments. Additionally, some limitations with the instruments occurred, as the temperature could not exceed 40 °C. The relatively short experimental duration limited the ability to observe long-term degradation, especially for benzene. Lastly, with the toxicology bioassay being conducted on collected bioremediated soil, while it mimics real-world engineering situations, it presents some unknowns, such as unidentified metabolites produced, the microbial community presence during and after the remediation, and any changes in geochemistry.

While this research provides insights into the interactions among temperature, microbial degradation, and ecological responses in BTEX-contaminated environments, several areas warrant further investigation. Future work should move beyond laboratory conditions and implement a field-scale pilot study to evaluate the feasibility of coupling geothermal heat pump (GHP) systems with bioremediation under realistic subsurface conditions. A testable next step would be to install a pilot GHP system at a BTEX-contaminated site and operate it under controlled cyclic temperature regimes that mimic seasonal heating and cooling. Groundwater monitoring wells should be installed to measure key parameters, such as BTEX concentrations, dissolved oxygen, redox conditions, pH, and microbial activity, over time. This design would allow direct comparison between steady-state and cyclic thermal conditions to quantify how temperature fluctuations influence contaminant degradation rates, plume dynamics, and microbial community responses.

A second recommendation is to further conduct metabolite toxicity assays. Investigating the metabolic degradation pathways of BTEX in plant systems, in the silty loam soil used in the germination assay, and in other soil types, would help determine whether the transformation products observed in groundwater are similarly produced in soil or if additional metabolites are formed due to plant–microbe interactions and soil-specific properties. Furthermore, evaluating how factors such as soil type, organic matter content, and microbial community composition influence degradation pathways would improve understanding of contaminant fate, persistence, and potential toxicity. Such research would also support the development of more effective phytoremediation and soil remediation strategies by identifying conditions that promote complete mineralization versus the accumulation of intermediate by-products.

Additionally, future research should develop and validate coupled reactive transport models that integrate thermal, hydraulic, and biogeochemical processes. A practical next step would be to calibrate a modelling

framework using tools such as MODFLOW for groundwater flow, MT3DMS for contaminant transport, and RT3D for biodegradation kinetics. These models should be parameterized using laboratory- and pilot-scale data, then used to simulate different operating scenarios, including temperature-cycling frequency, flow velocity, and soil heterogeneity. Model outputs can be validated against field observations to improve predictive accuracy and guide system design.

Despite the technical potential, several policy and implementation barriers may limit the adoption of GHP-assisted bioremediation. Regulatory frameworks for contaminated site management and geothermal energy systems are often developed independently, creating uncertainty around permitting and liability. Additionally, there may be limited guidance on how to account for remediation benefits within existing environmental assessment protocols. High upfront installation costs on brownfield sites, lack of standardized design guidelines, and uncertainty in long-term performance may also deter stakeholders. Addressing these barriers will require the development of integrated regulatory pathways, economic motivations, and clear design and monitoring standards to support the safe and effective implementation of combined geothermal–bioremediation systems.

References

Adam, G., & Duncan, H. (2002). Influence of diesel fuel on seed germination. *Environmental Pollution*, *120*(2), 363–370. https://doi.org/10.1016/S0269-7491(02)00119-7

Agency for Toxic Substances and Disease Registry (US). (2007). *Toxicological Profile for Benzene*. https://www.ncbi.nlm.nih.gov/books/NBK591287/

Alexander, M. (2000). Aging, Bioavailability, and Overestimation of Risk from Environmental Pollutants. *Environmental Science & Technology*, *34*(20), 4259–4265. https://doi.org/10.1021/es001069+

Allouhi, A., Saadani, R., Kousksou, T., Saidur, R., Jamil, A., & Rahmoune, M. (2016). Grid-connected PV systems installed on institutional buildings: Technology comparison, energy analysis and economic performance. *Energy and Buildings*, *130*, 188–201. https://doi.org/10.1016/j.enbuild.2016.08.054

Amanambu, A. C., Obarein, O. A., Mossa, J., Li, L., Ayeni, S. S., Balogun, O., Oyebamiji, A., & Ochege, F. U. (2020). Groundwater system and climate change: Present status and future considerations. *Journal of Hydrology*, *589*, 125163. https://doi.org/10.1016/j.jhydrol.2020.125163

Ambaye, T. G., Chebbi, A., Formicola, F., Prasad, S., Gomez, F. H., Franzetti, A., & Vaccari, M. (2022). Remediation of soil polluted with petroleum hydrocarbons and its reuse for agriculture: Recent progress, challenges, and perspectives. *Chemosphere*, *293*, 133572. https://doi.org/10.1016/j.chemosphere.2022.133572

Aronson, D., Howard, P., Citra, M., Shuler, K., & Printup, H. (1999). Aerobic Biodegradation of Organic Chemicals in Environmental Media: A Summary of Field and Laboratory Studies. *Environmental Science Center, Syracuse Research Corporation for American Petroleum Institute*.

Arthurs, P., Stiver, W. H., & Zytner, R. G. (1995). Passive volatilization of gasoline from soil. *Journal of Soil Contamination*, *4*(2), 123–135. https://doi.org/10.1080/15320389509383487

Asejeje, G. I., Ipeaiyeda, A. R., & Onianwa, P. C. (2021). Occurrence of BTEX from petroleum hydrocarbons in surface water, sediment, and biota from Ubeji Creek of Delta State, Nigeria. *Environmental Science and Pollution Research*, *28*(12), 15361–15379. https://doi.org/10.1007/s11356-020-11196-y

Atawneh, D. Al, Cartwright, N., & Bertone, E. (2021). Climate change and its impact on the projected values of groundwater recharge: A review. *Journal of Hydrology*, *601*, 126602. https://doi.org/10.1016/j.jhydrol.2021.126602

Aydinalp Koksal, M., Rowlands, I. H., & Parker, P. (2015). Energy, cost, and emission end-use profiles of homes: An Ontario (Canada) case study. *Applied Energy*, *142*, 303–316. https://doi.org/10.1016/j.apenergy.2014.12.077

Bakhtiari, R., Hadei, M., Hopke, P. K., Shahsavani, A., Rastkari, N., Kermani, M., Yarahmadi, M., & Ghaderpoori, A. (2018). Investigation of in-cabin volatile organic compounds (VOCs) in taxis; influence of vehicle's age, model, fuel, and refueling. *Environmental Pollution*, *237*, 348–355. https://doi.org/https://doi.org/10.1016/j.envpol.2018.02.063

Bala, S., Garg, D., Thirumalesh, B. V., Sharma, M., Sridhar, K., Inbaraj, B. S., & Tripathi, M. (2022). Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics*, *10*(8). https://doi.org/10.3390/toxics10080484

Banks, D. (2012). *An Introduction to Thermogeology: Ground Source Heating and Cooling* (2nd ed.). John Wiley & Sons.

Banks, M. K., & Schultz, K. E. (2005). Comparison of Plants for Germination Toxicity Tests in Petroleum-Contaminated Soils. *Water, Air, and Soil Pollution*, *167*(1–4), 211–219. https://doi.org/10.1007/s11270-005-8553-4

Barbaro, J. R., Barker, J. F., Lemon, L. A., & Mayfield, C. I. (1992). Biotransformation of BTEX under anaerobic, denitrifying conditions: Field and laboratory observations. *Journal of Contaminant Hydrology*, *11*(3–4), 245–272. https://doi.org/10.1016/0169-7722(92)90019-B

Barbieri, M., Barberio, M. D., Banzato, F., Billi, A., Boschetti, T., Franchini, S., Gori, F., & Petitta, M. (2023). Climate change and its effect on groundwater quality. *Environmental Geochemistry and Health*, *45*(4), 1133–1144. https://doi.org/10.1007/s10653-021-01140-5

Barla, M., Di Donna, A., & Baralis, M. (2020). City-scale analysis of subsoil thermal conditions due to geothermal exploitation. *Environmental Geotechnics*, *7*(4), 306–316. https://doi.org/10.1680/jenge.17.00087

Barnes, I. (2015). TROPOSPHERIC CHEMISTRY AND COMPOSITION | Aromatic Hydrocarbons. In G. R. North, J. Pyle, & F. Zhang (Eds.), *Encyclopedia of Atmospheric Sciences (Second Edition)* (pp. 204–213). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-382225-3.00424-2

Barron, M. G. (2012). Ecological Impacts of the Deepwater Horizon Oil Spill: Implications for Immunotoxicity. *Toxicologic Pathology*, *40*(2), 315–320. https://doi.org/10.1177/0192623311428474

Basu, A., Dixit, S. S., & Phale, P. S. (2003). Metabolism of benzyl alcohol via catechol ortho -pathway in methylnaphthalene-degrading Pseudomonas putida CSV86. *Applied Microbiology and Biotechnology*, *62*(5–6), 579–585. https://doi.org/10.1007/s00253-003-1305-8

Bates, B. C. ., Kundzewicz, Zbigniew., Wu, Shaohong., & Palutikof, Jean. (2013). *Climate change and water*. Intergovernmental Panel on Climate Change. https://www.taccire.sua.ac.tz/handle/123456789/552

Baud-Grasset, F., Baud-Grasset, S., & Safferman, S. I. (1993). Evaluation of the bioremediation of a contaminated soil with phytotoxicity tests. *Chemosphere*, *26*(7), 1365–1374. https://doi.org/10.1016/0045-6535(93)90187-A

Beller, H. R., & Spormann, A. M. (1998). Analysis of the novel benzylsuccinate synthase reaction for anaerobic toluene activation based on structural studies of the product. *Journal of Bacteriology*, *180*(20), 5454–5457. https://doi.org/10.1128/JB.180.20.5454-5457.1998

BenIsrael, M., Obregon, D., Wanner, P., Fernandes, J., Burken, J. G., Aravena, R., Parker, B. L., Haack, E. A., Tsao, D. T., & Dunfield, K. E. (2025). Active phytoextraction of toluene shifts the microbiome and enhances degradation capacity in hybrid poplar. *Journal of Environmental Management*, *373*, 123910. https://doi.org/10.1016/j.jenvman.2024.123910

Bethke, C. M., Sanford, R. A., Kirk, M. F., Jin, Q., & Flynn, T. M. (2011). The thermodynamic ladder in geomicrobiology. *American Journal of Science*, *311*(3), 183–210. https://doi.org/10.2475/03.2011.01

Bolden, A. L., Kwiatkowski, C. F., & Colborn, T. (2015a). New Look at BTEX: Are Ambient Levels a Problem? *Environmental Science & Technology*, *49*(9), 5261–5276. https://doi.org/10.1021/es505316f

Bolden, A. L., Kwiatkowski, C. F., & Colborn, T. (2015b). New Look at BTEX: Are Ambient Levels a Problem? *Environmental Science & Technology*, *49*(9), 5261–5276. https://doi.org/10.1021/es505316f

Bonte, M., Röling, W. F. M., Zaura, E., van der Wielen, P. W. J. J., Stuyfzand, P. J., & van Breukelen, B. M. (2013). Impacts of Shallow Geothermal Energy Production on Redox Processes and Microbial Communities. *Environmental Science & Technology*, *47*(24), 14476–14484. https://doi.org/10.1021/es4030244

Bonte, M., Stuyfzand, P. J., & Breukelen, B. M. V. (2014). Reactive transport modeling of thermal column experiments to investigate the impacts of aquifer thermal energy storage on groundwater quality. *Environmental Science and Technology*, *48*(20), 12099–12107. https://doi.org/10.1021/es502477m

Bonte, M., Stuyfzand, P. J., van den Berg, G. A., & Hijnen, W. A. M. (2011). Effects of aquifer thermal energy storage on groundwater quality and the consequences for drinking water production: a case study from the Netherlands. *Water Science and Technology*, *63*(9), 1922–1931. https://doi.org/10.2166/wst.2011.189

Bookstaver, M., Godfrin, M. P., Bose, A., & Tripathi, A. (2015). An insight into the growth of Alcanivorax borkumensis under different inoculation conditions. *Journal of Petroleum Science and Engineering*, *129*, 153–158. https://doi.org/10.1016/j.petrol.2015.02.038

Borch, T., Kretzschmar, R., Kappler, A., Cappellen, P. Van, Ginder-Vogel, M., Voegelin, A., & Campbell, K. (2010). Biogeochemical Redox Processes and their Impact on Contaminant Dynamics. *Environmental Science & Technology*, *44*(1), 15–23. https://doi.org/10.1021/es9026248

Boyd, E. M., Meharg, A. A., Wright, J., & Killham, K. (1997). Assessment of toxicological interactions of benzene and its primary degradation products (catechol and phenol) using a lux-modified bacterial bioassay. *Environmental Toxicology and Chemistry*, *16*(5), 849–856. https://doi.org/10.1002/etc.5620160503

Braddock, J. F., & McCarthy, K. A. (1996). Hydrologic and Microbiological Factors Affecting Persistence and Migration of Petroleum Hydrocarbons Spilled in a Continuous-Permafrost Region. *Environmental Science & Technology*, *30*(8), 2626–2633. https://doi.org/10.1021/es960070z

Brielmann, H., Griebler, C., Schmidt, S. I., Michel, R., & Lueders, T. (2009). Effects of thermal energy discharge on shallow groundwater ecosystems. *FEMS Microbiology Ecology*, *68*(3), 273–286. https://doi.org/10.1111/j.1574-6941.2009.00674.x

Butcher, K. R., Nasto, M. K., Norton, J. M., & Stark, J. M. (2020). Physical mechanisms for soil moisture effects on microbial carbon-use efficiency in a sandy loam soil in the western United States. *Soil Biology and Biochemistry*, *150*, 107969. https://doi.org/10.1016/j.soilbio.2020.107969

Cai, Y., Wang, R., Rao, P., Wu, B., Yan, L., Hu, L., Park, S., Ryu, M., & Zhou, X. (2021). Bioremediation of Petroleum Hydrocarbons Using Acinetobacter sp. SCYY-5 Isolated from Contaminated Oil Sludge: Strategy and Effectiveness Study. *International Journal of Environmental Research and Public Health*, *18*(2), 819. https://doi.org/10.3390/ijerph18020819

Caliman, F. A., Robu, B. M., Smaranda, C., Pavel, V. L., & Gavrilescu, M. (2011). Soil and groundwater cleanup: benefits and limits of emerging technologies. *Clean Technologies and Environmental Policy*, *13*(2), 241–268. https://doi.org/10.1007/s10098-010-0319-z

Canada Energy Regulator. (2018). *Market Snapshot: Steady growth for heat pump technology*. https://www.cer-rec.gc.ca/en/data-analysis/energy-markets/market-snapshots/2018/market-snapshot-steady-growth-heat-pump-technology.html

Canada WaterPortal. (2024, November 29). *World Water Facts*. https://waterportal.ca/global-water-facts/

Casasso, A., & Sethi, R. (2015). Modelling thermal recycling occurring in groundwater heat pumps (GWHPs). *Renewable Energy*, *77*, 86–93. https://doi.org/10.1016/j.renene.2014.12.003

Casasso, A., & Sethi, R. (2019). Assessment and Minimization of Potential Environmental Impacts of Ground Source Heat Pump (GSHP) Systems. *Water*, *11*(8), 1573. https://doi.org/10.3390/w11081573

Chakraborty, R., O'Connor, S. M., Chan, E., & Coates, J. D. (2005). Anaerobic degradation of benzene, toluene, ethylbenzene, and xylene compounds by Dechloromonas strain RCB. *Applied and Environmental Microbiology*, *71*(12), 8649–8655. https://doi.org/10.1128/AEM.71.12.8649-8655.2005

Chandra, S., Sharma, R., Singh, K., & Sharma, A. (2013). Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. *Annals of Microbiology*, *63*(2), 417–431. https://doi.org/10.1007/s13213-012-0543-3

Chang, Y., Gu, Y., Zhang, L., Wu, C., & Liang, L. (2017). Energy and environmental implications of using geothermal heat pumps in buildings: An example from north China. *Journal of Cleaner Production*, *167*, 484–492. https://doi.org/10.1016/j.jclepro.2017.08.199

Chapelle, F. H. (2000). The significance of microbial processes in hydrogeology and geochemistry. *Hydrogeology Journal*, *8*(1), 41–46. https://doi.org/10.1007/PL00010973

Chen, W. S., Tong, L., Zheng, X. L., & Li, M. (2010). Influencing factors of BTEX volatilization. *2010 4th International Conference on Bioinformatics and Biomedical Engineering, ICBBE 2010*. https://doi.org/10.1109/ICBBE.2010.5516007

Chen, W., Tong, L., Zheng, X., & Li, M. (2010). Influencing Factors of BTEX Volatilization. *2010 4th International Conference on Bioinformatics and Biomedical Engineering*, 1–5. https://doi.org/10.1109/ICBBE.2010.5516007

Chesnaux, R. (2008). Analytical closed-form solutions for assessing pumping cycles, times, and costs required for NAPL remediation. *Environmental Geology*, *55*(7), 1381–1388. https://doi.org/10.1007/s00254-007-1088-9

Chicca, I., Becarelli, S., Dartiahl, C., La China, S., De Kievit, T., Petroni, G., Di Gregorio, S., & Levin, D. B. (2020). Degradation of BTEX mixture by a new Pseudomonas putida strain: role of the quorum sensing in the modulation of the upper BTEX oxidative pathway. *Environmental Science and Pollution Research*, *27*(29). https://doi.org/10.1007/s11356-020-09650-y

Chung, N., & Alexander, M. (1998). Differences in Sequestration and Bioavailability of Organic Compounds Aged in Dissimilar Soils. *Environmental Science & Technology*, *32*(7), 855–860. https://doi.org/10.1021/es970740g

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., & House, J. I. (2014). Carbon and other biogeochemical cycles. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Change* (pp. 465–570). chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://research-information.bris.ac.uk/files/34879270/WGIAR5_WGI_12Doc2b_FinalDraft_Chapter06_BGC_cycles.pdf

Coppotelli, B. M., Ibarrolaza, A., Dias, R. L., Del Panno, M. T., Berthe-Corti, L., & Morelli, I. S. (2010). Study of the Degradation Activity and the Strategies to Promote the Bioavailability of Phenanthrene by Sphingomonas paucimobilis Strain 20006FA. *Microbial Ecology*, *59*(2), 266–276. https://doi.org/10.1007/s00248-009-9563-3

Corseuil, H. X., & Weber, W. J. (1994). Potential biomass limitations on rates of degradation of monoaromatic hydrocarbons by indigenous microbes in subsurface soils. *Water Research*, *28*(6). https://doi.org/10.1016/0043-1354(94)90309-3

Council of Canadian Academics (CCA). (2009). *The Sustainable Management of Groundwater in Canada: The Expert Panel on Groundwater*. Council of Canadian Academies.

Cruz, J. M., Lopes, P. R. M., Montagnolli, R. N., Tamada, I. S., Silva, N. M. M. G., & Bidoia, E. D. (2013). Toxicity Assessment of Contaminated Soil Using Seeds as Bioindicators. *Journal of Applied Biotechnology*, *1*(1). https://doi.org/10.5296/jab.v1i1.3408

Daemi, N., & Krol, M. M. (2019). Impact of building thermal load on the developed thermal plumes of a multi-borehole GSHP system in different canadian climates. *Renewable Energy*, *134*, 550–557. https://doi.org/10.1016/j.renene.2018.11.074

Das, N., & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International*, *2011*, 1–13. https://doi.org/10.4061/2011/941810

Davidson, C. J., Hannigan, J. H., & Bowen, S. E. (2021). Effects of inhaled combined Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX): Toward an environmental exposure model. *Environmental Toxicology and Pharmacology*, *81*, 103518. https://doi.org/10.1016/j.etap.2020.103518

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, *440*(7081), 165–173. https://doi.org/10.1038/nature04514

Deeb, R. A., & Alvarez-Cohen, L. (1999). Temperature effects and substrate interactions during the aerobic biotransformation of BTEX mixtures by toluene-enriched consortia andRhodococcus rhodochrous. *Biotechnology and Bioengineering*, *62*(5), 526–536. https://doi.org/10.1002/(SICI)1097-0290(19990305)62:5<526::AID-BIT4>3.0.CO;2-8

Deng, Q., Li, S., Yao, M., Liu, C., Zhang, Z., & Xiang, S. (2023). Study on the factors of hydrogen sulfide production from lignite bacterial sulfate reduction based on response surface method. *Scientific Reports*, *13*(1), 20537. https://doi.org/10.1038/s41598-023-47787-1

Deng, Q., Wu, X., Wang, Y., & Liu, M. (2018). Activity characteristics of sulfate reducing bacteria and formation mechanism of hydrogen sulfide. *Applied Ecology & Environmental Research*. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://aloki.hu/pdf/1605_63696383.pdf

Dos Santos, M. M., Cezario, L. F. C., Simões, I. M., Baptista, J. O., Araujo, C. P. de, de Mello, T., Mayard, H., Gonçalves, E. de O., Fontes, M. M. P., Schmildt, E. R., Lopes, J. C., Caldeira, M. V. W., & Alexandre, R. S. (2020). Disinfection protocol and in vitro germination of seeds of dalbergia nigra. *Cerne*, *26*(2), 238–246. https://doi.org/10.1590/01047760202026022714

Du, X., Sun, W., Liu, X., Chi, Z., Sheng, H., & Liu, F. (2025). Comprehensive Ecotoxicity of the Complex System of Polycyclic Aromatic Hydrocarbon-Contaminated Sites to Wheat (Triticum aestivum L.) During Microbial Remediation. *Sustainability*, *17*(9), 4127. https://doi.org/10.3390/su17094127

Egidio, E., De Luca, D. A., & Lasagna, M. (2024). How groundwater temperature is affected by climate change: A systematic review. *Heliyon*, *10*(6), e27762. https://doi.org/10.1016/j.heliyon.2024.e27762

El-Naas, M. H., Acio, J. A., & El Telib, A. E. (2014). Aerobic biodegradation of BTEX: Progresses and Prospects. *Journal of Environmental Chemical Engineering*, *2*(2), 1104–1122. https://doi.org/10.1016/j.jece.2014.04.009

Environment and Climate Change Canada (ECCC). (2013, September 9). *Water sources: groundwater*. GovernmentofCanada. https://www.canada.ca/en/environment-climate-change/services/water-overview/sources/groundwater.html

Environment and Climate Change Canada (ECCC). (2024). *Federal Environmental Quality Guidelines BTEX*.

Environment and Climate Change Canada, & Health Canada. (2016). *Screening Assessment Report:: Ethylbenzene*. https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/screening-assessment-report3.html

Environment Canada. (1984). *Benzene* .

Environmental Protection Act. (2012). *GROUND SOURCE HEAT PUMPS O. Reg. 98/12*. https://www.ontario.ca/laws/regulation/120098

EPA. (2004). *How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites*. https://www.epa.gov/ust/how-evaluate-alternative-cleanup-technologies-underground-storage-tank-sites-guide-corrective

EPA. (2025). *National Primary Drinking Water Regulations*. https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#:~:text=Table_title:%20Organic%20Chemicals%2C%20except%20for%20PFAS%20Table_content:,TT%201%20(%20mg/L%20)2:%200.04%20%7C

Farhadian, M., Vachelard, C., Duchez, D., & Larroche, C. (2008). In situ bioremediation of monoaromatic pollutants in groundwater: A review. *Bioresource Technology*, *99*(13), 5296–5308. https://doi.org/10.1016/j.biortech.2007.10.025

Farzanehkhameneh, P., Soltani, M., Moradi Kashkooli, F., & Ziabasharhagh, M. (2020). Optimization and energy-economic assessment of a geothermal heat pump system. *Renewable and Sustainable Energy Reviews*, *133*, 110282. https://doi.org/10.1016/j.rser.2020.110282

Fedrizzi, F., Cagliari, J., Teixeira, C. E., Finotti, A. R., & Filho, I. do N. (2013). The environmental aspects of the evaporation of BTEX from gasoline with and without ethanol. *International Journal of Environment and Waste Management*, *11*(2), 148. https://doi.org/10.1504/IJEWM.2013.051820

Feenstra, S., Mackay, D. M., & Cherry, J. A. (1991). A Method for Assessing Residual NAPL Based on Organic Chemical Concentrations in Soil Samples. *Groundwater Monitoring & Remediation*, *11*(2), 128–136. https://doi.org/https://doi.org/10.1111/j.1745-6592.1991.tb00374.x

Ferguson, G., & Woodbury, A. D. (2004). Subsurface heat flow in an urban environment. *Journal of Geophysical Research: Solid Earth*, *109*(B2). https://doi.org/10.1029/2003JB002715

Fetter, C. W. , Boving, T., & Kreamer, D. (2017). *Contaminant Hydrogeology: Third Edition*.

Firmino, P. I. M., Farias, R. S., Buarque, P. M. C., Costa, M. C., Rodríguez, E., Lopes, A. C., & dos Santos, A. B. (2015). Engineering and microbiological aspects of BTEX removal in bioreactors under sulfate-reducing conditions. *Chemical Engineering Journal*, *260*, 503–512. https://doi.org/10.1016/j.cej.2014.08.111

Fleuchaus, P., Godschalk, B., Stober, I., & Blum, P. (2018a). Worldwide application of aquifer thermal energy storage – A review. *Renewable and Sustainable Energy Reviews*, *94*, 861–876. https://doi.org/10.1016/j.rser.2018.06.057

Fleuchaus, P., Godschalk, B., Stober, I., & Blum, P. (2018b). Worldwide application of aquifer thermal energy storage – A review. *Renewable and Sustainable Energy Reviews*, *94*, 861–876. https://doi.org/10.1016/j.rser.2018.06.057

Flynn, T. M., Antonopoulos, D. A., Skinner, K. A., Brulc, J. M., Johnston, E., Boyanov, M. I., Kwon, M. J., Kemner, K. M., & O'Loughlin, E. J. (2021). Biogeochemical dynamics and microbial community development under sulfate- and iron-reducing conditions based on electron shuttle amendment. *PLOS ONE*, *16*(5), e0251883. https://doi.org/10.1371/journal.pone.0251883

Folkerts, E. J., Goss, G. G., & Blewett, T. A. (2021). Investigating the Potential Toxicity of Hydraulic Fracturing Flowback and Produced Water Spills to Aquatic Animals in Freshwater Environments: A North American Perspective. In P. de Voogt (Ed.), *Reviews of Environmental Contamination and Toxicology Volume 254* (pp. 1–56). Springer International Publishing. https://doi.org/10.1007/398_2020_43

Fonkwe, M. L. D., & Trapp, S. (2016). Analyzing tree cores to detect petroleum hydrocarbon-contaminated groundwater at a former landfill site in the community of Happy Valley-Goose Bay, eastern Canadian subarctic. *Environmental Science and Pollution Research*, *23*(16), 16137–16151. https://doi.org/10.1007/s11356-016-6802-2

Fonseca, P., Moreno, R., & Rojo, F. (2011a). Growth of Pseudomonas putida at low temperature: Global transcriptomic and proteomic analyses. *Environmental Microbiology Reports*, *3*(3). https://doi.org/10.1111/j.1758-2229.2010.00229.x

Fonseca, P., Moreno, R., & Rojo, F. (2011b). Growth of Pseudomonas putida at low temperature: global transcriptomic and proteomic analyses. *Environmental Microbiology Reports*, *3*(3), 329–339. https://doi.org/10.1111/j.1758-2229.2010.00229.x

Freedman, V. L., Waichler, S. R., Mackley, R. D., & Horner, J. A. (2012). Assessing the thermal environmental impacts of an groundwater heat pump in southeastern Washington State. *Geothermics*, *42*, 65–77. https://doi.org/10.1016/j.geothermics.2011.10.004

Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. *Geoderma*, *122*(2–4), 109–119. https://doi.org/10.1016/j.geoderma.2004.01.002

Gao, W., Huo, X., & Yu, T. (2023). Acetophenone preparation via continuous bubbling of ozone for catalyst-free ethylbenzene oxidation. *Chemical Engineering and Processing - Process Intensification*, *193*, 109545. https://doi.org/10.1016/j.cep.2023.109545

Garnier, F., Lesueur, H., Motelica-Heino, M., & Ignatiadis, I. (2011). *AQUIFER BIOTHERMOREMEDIATION USING HEAT PUMPS: SOUND THEORETICAL BASIS AND RESULTS ON THERMAL, GEOCHEMICAL AND BIOLOGICAL IMPACTS ON AQUIFERS*.

Gilbert, R. J., Stringer, M. F., & Peace, T. C. (1974). The survival and growth of Bacillus cereus in boiled and fried rice in relation to outbreaks of food poisoning. *Journal of Hygiene*, *73*(3). https://doi.org/10.1017/S0022172400042790

Gissi, F., Strzelecki, J., Binet, M. T., Golding, L. A., Adams, M. S., Elsdon, T. S., Robertson, T., & Hook, S. E. (2021). A Comparison of Short-Term and Continuous Exposures in Toxicity Tests of Produced Waters, Condensate, and Crude Oil to Marine Invertebrates and Fish. *Environmental Toxicology and Chemistry*, *40*(9), 2587–2600. https://doi.org/10.1002/etc.5129

Gong, P., Wilke, B.-M., Strozzi, E., & Fleischmann, S. (2001). Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. *Chemosphere*, *44*(3), 491–500. https://doi.org/10.1016/S0045-6535(00)00280-0

Government of Canada. (2025). *Water Quality - Reports and Publications*.

Government of Canada, Environment Canada, & Health Canada. (1993). *Xylenes / Government of Canada, Environment Canada, Health Canada*. https://publications.gc.ca/site/eng/9.863462/publication.html

Government of Canada, H. and W. C. E. C. (1992). *PRIORITY SUBSTANCES LIST ASSESSMENT REPORT NO. 4: TOLUENE*. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.canada.ca/content/dam/hc-

sc/migration/hc-sc/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl1-lsp1/toulene/toluene-eng.pdf

Grains Research & Development Corporation. (2016). *SECTION 4 PLANT GROWTH AND PHYSIOLOGY*. http://www.grdc.com.au/uploads/documents/GRDC%20

Grasby, S. E., Allen, D. M., Bell, S., Chen, Z., Ferguson, G., Jessop, A., Kelman, M., Ko, M., Majorowicz, J., Moore, M., Raymond, J., & Therrien, R. (2012). *Geothermal energy resource potential of Canada*. https://doi.org/10.4095/291488

Griebler, C., Brielmann, H., Haberer, C. M., Kaschuba, S., Kellermann, C., Stumpp, C., Hegler, F., Kuntz, D., Walker-Hertkorn, S., & Lueders, T. (2016). Potential impacts of geothermal energy use and storage of heat on groundwater quality, biodiversity, and ecosystem processes. *Environmental Earth Sciences*, *75*(20). https://doi.org/10.1007/s12665-016-6207-z

Guerra, K., & Drewes, J. (2008). Produced Water in the Western United States: Geographical Distribution, Occurrence, and Composition. *Environmental Engineering Science - ENVIRON ENG SCI*, *25*, 239–246. https://doi.org/10.1089/ees.2007.0026

Haider, F. U., Ejaz, M., Cheema, S. A., Khan, M. I., Zhao, B., Liqun, C., Salim, M. A., Naveed, M., Khan, N., Núñez-Delgado, A., & Mustafa, A. (2021). Phytotoxicity of petroleum hydrocarbons: Sources, impacts and remediation strategies. *Environmental Research*, *197*, 111031. https://doi.org/10.1016/j.envres.2021.111031

Han, Y., Sheng, Y., Zhao, J., & Zhu, L. (2024). Prediction of BTEX volatilization in polluted soil based on the sorption potential energy theory. *Environmental Pollution*, *360*, 124624. https://doi.org/10.1016/j.envpol.2024.124624

Han, Z., Jiao, W., Tian, Y., Jian HU, & Denglun Han. (2020). Lab-scale removal of PAHs in contaminated soil using electrical resistance heating: Removal efficiency and alteration of soil properties. *Chemosphere*, *239*, 124496. https://doi.org/10.1016/j.chemosphere.2019.124496

Handayani, S., Safitri, R., Surono, W., Astika, H., Damayanti, R., Agung, M., & Rukiah. (2019). Biodegradation of BTEX by indigenous microorganisms isolated from UCG project area, South Sumatra. *IOP Conference Series: Earth and Environmental Science*, *308*(1). https://doi.org/10.1088/1755-1315/308/1/012017

Headley, J. V., Goudey, S., Birkholz, D., Linton, L. R., & Dickson, L. C. (2001). Toxicity Screening of Benzene, Toluene, Ethylbenzene and Xylene (BTEX) Hydrocarbons in Groundwater at Sour-Gas Plants. *Canadian Water Resources Journal*, *26*(3), 345–358. https://doi.org/10.4296/cwrj2603345

Health Canada. (2015). *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Toluene, Ethylbenzene and the Xylenes*.

Heipieper, H. J., Neumann, G., Cornelissen, S., & Meinhardt, F. (2007). Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Applied Microbiology and Biotechnology*, *74*(5), 961–973. https://doi.org/10.1007/s00253-006-0833-4

Hemme, C. L., Tu, Q., Shi, Z., Qin, Y., Gao, W., Deng, Y., Nostrand, J. D. Van, Wu, L., He, Z., Chain, P. S. G., Tringe, S. G., Fields, M. W., Rubin, E. M., Tiedje, J. M., Hazen, T. C., Arkin, A. P., & Zhou, J. (2015). Comparative metagenomics reveals impact of contaminants on groundwater microbiomes. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.01205

Herbert, A., Arthur, S., & Chillingworth, G. (2013). Thermal modelling of large scale exploitation of ground source energy in urban aquifers as a resource management tool. *Applied Energy*, *109*, 94–103. https://doi.org/10.1016/j.apenergy.2013.03.005

Hernández-Ospina, D. A., Osorio-González, C. S., Miri, S., & Kaur Brar, S. (2024). New perspectives on the anaerobic degradation of BTEX: Mechanisms, pathways, and intermediates. *Chemosphere*, *361*, 142490. https://doi.org/10.1016/j.chemosphere.2024.142490

Hinchee, R. E., & Smith, L. A. (1992). *In-Situ Thermal Technologies for Site Remediation*. CRC Press. https://books.google.ca/books?hl=en&lr=&id=iOngcSyK60QC&oi=fnd&pg=PA1&ots=f1FeR-9bhd&sig=rRQLrislOWq2wRdgly8bWsSYwsE&redir_esc=y#v=onepage&q&f=false

Hinwood, A. L., Rodriguez, C., Runnion, T., Farrar, D., Murray, F., Horton, A., Glass, D., Sheppeard, V., Edwards, J. W., Denison, L., Whitworth, T., Eiser, C., Bulsara, M., Gillett, R. W., Powell, J., Lawson, S., Weeks, I., & Galbally, I. (2007). Risk factors for increased BTEX exposure in four Australian cities. *Chemosphere*, *66*(3), 533–541. https://doi.org/https://doi.org/10.1016/j.chemosphere.2006.05.040

Honda, M., & Suzuki, N. (2020). Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. In *International Journal of Environmental Research and Public Health* (Vol. 17, Number 4). MDPI AG. https://doi.org/10.3390/ijerph17041363

Huang, Y., Chen, Y., Wu, Q., Shi, P., Yang, B., & Xie, Y. (2024). Evaluation of BTEX Pollution and Health Risk for Sustainable Use of a Typical Chemical Pesticide Industrial Site. *Sustainability*, *16*(15), 6494. https://doi.org/10.3390/su16156494

Huo, C., Ahmed Dar, A., Nawaz, A., Hameed, J., albashar, G., Pan, B., & Wang, C. (2021). Groundwater contamination with the threat of COVID-19: Insights into CSR theory of Carroll's pyramid. *Journal of King Saud University - Science*, *33*(2), 101295. https://doi.org/10.1016/j.jksus.2020.101295

I., S., ABDULWAHID, I. A., MUDI, Z. R., YA'U, S. A., & RITA, S. M. (2022). Bioremediation On Oil Spills Using Bacterium (Pseudomonas Putida). *IRE Journals*, *5*(8).

Ikram, M., Naeem, M., Zahoor, M., Rahim, A., Hanafiah, M. M., Oyekanmi, A. A., Shah, A. B., Mahnashi, M. H., Al Ali, A., Jalal, N. A., Bantun, F., & Sadiq, A. (2022). Biodegradation of Azo Dye Methyl Red by Pseudomonas aeruginosa: Optimization of Process Conditions. *International Journal of Environmental Research and Public Health*, *19*(16), 9962. https://doi.org/10.3390/ijerph19169962

Illangasekare, T. H., III, E. J. A., & Yates, D. N. (1995). Non-Aqueous-Phase Fluids in Heterogeneous Aquifers—Experimental Study. *Journal of Environmental Engineering*, *121*(8), 571–579. https://doi.org/10.1061/(ASCE)0733-9372(1995)121:8(571)

Indigenous Services Canada. (2013). *Evaluation of the First Nations Water and Wastewater Action Plan*.

International Organization for Standardization (ISO). (1993). *ISO 11269-2:2005, Ed. 1, Soil quality—Determination of the effects of pollutants on soil flora—Part 2: Effects of chemicals on the growth of higher plants.*

Jesußek, A., Grandel, S., & Dahmke, A. (2013). Impacts of subsurface heat storage on aquifer hydrogeochemistry. *Environmental Earth Sciences*, *69*(6), 1999–2012. https://doi.org/10.1007/s12665-012-2037-9

Jin, H. M., Choi, E. J., & Jeon, C. O. (2013). Isolation of a BTEX-degrading bacterium, Janibacter sp. SB2, from a sea-tidal flat and optimization of biodegradation conditions. *Bioresource Technology*, *145*, 57–64. https://doi.org/10.1016/j.biortech.2013.02.004

Jin, Q., & Kirk, M. F. (2016). Thermodynamic and Kinetic Response of Microbial Reactions to High CO2. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01696

Jo, M.-S., Rene, E. R., Kim, S.-H., & Park, H.-S. (2008). An analysis of synergistic and antagonistic behavior during BTEX removal in batch system using response surface methodology. *Journal of Hazardous Materials*, *152*(3), 1276–1284. https://doi.org/10.1016/j.jhazmat.2007.08.002

John V. Headley S. Lee Barbour, R. W. T., & Gong, Y. (2000). An Evaluation of the Ideality of Benzene, Toluene, Ethylbenzene, and Xylene on Activity Coefficients in Gas Condensate and the Implications for Dissolution in Groundwater . *Canadian Water Resources Journal / Revue Canadienne Des Ressources Hydriques*, *25*(1), 67–79. https://doi.org/10.4296/cwrj2501067

Kadri, T., Magdouli, S., Rouissi, T., & Brar, S. K. (2018). Ex-situ biodegradation of petroleum hydrocarbons using Alcanivorax borkumensis enzymes. *Biochemical Engineering Journal*, *132*, 279–287. https://doi.org/10.1016/j.bej.2018.01.014

Kang, B., Grancher, N., Koyffmann, V., Lardemer, D., Burney, S., & Ahmad, M. (2008). Multiple interactions between cryptochrome and phototropin blue-light signalling pathways in Arabidopsis thaliana. *Planta*, *227*(5), 1091–1099. https://doi.org/10.1007/s00425-007-0683-z

Kaplan, L. A., & Newbold, J. D. (2000). Surface and Subsurface Dissolved Organic Carbon. In *Streams and Ground Waters* (pp. 237–258). Elsevier. https://doi.org/10.1016/B978-012389845-6/50011-9

Kappler, A., Bryce, C., Mansor, M., Lueder, U., Byrne, J. M., & Swanner, E. D. (2021). An evolving view on biogeochemical cycling of iron. *Nature Reviews Microbiology*, *19*(6), 360–374. https://doi.org/10.1038/s41579-020-00502-7

Kassotis, C. D., Tillitt, D. E., Lin, C.-H., McElroy, J. A., & Nagel, S. C. (2016). Endocrine-Disrupting Chemicals and Oil and Natural Gas Operations: Potential Environmental Contamination and Recommendations to Assess Complex Environmental Mixtures. *Environmental Health Perspectives*, *124*(3), 256–264. https://doi.org/10.1289/ehp.1409535

Kaur, G. (2026). *Coupling Geothermal Heating with BTEX Bioremediation in the Subsurface*.

Kaur, G., Kaur, G., Krol, M., & Kaur Brar, S. (2022). Unraveling the mystery of subsurface microorganisms in bioremediation. In *Current Research in Biotechnology* (Vol. 4). https://doi.org/10.1016/j.crbiot.2022.07.001

Kaur, G., Krol, M., & Brar, S. K. (2021). Geothermal heating: Is it a boon or a bane for bioremediation? *Environmental Pollution*, *287*. https://doi.org/10.1016/j.envpol.2021.117609

Kaur, G., Lecka, J., Krol, M., & Brar, S. K. (2023). Novel BTEX-degrading strains from subsurface soil: Isolation, identification and growth evaluation. *Environmental Pollution*, *335*, 122303. https://doi.org/10.1016/j.envpol.2023.122303

Kaur, G., Verma, S., Krol, M., & Brar, S. K. (2025). Analysis of benzene, toluene, ethylbenzene, xylene(s) biodegradation under anoxic conditions using response surface methodology. *International Biodeterioration & Biodegradation*, *198*, 105973. https://doi.org/10.1016/j.ibiod.2024.105973

Khaeim, H., Kende, Z., Balla, I., Gyuricza, C., Eser, A., & Tarnawa, Á. (2022). The Effect of Temperature and Water Stresses on Seed Germination and Seedling Growth of Wheat (Triticum aestivum L.). *Sustainability*, *14*(7), 3887. https://doi.org/10.3390/su14073887

Khan, M. A. I., Biswas, B., Smith, E., Naidu, R., & Megharaj, M. (2018). Toxicity assessment of fresh and weathered petroleum hydrocarbons in contaminated soil- a review. *Chemosphere*, *212*, 755–767. https://doi.org/10.1016/j.chemosphere.2018.08.094

Kim, J. M., & Jeon, C. O. (2009). Isolation and Characterization of a New Benzene, Toluene, and Ethylbenzene Degrading Bacterium, Acinetobacter sp. B113. *Current Microbiology*, *58*(1), 70–75. https://doi.org/10.1007/s00284-008-9268-8

Knoke, K. L., Marwood, T. M., Cassidy, M. B., Liu, D., Seech, A. G., Lee, H., & Trevors, J. T. (1999). *A COMPARISON OF FIVE BIOASSAYS TO MONITOR TOXICITY DURING BIOREMEDIATION OF PENTACHLOROPHENOL-CONTAMINATED SOIL*.

Koshlaf, E., & S Ball, A. (2017). Soil bioremediation approaches for petroleum hydrocarbon polluted environments. *AIMS Microbiology*, *3*(1), 25–49. https://doi.org/10.3934/microbiol.2017.1.25

Krol, M. M., Sleep, B. E., & Johnson, R. L. (2011). Impact of low-temperature electrical resistance heating on subsurface flow and transport. *Water Resources Research*, *47*(5). https://doi.org/10.1029/2010WR009675

Kumar, M., León, V., De Sisto Materano, A., Ilzins, O. A., & Luis, L. (2008). Biosurfactant production and hydrocarbon-degradation by halotolerant and thermotolerant Pseudomonas sp. *World Journal of Microbiology and Biotechnology*, *24*(7), 1047–1057. https://doi.org/10.1007/s11274-007-9574-5

Kuo, Y. C., Cheng, S. F., Liu, P. W. G., Chiou, H. Y., & Kao, C. M. (2012). Application of enhanced bioremediation for TCE-contaminated groundwater: a pilot-scale study. *Desalination and Water Treatment*, *41*(1–3), 364–371. https://doi.org/10.1080/19443994.2012.664742

Kurylyk, B. L., MacQuarrie, K. T. B., & Voss, C. I. (2014). Climate change impacts on the temperature and magnitude of groundwater discharge from shallow, unconfined aquifers. *Water Resources Research*, *50*(4), 3253–3274. https://doi.org/10.1002/2013WR014588

LaBauve, A. E., & Wargo, M. J. (2012). Growth and Laboratory Maintenance of *Pseudomonas aeruginosa*. *Current Protocols in Microbiology*, *25*(1). https://doi.org/10.1002/9780471729259.mc06e01s25

Ladino-Orjuela, G., Gomes, E., da Silva, R., Salt, C., & Parsons, J. R. (2016). *Metabolic Pathways for Degradation of Aromatic Hydrocarbons by Bacteria* (pp. 105–121). https://doi.org/10.1007/978-3-319-23573-8_5

Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, *54*(3), 305–315. https://doi.org/10.1128/mr.54.3.305-315.1990

Lee, J.-Y. (2009). Current status of ground source heat pumps in Korea. *Renewable and Sustainable Energy Reviews*, *13*(6–7), 1560–1568. https://doi.org/10.1016/j.rser.2008.10.005

Lekmine, G., Bastow, T. P., Johnston, C. D., & Davis, G. B. (2014). Dissolution of multi-component LNAPL gasolines: The effects of weathering and composition. *Journal of Contaminant Hydrology*, *160*. https://doi.org/10.1016/j.jconhyd.2014.02.003

Leuthner, B., Leutwein, C., Schulz, H., Hörth, P., Haehnel, W., Schiltz, E., Schägger, H., & Heider, J. (1998). Biochemical and genetic characterization of benzylsuccinate synthase from *Thauera aromatica* : a new glycyl radical enzyme catalysing the first step in anaerobic toluene metabolism. *Molecular Microbiology*, *28*(3), 615–628. https://doi.org/10.1046/j.1365-2958.1998.00826.x

Li, L., Zhu, P., Wang, X., & Zhang, Z. (2020). Phytoremediation effect of Medicago sativa colonized by Piriformospora indica in the phenanthrene and cadmium co-contaminated soil. *BMC Biotechnology*, *20*(1), 20. https://doi.org/10.1186/s12896-020-00613-2

Li, Q., Yin, J., Wu, L., Fu, R., & Chen, L. (2023). Ecotoxicity Assessment of the Water Extracts from Metal-Contaminated and Hydrocarbon-Contaminated Soils. *Water*, *15*(23), 4061. https://doi.org/10.3390/w15234061

Lim, M. W., Lau, E. Von, & Poh, P. E. (2016). A comprehensive guide of remediation technologies for oil contaminated soil — Present works and future directions. *Marine Pollution Bulletin*, *109*(1), 14–45. https://doi.org/10.1016/j.marpolbul.2016.04.023

Lins, E. A. M. de S. R. B. R. (2019). Btex contaminated area diagnosis and mapping: Case study. *International Journal of Development Research*, *9*, 31548–31553.

Liu, A., Hong, N., Zhu, P., & Guan, Y. (2018). Characterizing benzene series (BTEX) pollutants build-up process on urban roads: Implication for the importance of temperature. *Environmental Pollution*, *242*. https://doi.org/10.1016/j.envpol.2018.07.045

Liu, F., & Wang, D. (2022). Dissolved organic carbon concentration and biodegradability across the global rivers: A meta-analysis. *Science of The Total Environment*, *818*, 151828. https://doi.org/10.1016/j.scitotenv.2021.151828

Liu, Z., Lin, X., Sun, M., Ma, S., Liu, J., & Zhang, S. (2024). Microbial Community Dynamics in Groundwater of a Petrochemical Refinery: Influence of BTEX and Dichloroethane Contamination. *Water*, *16*(22), 3275. https://doi.org/10.3390/w16223275

Lovley, D. R. (1997). Potential for anaerobic bioremediation of BTEX in petroleum-contaminated aquifers. *Journal of Industrial Microbiology and Biotechnology*, *18*(2–3), 75–81. https://doi.org/10.1038/sj.jim.2900246

Lovley, D. R. (2001). Anaerobes to the Rescue. *Science*, *293*(5534), 1444–1446. https://doi.org/10.1126/science.1063294

Lovley, D. R. (2003). Cleaning up with genomics: applying molecular biology to bioremediation. *Nature Reviews Microbiology*, *1*(1), 35–44. https://doi.org/10.1038/nrmicro731

Lueders, T. (2017). The ecology of anaerobic degraders of BTEX hydrocarbons in aquifers. *FEMS Microbiology Ecology*, *93*(1), fiw220. https://doi.org/10.1093/femsec/fiw220

Lund, J. W., & Toth, A. N. (2021). Direct utilization of geothermal energy 2020 worldwide review. *Geothermics*, *90*, 101915. https://doi.org/10.1016/j.geothermics.2020.101915

Maciel, H., Mathis, H., Lopes Ferreira, N., Lyew, D., Guiot, S., Monot, F., Greer, C. W., & Fayolle-Guichard, F. (2008). Use of Mycobacterium austroafricanum IFP 2012 in a MTBE-degrading bioreactor. *Microbial Physiology*, *15*(2–3), 190–198. https://doi.org/10.1159/000121330

Madsen, E. L. (2011). Microorganisms and their roles in fundamental biogeochemical cycles. *Current Opinion in Biotechnology*, *22*(3), 456–464. https://doi.org/10.1016/j.copbio.2011.01.008

Marazuela, M. A., & García-Gil, A. (2022). Frontier Research of Engineering: Geothermal Energy Utilization and Groundwater Heat Pump Systems. *Sustainability*, *14*(21), 13745. https://doi.org/10.3390/su142113745

Margesin, R., & Schinner, F. (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. *Applied Microbiology and Biotechnology*, *56*(5–6), 650–663. https://doi.org/10.1007/s002530100701

Marzi, D., Mazzurco-Miritana, V., Passatore, L., Zacchini, M., Pietrini, F., Carloni, S., Peruzzi, E., Louro, M., Sousa Silva, M., Cordeiro, C., & Nogués, I. (2025). Assessment of phyto-compatibility after soil remediation: Insights from plant growth, physiological, and metabolomic analyses. *Journal of Environmental Management*, *393*, 127239. https://doi.org/10.1016/j.jenvman.2025.127239

Masakorala, K., Yao, J., Chandankere, R., Yuan, H., Liu, H., Yu, C., & Cai, M. (2013). Effects of Petroleum Hydrocarbon Contaminated Soil on Germination, Metabolism and Early Growth of Green Gram, Vigna radiata L. *Bulletin of Environmental Contamination and Toxicology*, *91*(2), 224–230. https://doi.org/10.1007/s00128-013-1042-3

McDonough, L. K., Santos, I. R., Andersen, M. S., O'Carroll, D. M., Rutlidge, H., Meredith, K., Oudone, P., Bridgeman, J., Gooddy, D. C., Sorensen, J. P. R., Lapworth, D. J., MacDonald, A. M., Ward, J., & Baker, A. (2020). Changes in global groundwater organic carbon driven by climate change and urbanization. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-14946-1

McGenity, T. J. (2014). Hydrocarbon biodegradation in intertidal wetland sediments. *Current Opinion in Biotechnology*, *27*, 46–54. https://doi.org/10.1016/j.copbio.2013.10.010

Metzgar, D. (2004). Acinetobacter sp. ADP1: an ideal model organism for genetic analysis and genome engineering. *Nucleic Acids Research*, *32*(19), 5780–5790. https://doi.org/10.1093/nar/gkh881

Ministry of the Environment. (2011). *Soil, ground water and sediment standards for use under Part XV.1 of the Environmental Protection Act*.

Miri, S., Davoodi, S. M., Robert, T., Brar, S. K., Martel, R., & Rouissi, T. (2022). Enzymatic biodegradation of highly p-xylene contaminated soil using cold-active enzymes: A soil column study. *Journal of Hazardous Materials*, *423*. https://doi.org/10.1016/j.jhazmat.2021.127099

Mitra, S., & Roy, P. (2011). BTEX : A Serious Ground-water Contaminant. *Research Journal of Environmental Sciences*, *5*(5). https://doi.org/10.3923/rjes.2011.394.398

Mohamad Shahimin, M. F., & Siddique, T. (2017). Sequential biodegradation of complex naphtha hydrocarbons under methanogenic conditions in two different oil sands tailings. *Environmental Pollution*, *221*, 398–406. https://doi.org/10.1016/j.envpol.2016.12.002

Mohanta, S., Pradhan, B., & Behera, I. D. (2024). Impact and Remediation of Petroleum Hydrocarbon Pollutants on Agricultural Land: A Review. *Geomicrobiology Journal*, *41*(4), 345–359. https://doi.org/10.1080/01490451.2023.2243925

Moore, B. J., Headley, J. V., Dupont, R. R., Doucette, W. D., & Armstrong, J. E. (2002). ABATEMENT OF GAS-CONDENSATE HYDROCARBONS IN A NATURAL WETLAND. *Journal of Environmental Science and Health, Part A*, *37*(4), 425–438. https://doi.org/10.1081/ESE-120003225

Moore, J. W., & Ramamoorthy, S. (1984). Aromatic Hydrocarbons—Polycyclics. In J. W. Moore & S. Ramamoorthy (Eds.), *Organic Chemicals in Natural Waters: Applied Monitoring and Impact Assessment* (pp. 67–87). Springer New York. https://doi.org/10.1007/978-1-4613-9538-6_5

Moradi, A., M. Smits, K., & O. Sharp, J. (2018). Coupled Thermally-Enhanced Bioremediation and Renewable Energy Storage System: Conceptual Framework and Modeling Investigation. *Water*, *10*(10), 1288. https://doi.org/10.3390/w10101288

Morgan, P., Lewis, S. T., & Watkinson, R. J. (1993). Biodegradation of benzene, toluene, ethylbenzene and xylenes in gas-condensate-contaminated ground-water. *Environmental Pollution*, *82*(2), 181–190. https://doi.org/10.1016/0269-7491(93)90115-5

Morya, R., Salvachúa, D., & Thakur, I. S. (2020). Burkholderia: An Untapped but Promising Bacterial Genus for the Conversion of Aromatic Compounds. *Trends in Biotechnology*, *38*(9), 963–975. https://doi.org/10.1016/j.tibtech.2020.02.008

Mustafa Omer, A. (2008). Ground-source heat pumps systems and applications. *Renewable and Sustainable Energy Reviews*, *12*(2), 344–371. https://doi.org/10.1016/j.rser.2006.10.003

Nagpal, N. K. . (2003). *Ambient water quality guidelines for chloride: Overview*. Water, Air and Climate Branch.

Nakamura, T., Senior, C. L., Burns, E. G., & Bell, M. D. (2000). Solar-powered soil vapor extraction for removal of dense nonaqueous phase organics from soil. *Journal of Environmental Science and Health, Part A*, *35*(6), 795–816. https://doi.org/10.1080/10934520009377004

Namiki, S. (2022). Studies on the abilities of uptake and translocation from root to shoot of pesticides in soil. *Journal of Pesticide Science*, *47*(3), 131–138. https://doi.org/10.1584/jpestics.J22-01

National Research Council. (2000). *Natural Attenuation for Groundwater Remediation*. National Academies Press. https://doi.org/10.17226/9792

National Research Council. (2003). *Bioavailability of Contaminants in Soils and Sediments*. National Academies Press. https://doi.org/10.17226/10523

National Research Council. (2013). *Alternatives for Managing the Nation's Complex Contaminated Groundwater Sites*. National Academies Press. https://doi.org/10.17226/14668

National Research Council (US) Committee on Pyrene and Selected Analogues. (1983). POLYCYCLIC AROMATIC HYDROCARBONS FROM NATURAL AND STATIONARY ANTHROPOGENIC SOURCES AND THEIR ATMOSPHERIC CONCENTRATIONS. In *Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects* (p. 2).

Nguyen, T. T., & Marschner, P. (2016). Sorption of Water-Extractable Organic Carbon in Various Clay Subsoils: Effects of Soil Properties. *Pedosphere*, *26*(1), 55–61. https://doi.org/10.1016/S1002-0160(15)60022-4

Ni, Z., van Gaans, P., Smit, M., Rijnaarts, H., & Grotenhuis, T. (2016). Combination of aquifer thermal energy storage and enhanced bioremediation: resilience of reductive dechlorination to redox changes. *Applied Microbiology and Biotechnology*, *100*(8), 3767–3780. https://doi.org/10.1007/s00253-015-7241-6

Ni, Z., Wang, Y., Wang, Y., Chen, S., Xie, M., Grotenhuis, T., & Qiu, R. (2020). Comparative Life-Cycle Assessment of Aquifer Thermal Energy Storage Integrated with in Situ Bioremediation of Chlorinated Volatile Organic Compounds. *Environmental Science and Technology*, *54*(5), 3039–3049. https://doi.org/10.1021/acs.est.9b07020

Nicholls, J. W. F., Chin, J. P., Williams, T. A., Lenton, T. M., O'Flaherty, V., & McGrath, J. W. (2023). On the potential roles of phosphorus in the early evolution of energy metabolism. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1239189

Nicole, W. (2022). Skin Protection Dilemma: Testing Detects Benzene in Some Sun Care Products. *Environmental Health Perspectives*, *130*(5), 54002. https://doi.org/10.1289/EHP11423

Njobuenwu, D. O., Amadi, S. A., & Ukpaka, P. C. (2005). Dissolution Rate of BTEX Contaminants in Water. *The Canadian Journal of Chemical Engineering*, *83*(6), 985–989. https://doi.org/10.1002/cjce.5450830608

OECD. (2006). *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test*. OECD. https://doi.org/10.1787/9789264070066-en

Ossai, I. C., Ahmed, A., Hassan, A., & Hamid, F. S. (2020). Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environmental Technology & Innovation*, *17*, 100526. https://doi.org/10.1016/j.eti.2019.100526

Pal, V. K., Lee, S., & Kannan, K. (2023). Occurrence of and dermal exposure to benzene, toluene and styrene in sunscreen products marketed in the United States. *Science of The Total Environment*, *888*, 164196. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.164196

Pal, V. K., Lee, S., Naidu, M., Lee, C., & Kannan, K. (2022). Occurrence of and dermal exposure to benzene, toluene and styrene found in hand sanitizers from the United States. *Environment International*, *167*, 107449. https://doi.org/https://doi.org/10.1016/j.envint.2022.107449

Palleroni*, N. J., Pieper, D. H., & Moore, E. R. B. (2010). Microbiology of Hydrocarbon-Degrading Pseudomonas. In *Handbook of Hydrocarbon and Lipid Microbiology* (pp. 1787–1798). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-77587-4_129

Pandolfo, E., Barra Caracciolo, A., & Rolando, L. (2023). Recent Advances in Bacterial Degradation of Hydrocarbons. *Water*, *15*(2), 375. https://doi.org/10.3390/w15020375

Parkinson, D., Brown, N., Sorensen, E., Eischen, C., Kupar, J., & Kmetz, T. (2004). Remediation of a chlorinated solvent-contaminated site using steam injection and extraction. *Environmental Geosciences*, *11*(4), 239–253. https://doi.org/10.1306/eg.06150404016

Parthipan, P., Preetham, E., Machuca, L. L., Rahman, P. K. S. M., Murugan, K., & Rajasekar, A. (2017). Biosurfactant and Degradative Enzymes Mediated Crude Oil Degradation by Bacterium Bacillus subtilis A1. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00193

Pathak, P., Ghosh, P., Swaraj, A., Yu, T.-L., & Shen, C.-C. (2022). Role of carbon and sulfur biogeochemical cycles on the seasonal arsenic mobilization process in the shallow groundwater of the Bengal aquifer. *Applied Geochemistry*, *141*, 105322. https://doi.org/10.1016/j.apgeochem.2022.105322

Patterson, B. M., Pribac, F., Barber, C., Davis, G. B., & Gibbs, R. (1993). Biodegradation and retardation of PCE and BTEX compounds in aquifer material from Western Australia using large-scale columns. *Journal of Contaminant Hydrology*, *14*(3–4), 261–278. https://doi.org/10.1016/0169-7722(93)90028-Q

Pawar, R. M. (2012). *The effect of soil pH on degradation of polycyclic aromatic hydrocarbons (PAHs)*.

Piga, B., Casasso, A., Pace, F., Godio, A., & Sethi, R. (2017). Thermal Impact Assessment of Groundwater Heat Pumps (GWHPs): Rigorous vs. Simplified Models. *Energies*, *10*(9), 1385. https://doi.org/10.3390/en10091385

Powers, S. E., Hunt, C. S., Heermann, S. E., Corseuil, H. X., Rice, D., & Alvarez, P. J. J. (2001). The Transport and Fate of Ethanol and BTEX in Groundwater Contaminated by Gasohol. *Critical Reviews in Environmental Science and Technology*, *31*(1), 79–123. https://doi.org/10.1080/20016491089181

Price, S. L., Kasevich, R. S., Johnson, M. A., Wiberg, D., & Marley, M. C. (1999). Radio Frequency Heating for Soil Remediation. *Journal of the Air & Waste Management Association*, *49*(2), 136–145. https://doi.org/10.1080/10473289.1999.10463796

Rahman, K. S. M., Thahira-Rahman, J., Lakshmanaperumalsamy, P., & Banat, I. M. (2002). Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresource Technology*, *85*(3), 257–261. https://doi.org/10.1016/S0960-8524(02)00119-0

Rajendiran, T., Sabarathinam, C., Panda, B., & Elumalai, V. (2023). Influence of Dissolved Oxygen, Water Level and Temperature on Dissolved Organic Carbon in Coastal Groundwater. *Hydrology*, *10*(4), 85. https://doi.org/10.3390/hydrology10040085

Ranucci, E., Treccani, S., Ferruti, P., & Alongi, J. (2024). The Seed Germination Test as a Valuable Tool for the Short-Term Phytotoxicity Screening of Water-Soluble Polyamidoamines. *Polymers*, *16*(12). https://doi.org/10.3390/polym16121744

Ravi, A., Ravuri, M., Krishnan, R., Narenkumar, J., Anu, K., Alsalhi, M. S., Devanesan, S., Kamala-Kannan, S., & Rajasekar, A. (2022). Characterization of petroleum degrading bacteria and its optimization conditions on effective utilization of petroleum hydrocarbons. *Microbiological Research*, *265*, 127184. https://doi.org/10.1016/j.micres.2022.127184

Richardson, A. E., & Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology*, *156*(3), 989–996. https://doi.org/10.1104/pp.111.175448

Riedel, T. (2019). Temperature-associated changes in groundwater quality. *Journal of Hydrology*, *572*, 206–212. https://doi.org/10.1016/j.jhydrol.2019.02.059

Rivett, M. O., Buss, S. R., Morgan, P., Smith, J. W. N., & Bemment, C. D. (2008). Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. *Water Research*, *42*(16), 4215–4232. https://doi.org/10.1016/j.watres.2008.07.020

Robinson, C., Brovelli, A., Barry, D. A., & Li, L. (2009). Tidal influence on BTEX biodegradation in sandy coastal aquifers. *Advances in Water Resources*, *32*(1), 16–28. https://doi.org/https://doi.org/10.1016/j.advwatres.2008.09.008

Robson, D. B., Germida, J. J., Farrell, R. E., & Knight, D. J. (2004). Hydrocarbon Tolerance Correlates with Seed Mass and Relative Growth Rate. *Bioremediation Journal*, *8*(3–4), 185–199. https://doi.org/10.1080/10889860490887536

Roohidehkordi, I., & Krol, M. M. (2021). Applicability of ground source heat pumps as a bioremediation-enhancing technology for monoaromatic hydrocarbon contaminants. *Science of The Total Environment*, *778*, 146235. https://doi.org/10.1016/j.scitotenv.2021.146235

Roques, O., Bayard, R., Le Maux, J., Patureau, D., Nélieu, S., Lamy, I., & Bedell, J.-P. (2023). Assessing the chronic toxicity of spreading organic amendments on agricultural soil: Tests on earthworms and plants. *Ecotoxicology and Environmental Safety*, *265*, 115504. https://doi.org/10.1016/j.ecoenv.2023.115504

Ruiz-Aguilar, G. M. L., O'Reilly, K., & Alvarez, P. J. J. (2003). A Comparison of Benzene and Toluene Plume Lengths for Sites Contaminated with Regular vs. Ethanol-Amended Gasoline. *Groundwater Monitoring & Remediation*, *23*(1), 48–53. https://doi.org/10.1111/j.1745-6592.2003.tb00782.x

Salisbury, F. B., Bugbee, B., & Bubenheim, D. (1987). Wheat production in controlled environments. *Advances in Space Research*, *7*(4), 123–132. https://doi.org/10.1016/0273-1177(87)90043-3

Sathesh-Prabu, C., Woo, J., Kim, Y., Kim, S. M., Lee, S. B., Jeon, C. O., Kim, D., & Lee, S. K. (2023). Comparative Genomic Analysis and BTEX Degradation Pathways of a Thermotolerant Cupriavidus cauae PHS1. *Journal of Microbiology and Biotechnology*, *33*(7), 875–885. https://doi.org/10.4014/jmb.2301.01011

Sauer, D., & Burroughs, R. (1986). Disinfection of Seed Surfaces with Sodium Hypochlorite. *Phytopathology*, *76*(7), 745–749.

Saunders, P. J., McCoy, D., Goldstein, R., Saunders, A. T., & Munroe, A. (2018). A review of the public health impacts of unconventional natural gas development. *Environmental Geochemistry and Health*, *40*(1), 1–57. https://doi.org/10.1007/s10653-016-9898-x

Savoie, J. G., Lyford, F. P., & Clifford, S. (1998). Potential for Advection of Volatile Organic Compounds in Ground Water to the Cochato River, Baird & McGuire Superfund Site, Holbrook, Massachusetts, March and April 1998. In *Water-resources investigations report* (Vol. 98, Number 4257).

Schönsee, C. D., Wettstein, F. E., & Bucheli, T. D. (2021). Phytotoxin sorption to clay minerals. *Environmental Sciences Europe*, *33*(1), 36. https://doi.org/10.1186/s12302-021-00469-z

Seagren, E. A., & Becker, J. G. (2002). Review of Natural Attenuation of BTEX and MTBE in Groundwater. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, *6*(3), 156–172. https://doi.org/10.1061/(ASCE)1090-025X(2002)6:3(156)

Sharma, I. (2021). Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects. In *Trace Metals in the Environment - New Approaches and Recent Advances*. IntechOpen. https://doi.org/10.5772/intechopen.90453

Shores, A., Laituri, M., & Butters, G. (2017). Produced Water Surface Spills and the Risk for BTEX and Naphthalene Groundwater Contamination. *Water, Air, & Soil Pollution*, *228*(11), 435. https://doi.org/10.1007/s11270-017-3618-8

Siddique, T., Mohamad Shahimin, M. F., Zamir, S., Semple, K., Li, C., & Foght, J. M. (2015). Long-Term Incubation Reveals Methanogenic Biodegradation of C5 and C6iso-Alkanes in Oil Sands Tailings. *Environmental Science & Technology*, *49*(24), 14732–14739. https://doi.org/10.1021/acs.est.5b04370

Siddiqui, S. C. F. M. A. A. (2008). Germination behavior of wheat (triticum aestivum) varieties to artificial ageing under varying temperature and humidity. *Pakistan Journal of Botany*, *70*(3), 1121–1127.

Simmer, R. A., & Schnoor, J. L. (2022). Phytoremediation, Bioaugmentation, and the Plant Microbiome. *Environmental Science & Technology*, *56*(23), 16602–16610. https://doi.org/10.1021/acs.est.2c05970

Soltani, M., M. Kashkooli, F., Dehghani-Sanij, A. R., Kazemi, A. R., Bordbar, N., Farshchi, M. J., Elmi, M., Gharali, K., & B. Dusseault, M. (2019). A comprehensive study of geothermal heating and cooling systems. *Sustainable Cities and Society*, *44*, 793–818. https://doi.org/10.1016/j.scs.2018.09.036

Somboon, K., Doble, A., Bulmer, D., Baslé, A., Khalid, S., & van den Berg, B. (2020). Uptake of monoaromatic hydrocarbons during biodegradation by FadL channel-mediated lateral diffusion. *Nature Communications*, *11*(1), 6331. https://doi.org/10.1038/s41467-020-20126-y

Sommer, W., Drijver, B., Sommer, W., Verburg, R., Slenders, H., De Vries, E., Dinkla, I., Leusbrock, I., & Grotenhuis, T. (2013). Combining shallow geothermal energy and groundwater remediation. In *European Geothermal Congress*. www.meermetbodemenergie.nl

Sparnins, V. L., Chapman, P. J., & Dagley, S. (1974). Bacterial Degradation of 4-Hydroxyphenylacetic Acid and Homoprotocatechuic Acid. *Journal of Bacteriology*, *120*(1), 159–167. https://doi.org/10.1128/jb.120.1.159-167.1974

Srivastava, J., Naraian, R., Kalra, S. J. S., & Chandra, H. (2014). Advances in microbial bioremediation and the factors influencing the process. *International Journal of Environmental Science and Technology*, *11*(6), 1787–1800. https://doi.org/10.1007/s13762-013-0412-z

Statistics Canada. (2025). *Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units*. https://doi.org/10.25318/3210035901-eng

Steliga, T., & Kluk, D. (2020). Application of Festuca arundinacea in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. *Ecotoxicology and Environmental Safety*, *194*, 110409. https://doi.org/10.1016/j.ecoenv.2020.110409

Steliga, T., & Kluk, D. (2021). Assessment of the Suitability of Melilotus officinalis for Phytoremediation of Soil Contaminated with Petroleum Hydrocarbons (TPH and PAH), Zn, Pb and Cd Based on Toxicological Tests. *Toxics*, *9*(7), 148. https://doi.org/10.3390/toxics9070148

Stevenazzi, S., Bonfanti, M., Masetti, M., Nghiem, S. V., & Sorichetta, A. (2017). A versatile method for groundwater vulnerability projections in future scenarios. *Journal of Environmental Management*, *187*, 365–374. https://doi.org/10.1016/j.jenvman.2016.10.057

Szulc, A., Ambrożewicz, D., Sydow, M., Ławniczak, Ł., Piotrowska-Cyplik, A., Marecik, R., & Chrzanowski, Ł. (2014). The influence of bioaugmentation and biosurfactant addition on bioremediation efficiency of diesel-oil contaminated soil: Feasibility during field studies. *Journal of Environmental Management*, *132*, 121–128. https://doi.org/10.1016/j.jenvman.2013.11.006

Tang, J., Wang, R., Niu, X., & Zhou, Q. (2010). Enhancement of soil petroleum remediation by using a combination of ryegrass (Lolium perenne) and different microorganisms. *Soil and Tillage Research*, *110*(1), 87–93. https://doi.org/10.1016/j.still.2010.06.010

Tang, Y., Nakashima, S., Saiki, S., Myoi, Y., Abe, N., Kuwazuru, S., Zhu, B., Ashida, H., Murata, Y., & Nakamura, Y. (2016a). 3,4-Dihydroxyphenylacetic acid is a predominant biologically-active catabolite of quercetin glycosides. *Food Research International*, *89*, 716–723. https://doi.org/10.1016/j.foodres.2016.09.034

Tang, Y., Nakashima, S., Saiki, S., Myoi, Y., Abe, N., Kuwazuru, S., Zhu, B., Ashida, H., Murata, Y., & Nakamura, Y. (2016b). 3,4-Dihydroxyphenylacetic acid is a predominant biologically-active catabolite of quercetin glycosides. *Food Research International*, *89*, 716–723. https://doi.org/10.1016/j.foodres.2016.09.034

Taylor, R. G., Scanlon, B., Döll, P., Rodell, M., van Beek, R., Wada, Y., Longuevergne, L., Leblanc, M., Famiglietti, J. S., Edmunds, M., Konikow, L., Green, T. R., Chen, J., Taniguchi, M., Bierkens, M. F. P., MacDonald, A., Fan, Y., Maxwell, R. M., Yechieli, Y., … Treidel, H. (2013). Ground water and climate change. *Nature Climate Change*, *3*(4), 322–329. https://doi.org/10.1038/nclimate1744

Treasury Board of Canada Secretariat. (2024). *Federal Contaminated Sites Inventory*. Government of Canada.

Ugrekhelidze, D., Korte, F., & Kvesitadze, G. (1997). Uptake and Transformation of Benzene and Toluene by Plant Leaves. *Ecotoxicology and Environmental Safety*, *37*(1), 24–29. https://doi.org/10.1006/eesa.1996.1512

Ulrich, A. C., Tappenden, K., Armstrong, J., & Biggar, K. W. (2010). Effect of cold temperature on the rate of natural attenuation of benzene, toluene, ethylbenzene, and the three isomers of xylene (BTEX). *Canadian Geotechnical Journal*, *47*(5), 516–527. https://doi.org/10.1139/T09-114

UNESCO World Water Assessment Programme. (2022). *Groundwater : making the invisible visible*. United Nations Educational, Scientific and Cultural Organization. https://unesdoc.unesco.org/ark:/48223/pf0000380721

United States Environmental Protection Agency. (1998). *Guidelines for Ecological Risk Assessment*.

US EPA. (1996). *Ecological Effects Test Guidelines: Seed Germination/Root Elongation Toxicity Test*.

US EPA. (2021). *Oxygen Demand, Chemical Method 8000*.

US EPA Office of Water. (2001). *Method 1684: Total, Fixed, and Volatile Solids in Water, Solid, and Biosolids*.

Valerio, M. E., García, J. F., & Peinado, F. M. (2007). Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (Lactuca sativa L.). *Science of The Total Environment*, *378*(1–2), 63–66. https://doi.org/10.1016/j.scitotenv.2007.01.007

Van Stempvoort, D., & Biggar, K. (2008). Potential for bioremediation of petroleum hydrocarbons in groundwater under cold climate conditions: A review. *Cold Regions Science and Technology*, *53*(1), 16–41. https://doi.org/10.1016/J.COLDREGIONS.2007.06.009

Varasteh, K., Babaei, A., & Abdoli, M. (2015). The Effect of Different Sodium Hypochlorite Concentrations on Seed Germination of Dracocephalum Moldavica L. *Austin Journal of Plant Biology*. www.austinpublishinggroup.com

Vidali, M. (2001). Bioremediation. An overview. *Pure and Applied Chemistry*, *73*(7). https://doi.org/10.1351/pac200173071163

Vienken, T., Kreck, M., & Dietrich, P. (2019). Monitoring the impact of intensive shallow geothermal energy use on groundwater temperatures in a residential neighborhood. *Geothermal Energy*, *7*(1), 8. https://doi.org/10.1186/s40517-019-0123-x

Wan, J., & Winn, L. M. (2004). The effects of benzene and the metabolites phenol and catechol on c-Myb and Pim-1 signaling in HD3 cells. *Toxicology and Applied Pharmacology*, *201*(2), 194–201. https://doi.org/10.1016/j.taap.2004.05.010

Wang, L., Cheng, Y., Wu, C., Luo, F., Lin, Z., & Naidu, R. (2023). Rapid on-site detection of underground petroleum pipeline leaks and risk assessment using portable gas chromatography-mass spectrometry and solid phase microextraction. *Journal of Chromatography A*, *1696*, 463980. https://doi.org/10.1016/j.chroma.2023.463980

Wang, M., Dowling, D. N., & Germaine, K. J. (2025). Bioremediation of Diesel-Contaminated Soil: Hydrocarbon Degradation and Soil Toxicity Reduction by Constructed Bacterial Consortia. *Applied Sciences*, *15*(18), 10143. https://doi.org/10.3390/app151810143

Wang, Q., Guo, S., Ali, M., Song, X., Tang, Z., Zhang, Z., Zhang, M., & Luo, Y. (2022). Thermally enhanced bioremediation: A review of the fundamentals and applications in soil and groundwater remediation. In *Journal of Hazardous Materials* (Vol. 433). https://doi.org/10.1016/j.jhazmat.2022.128749

Wang, W. (1991). Literature review on higher plants for toxicity testing. *Water, Air, and Soil Pollution*, *59*(3–4), 381–400. https://doi.org/10.1007/BF00211845

Wang, Z., Yang, C., Yang, Z., Brown, C. E., Hollebone, B. P., & Stout, S. A. (2016). 4 - Petroleum biomarker fingerprinting for oil spill characterization and source identification. In S. A. Stout & Z. Wang (Eds.), *Standard Handbook Oil Spill Environmental Forensics (Second Edition)* (pp. 131–254). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-803832-1.00004-0

Wanjala, P. M., Opoku, B. K., Ibisime, E., & Wafula, E. N. (2025). Assessment of the impact of benzene, toluene, ethylbenzene, and xylene (BTEX) on soil microbial population in selected areas of Port Harcourt City, Nigeria. *Scientific African*, *27*, e02525. https://doi.org/10.1016/j.sciaf.2024.e02525

Watanabe, M. D. B., & Ortega, E. (2011). Ecosystem services and biogeochemical cycles on a global scale: valuation of water, carbon and nitrogen processes. *Environmental Science & Policy*, *14*(6), 594–604. https://doi.org/10.1016/j.envsci.2011.05.013

Weber, K. A., Achenbach, L. A., & Coates, J. D. (2006). Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology*, *4*(10), 752–764. https://doi.org/10.1038/nrmicro1490

Weelink, S. A. B., van Eekert, M. H. A., & Stams, A. J. M. (2010). Degradation of BTEX by anaerobic bacteria: physiology and application. *Reviews in Environmental Science and Bio/Technology*, *9*(4), 359–385. https://doi.org/10.1007/s11157-010-9219-2

Wei, H., Song, S., Tian, H., & Liu, T. (2013). Effects of phenanthrene on seed germination and some physiological activities of wheat seedling. *Comptes Rendus. Biologies*, *337*(2), 95–100. https://doi.org/10.1016/j.crvi.2013.11.005

Werder, E. J., Engel, L. S., Blair, A., Kwok, R. K., McGrath, J. A., & Sandler, D. P. (2019). Blood BTEX levels and neurologic symptoms in Gulf states residents. *Environmental Research*, *175*, 100–107. https://doi.org/https://doi.org/10.1016/j.envres.2019.05.004

Weschler, C. J., & Shields, H. C. (1997). Potential reactions among indoor pollutants. *Atmospheric Environment*, *31*(21), 3487–3495. https://doi.org/https://doi.org/10.1016/S1352-2310(97)00219-7

Wilson, L. P., & Bouwer, E. J. (1997). Biodegradation of aromatic compounds under mixed oxygen/denitrifying conditions: a review. *Journal of Industrial Microbiology and Biotechnology*, *18*(2–3), 116–130. https://doi.org/10.1038/sj.jim.2900288

Wolf, H. B. (1980). *Geothermal heating and cooling system*. https://patents.google.com/patent/US4325228A/en

Wongbunmak, A., Khiawjan, S., Suphantharika, M., & Pongtharangkul, T. (2017). BTEX- and naphthalene-degrading bacterium Microbacterium esteraromaticum strain SBS1-7 isolated from estuarine sediment. *Journal of Hazardous Materials*, *339*. https://doi.org/10.1016/j.jhazmat.2017.06.016

Wongbunmak, A., Khiawjan, S., Suphantharika, M., & Pongtharangkul, T. (2020). BTEX biodegradation by Bacillus amyloliquefaciens subsp. plantarum W1 and its proposed BTEX biodegradation pathways. *Scientific Reports*, *10*(1), 17408. https://doi.org/10.1038/s41598-020-74570-3

World Health Organization. (2019, June 18). *1 in 3 people globally do not have access to safe drinking water – UNICEF, WHO*.

Wu, H., Du, X., Wu, W., Zheng, J., Song, J., & Xie, J. (2023). Metagenomic analysis reveals specific BTEX degrading microorganisms of a bacterial consortium. *AMB Express*, *13*(1), 48. https://doi.org/10.1186/s13568-023-01541-y

Xi, Y., Song, Y., Johnson, D. M., Li, M., Liu, H., & Huang, Y. (2018). Se enhanced phytoremediation of diesel in soil by Trifolium repens. *Ecotoxicology and Environmental Safety*, *154*, 137–144. https://doi.org/10.1016/j.ecoenv.2018.01.061

Xiao, M., & Zytner, R. G. (2019). The effect of age on petroleum hydrocarbon contaminants in soil for bioventing remediation. *Bioremediation Journal*, *23*(4), 311–325. https://doi.org/10.1080/10889868.2019.1671306

Xu, C., Lin, X., Yin, S., Liu, K., & Liu, W. (2019). Spatio-vertical characterization of the BTEXS group of VOCs in Chinese agricultural soils. *Science of The Total Environment*, *694*, 133631. https://doi.org/10.1016/j.scitotenv.2019.133631

Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., & Ma, Z. (2009). Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. *Plant Physiology*, *149*(2), 760–774. https://doi.org/10.1104/pp.108.132217

Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., & Yu, H. (2018). Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02885

Xue, S., Jiang, X., Wu, C., Hartley, W., Qian, Z., Luo, X., & Li, W. (2020). Microbial driven iron reduction affects arsenic transformation and transportation in soil-rice system. *Environmental Pollution*, *260*, 114010. https://doi.org/10.1016/j.envpol.2020.114010

Yadav, B. K., & Gupta, P. K. (2022). Thermally Enhanced Bioremediation of NAPL Polluted Soil-Water Resources. *Pollutants*, *2*(1), 32–41. https://doi.org/10.3390/pollutants2010005

Yadav, B. K., Shrestha, S. R., & Hassanizadeh, S. M. (2012). Biodegradation of toluene under seasonal and diurnal fluctuations of soil-water temperature. *Water, Air, and Soil Pollution*, *223*(7). https://doi.org/10.1007/s11270-011-1052-x

Yates, M. V., Yates, S. R., & Gerba, C. P. (1988). Modeling microbial fate in the subsurface environment. *Critical Reviews in Environmental Control*, *17*(4), 307–344. https://doi.org/10.1080/10643388809388339

Yu, B., Yuan, Z., Yu, Z., & Xue-song, F. (2022). BTEX in the environment: An update on sources, fate, distribution, pretreatment, analysis, and removal techniques. In *Chemical Engineering Journal* (Vol. 435). https://doi.org/10.1016/j.cej.2022.134825

Yu, H., Kim, B. J., & Rittmann, B. E. (2001). The roles of intermediates in biodegradation of benzene, toluene, and p-xylene by Pseudomonas putida F1. *Biodegradation*, *12*(6), 455–463. https://doi.org/10.1023/A:1015008627732

Yu, H., Xiao, H., & Wang, D. (2014). Effects of soil properties and biosurfactant on the behavior of PAHs in soil-water systems. *Environmental Systems Research*, *3*(1), 6. https://doi.org/10.1186/2193-2697-3-6

Yuniati, M. D. (2018). Bioremediation of petroleum-contaminated soil: A Review. *IOP Conference Series: Earth and Environmental Science*, *118*, 012063. https://doi.org/10.1088/1755-1315/118/1/012063

Yuxuan, T., Xin, C., Ning, D., & Hong, L. (2023). Removal of BTEX from water system by synergistic flocculation of biosurfactant and iron salt. *Science of The Total Environment*, *892*, 164371. https://doi.org/10.1016/j.scitotenv.2023.164371

Zaman, W., Ayaz, A., & Puppe, D. (2025). Biogeochemical Cycles in Plant-Soil Systems: Significance for Agriculture, Interconnections, and Anthropogenic Disruptions. *Biology*, *14*(4). https://doi.org/10.3390/biology14040433

Zanello, V., Scherger, L. E., & Lexow, C. (2021). Assessment of groundwater contamination risk by BTEX from residual fuel soil phase. *SN Applied Sciences*, *3*(3), 307. https://doi.org/10.1007/s42452-021-04325-w

Zeman, N. R., Irianni Renno, M., Olson, M. R., Wilson, L. P., Sale, T. C., & De Long, S. K. (2014). Temperature impacts on anaerobic biotransformation of LNAPL and concurrent shifts in microbial community structure. *Biodegradation*, *25*(4), 569–585. https://doi.org/10.1007/s10532-014-9682-5

Zeng, Y., Wang, S., Huang, F., Luo, Q., Ren, B., Abo El-Maati, M. F., & El-Sappah, A. H. (2025). Fate of polycyclic aromatic hydrocarbons in the phytoremediation of different hydrocarbon contaminated soils with cotton, ryegrass, tall fescue, and wheat. *Frontiers in Plant Science*, *16*. https://doi.org/10.3389/fpls.2025.1550234

Zhang, K., Hu, Z., Zeng, F., Yang, X., Wang, J., Jing, R., Zhang, H., Li, Y., & Zhang, Z. (2019). Biodegradation of petroleum hydrocarbons and changes in microbial community structure in sediment under nitrate-, ferric-, sulfate-reducing and methanogenic conditions. *Journal of Environmental Management*, *249*, 109425. https://doi.org/10.1016/j.jenvman.2019.109425

Zhang, Z., Zhang, C., Yang, Y., Zhang, Z., Tang, Y., Su, P., & Lin, Z. (2022). A review of sulfate-reducing bacteria: Metabolism, influencing factors and application in wastewater treatment. *Journal of Cleaner Production*, *376*, 134109. https://doi.org/10.1016/j.jclepro.2022.134109

Zhao, L., Zhao, L. L., Zhang, Q., & Ding, G. L. (2003). Theoretical and basic experimental analysis on load adjustment of geothermal heat pump systems. *Energy Conversion and Management*, *44*(1). https://doi.org/10.1016/S0196-8904(02)00050-X

Zhao, S., Wang, J., & Zhu, W. (2023). Controlled-Release Materials for Remediation of Trichloroethylene Contamination in Groundwater. *Materials*, *16*(21), 7045. https://doi.org/10.3390/ma16217045

Zhao, X., Zeng, Y., & Zhao, D. (2015). Distributed solar photovoltaics in China: Policies and economic performance. *Energy*, *88*, 572–583. https://doi.org/10.1016/j.energy.2015.05.084

Zhu, B., Friedrich, S., Wang, Z., Táncsics, A., & Lueders, T. (2020). Availability of Nitrite and Nitrate as Electron Acceptors Modulates Anaerobic Toluene-Degrading Communities in Aquifer Sediments. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01867

Zuurbier, K. G., Hartog, N., Valstar, J., Post, V. E. A., & van Breukelen, B. M. (2013). The impact of low-temperature seasonal aquifer thermal energy storage (SATES) systems on chlorinated solvent contaminated groundwater: Modeling of spreading and degradation. *Journal of Contaminant Hydrology*, *147*, 1–13. https://doi.org/10.1016/j.jconhyd.2013.01.002

Zytner, R. G. (1994). Sorption of benzene, toluene, ethylbenzene and xylenes to various media. *Journal of Hazardous Materials*, *38*(1), 113–126. https://doi.org/10.1016/0304-3894(94)00027-1

Appendices

Appendix A : Supporting Information for Groundwater Characterization

Region of Waterloo

Workorder: 23C0955

			LAB #	23C0955-16
			MATRIX	Raw Observation Well
			SAMPLE ID	**314 WT-WC-MW6-11 -A**
	Method	**Units**	**Limit**	
General Chemistry (Water)				
Dissolved Organic Carbon	ROW6500	mg/L		0.66
Fluoride	ROW7500	mg/L		0.173
Chloride	ROW7500	mg/L		9.46
Nitrite-N	ROW7500	mg/L		<0.010
Nitrate-N	ROW7500	mg/L		<0.100
Sulphate	ROW7500	mg/L		76.6
Alkalinity (as CaCO3)	ROW7800	mg/L		243
pH (EST)	ROW7800			8.03
Metals (Water)				
Hardness (as CaCO3)	ROW9550	mg/L		327
Sodium	ROW9550	mg/L		4.53
Magnesium	ROW9550	mg/L		30.1
Calcium	ROW9550	mg/L		81.2
Manganese	ROW9550	mg/L		0.0184
Iron	ROW9550	mg/L		0.481
Arsenic	ROW9550	µg/L		3.24
Selenium	ROW9550	µg/L		1.07
Strontium	ROW9550	mg/L		0.225

Figure A. 1. Water quality analysis report from the Region of Waterloo for the 2022 groundwater sample

Workorder: 24E0562

Date of Issue: 2024-05-28

			LAB #	24E0562-14
			MATRIX	Raw Observation
				Well
			SAMPLE ID	314
				WT-WC-MW6-11-A
	Method	Units	Limit	
General Chemistry (Water)				
Dissolved Organic Carbon	ROW6500	mg/L		0.53
Fluoride	ROW7500	mg/L		0.171
Chloride	ROW7500	mg/L		9.68
Nitrite-N	ROW7500	mg/L	1	<0.010
Nitrate-N	ROW7500	mg/L	10	<0.100
Sulphate	ROW7500	mg/L		78.2
Alkalinity (as CaCO3)	ROW7800	mg/L		260
pH (EST)	ROW7800			8.05
Metals (Water)				
Hardness (as CaCO3)	ROW9550	mg/L		316
Sodium	ROW9550	mg/L	20	3.99
Magnesium	ROW9550	mg/L		28.0
Calcium	ROW9550	mg/L		80.2
Manganese	ROW9550	mg/L		0.0172
Iron	ROW9550	mg/L		0.449
Arsenic	ROW9550	µg/L	10	3.26
Selenium	ROW9550	µg/L	50	<0.200
Strontium	ROW9550	mg/L		0.213

Figure A. 2. Water quality analysis report from the Region of Waterloo for the 2024 groundwater sample

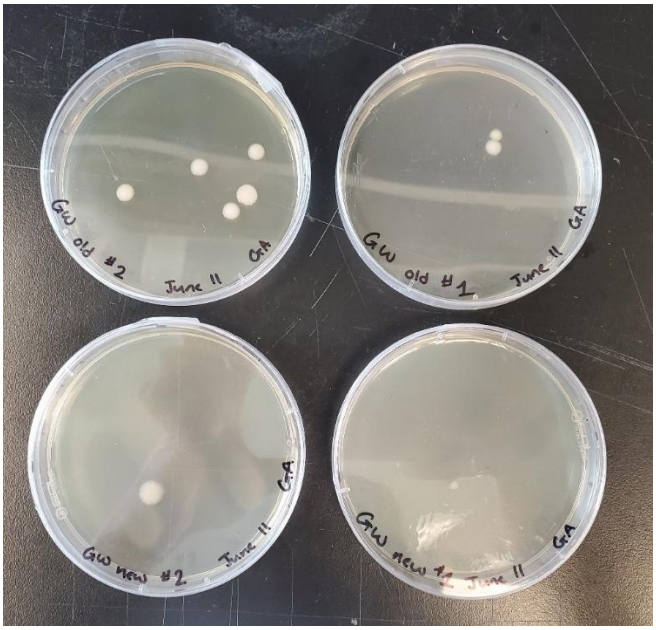


Figure A. 3. Plated groundwater results showing low microbial growth after 48 hours

Table A. 1. CFU counts for groundwater sample (CFU/mL)

		Sample 1	Sample 2
Raw Data	**Old groundwater**	2	5
	New groundwater	1	1
CFU Count CFU/mL =CFU/V x10 $^{\text{dilution factor}}$ V= 0.5 mL	**Old groundwater**	40 CFU/ml	100 CFU/ml
	New groundwater	20 CFU/ml	20 CFU/ml

Appendix B : Nutrient Selection for Groundwater Temperature Experiments

The following Appendix outlines the different nutrient medias used to grow *Microbacterium esteraromaticum* and *Bacillus infantis*. Some experiments did not include duplicate sampling, resulting in no standard deviation in Figures B.1-B.3.

The treatment set was incubated at 28°C and 200 RPM. There was no growth in unsterilized GW.

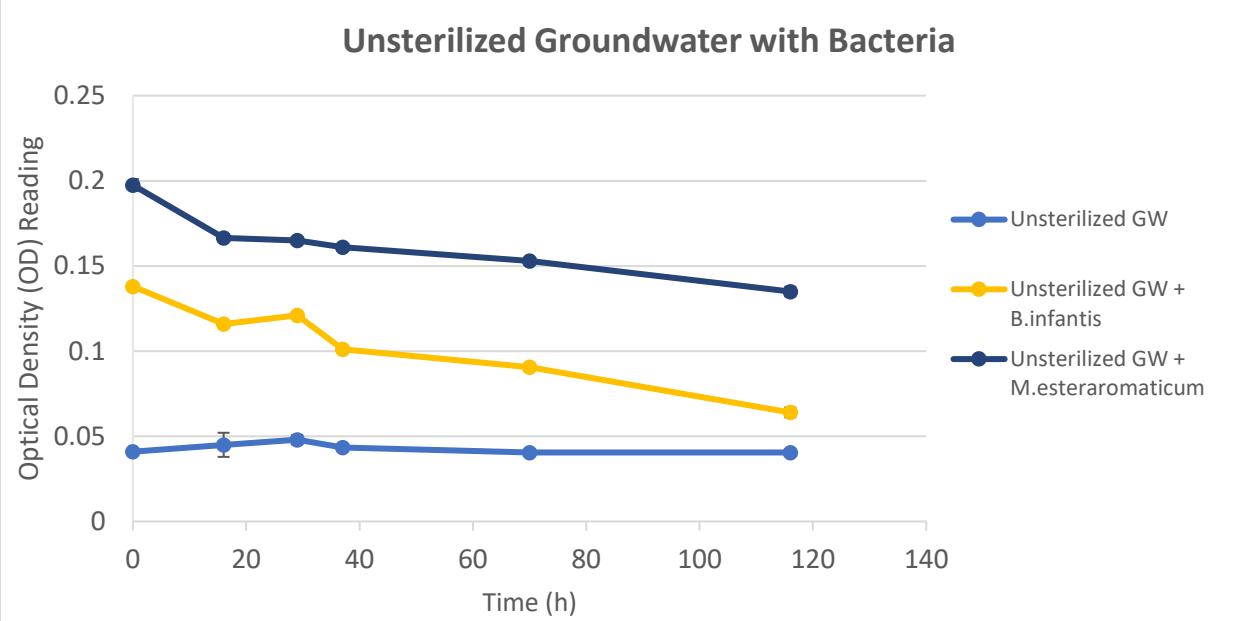


Figure B. 1. Bacterial growth of *Microbacterium esteraromaticum* and *Bacillus infantis* in unsterilized groundwater

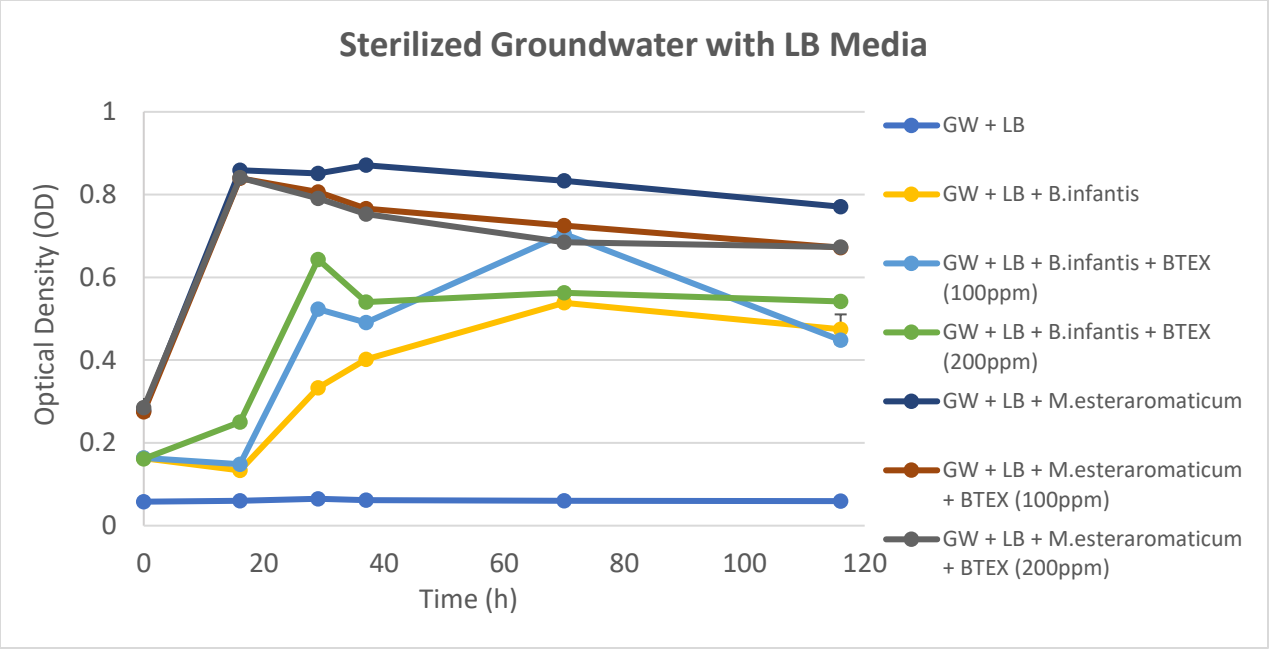


Figure B. 2. Bacterial growth of *Microbacterium esteraromaticum* and *Bacillus infantis* in sterilized groundwater supplemented with lysogeny broth, and different concentrations of BTEX

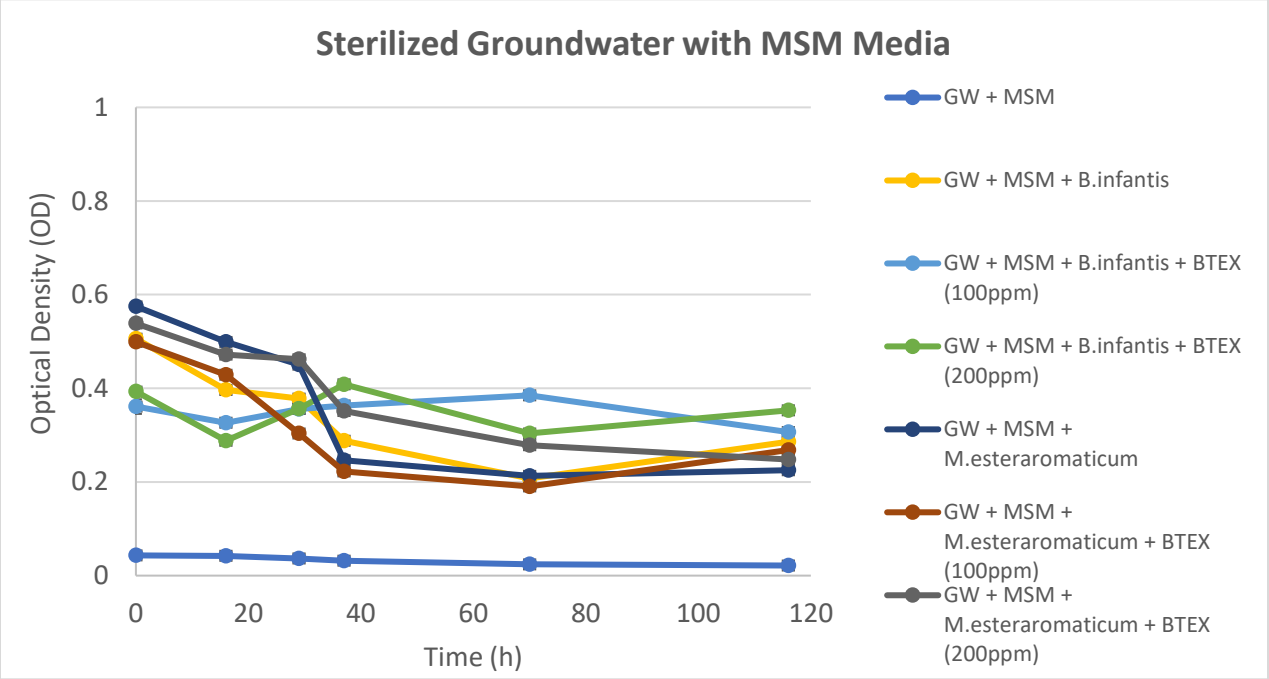


Figure B. 3. Bacterial growth of *Microbacterium esteraromaticum* and *Bacillus infantis* in sterilized groundwater supplemented with mineral salt media, and different concentrations of BTEX

Appendix C : BTEX Degradation Supplementary Material

Calibration curves for B, T, E, X in the Consortium experiment

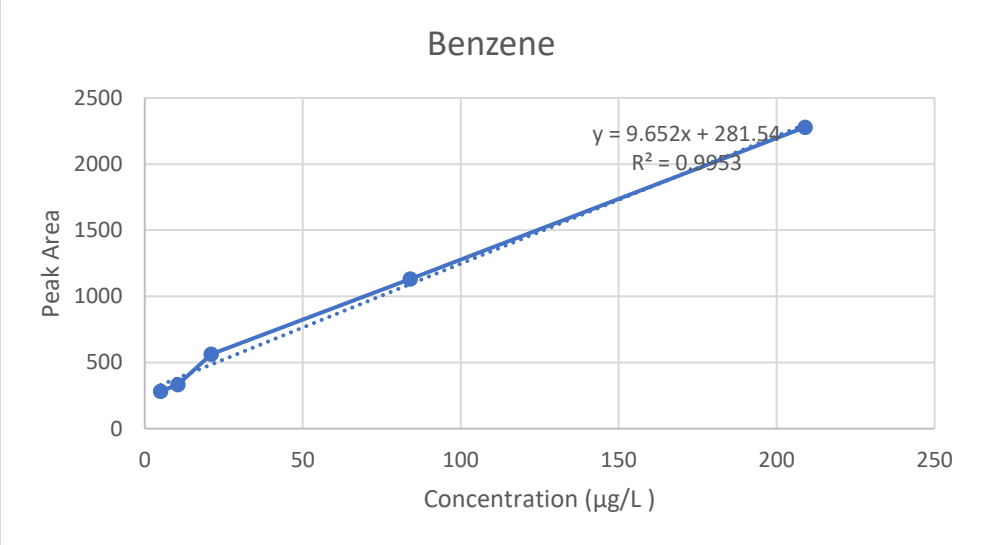


Figure C. 1. Benzene calibration curve

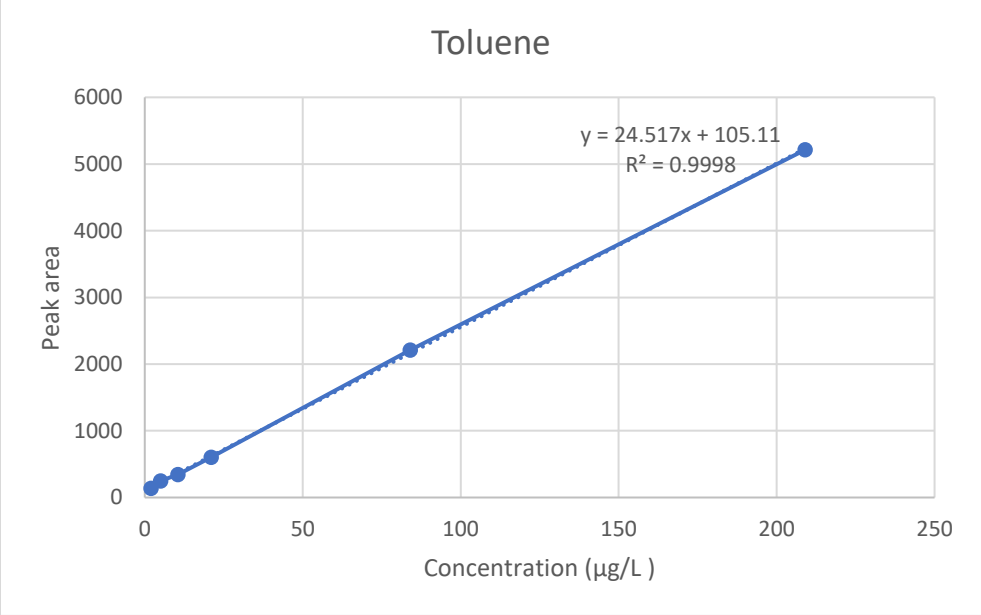


Figure C. 2. Toluene calibration curve

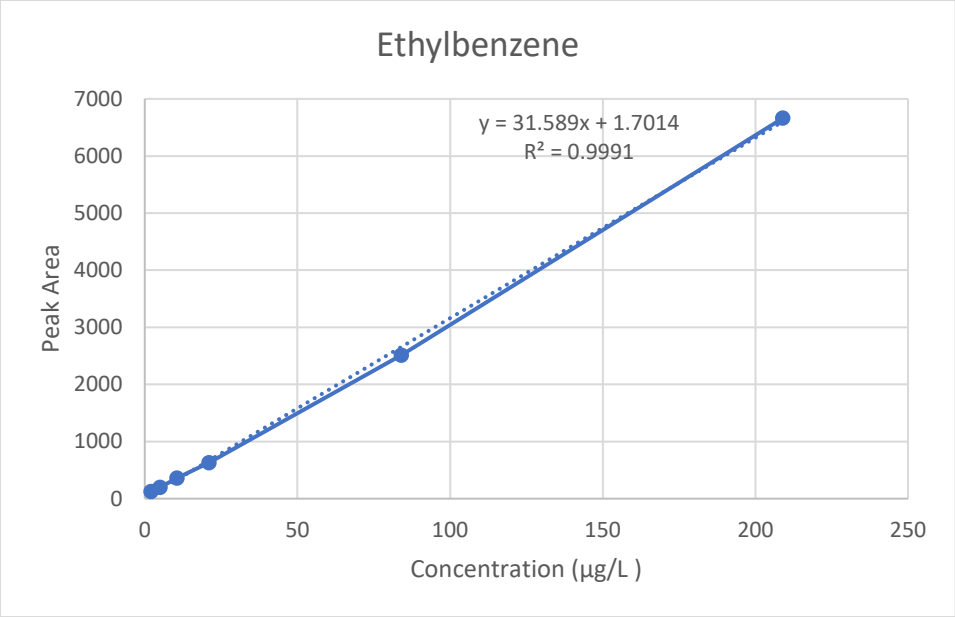


Figure C. 3. Ethylbenzene calibration curve

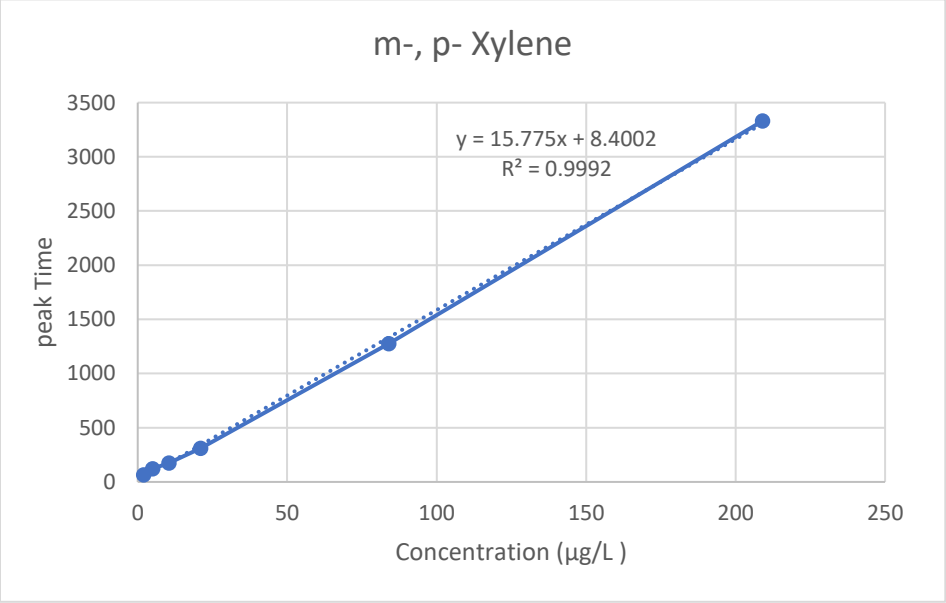


Figure C. 4. m-, p- Xylene calibration curve

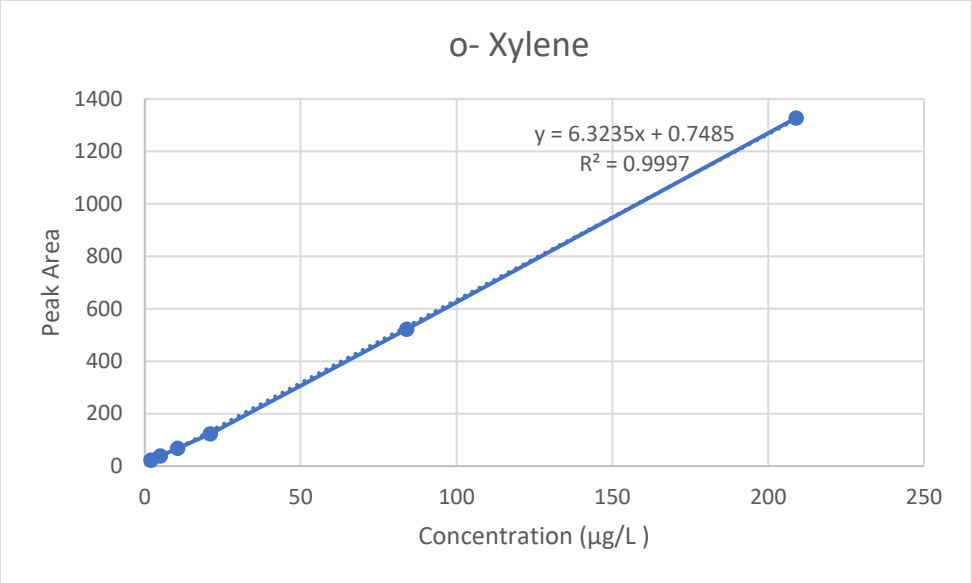


Figure C. 5. o- Xylene calibration curve

***Microbacterium esteraromaticum* degradation results**

The treatment sample contained groundwater, yeast extract, BTEX (200 mg/L), and *Microbacterium esteraromaticum*. Here are the BTEX degradation results for observations only, but due to instrumental errors, this data was not used to compare within the thesis.

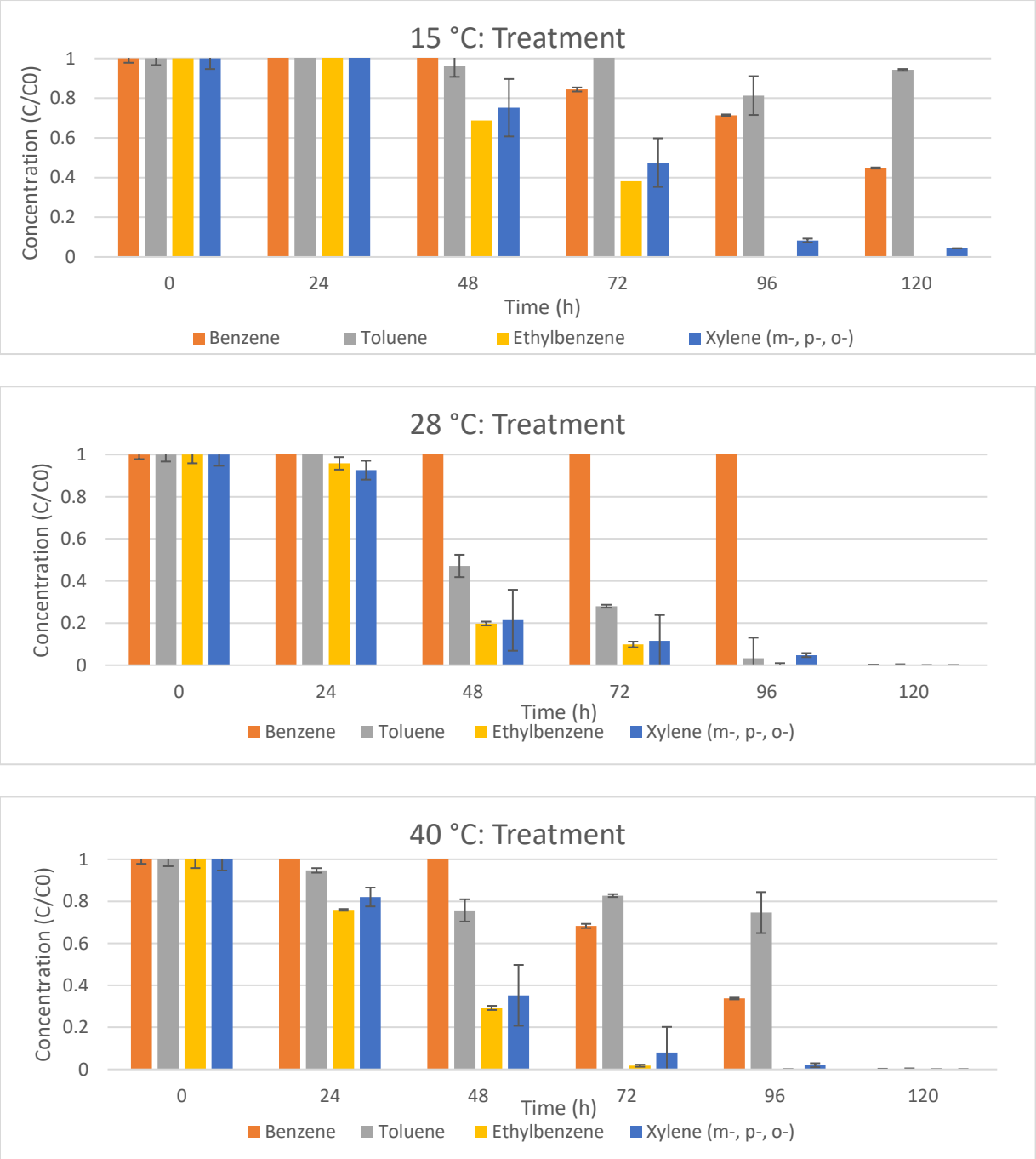


Figure C. 6. *Microbacterium esteraromaticum* BTEX degradation results at 15 °C, 28 °C, and 40 °C of the bacteria treatment

Bacillus infantis degradation results

The treatment sample contained groundwater, yeast extract, BTEX (200 mg/L), and *Bacillus infantis*. Here are the BTEX degradation results for observations only, but due to instrumental errors, this data was not used to compare within the thesis.

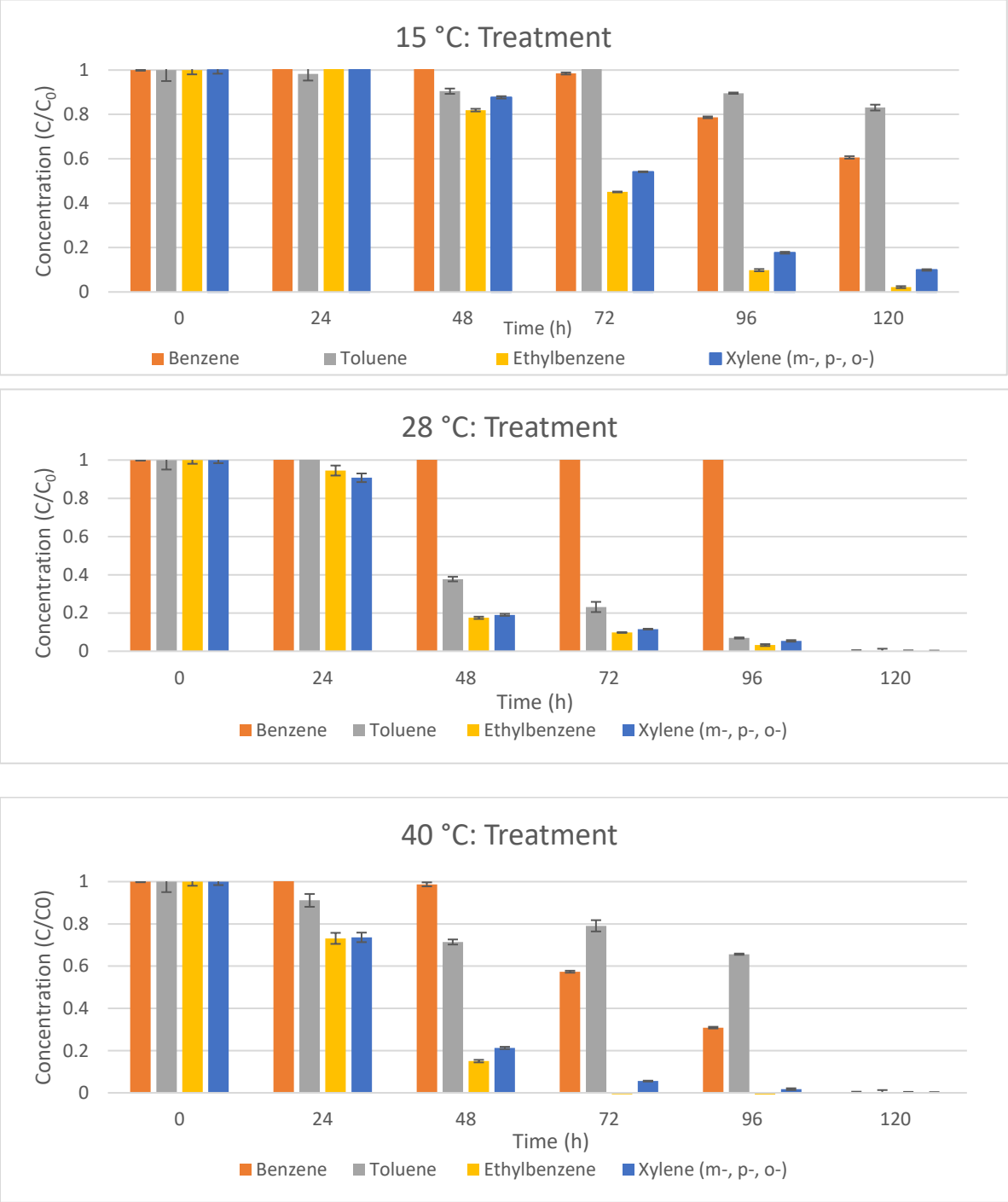


Figure C. 7. *Bacillus infantis* BTEX degradation results at 15 °C, 28 °C, and 40 °C of the bacteria treatment

Consortium degradation results

The treatment sample contained groundwater, yeast extract, BTEX (200 mg/L), and the consortium. Here are the BTEX degradation results at 40 °C for observations only. The results showed no volatilization, which was unexpected; this data was not used to compare within the thesis.

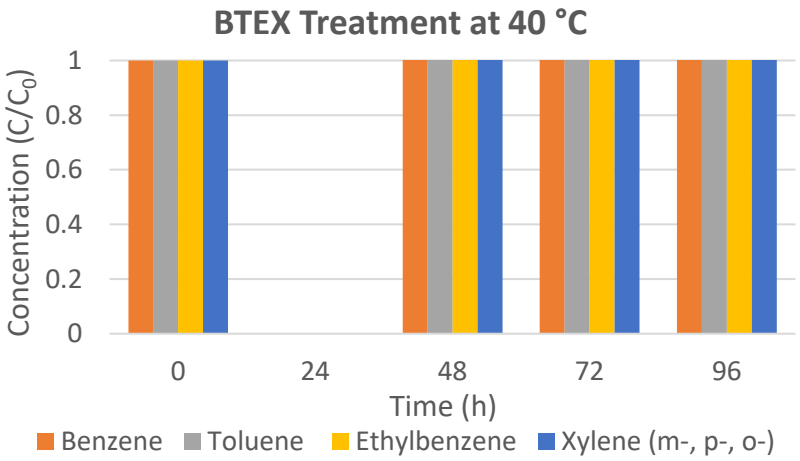


Figure C. 8. Consortium BTEX degradation results at 40 °C of the bacteria treatment

BTEX control used for experiments

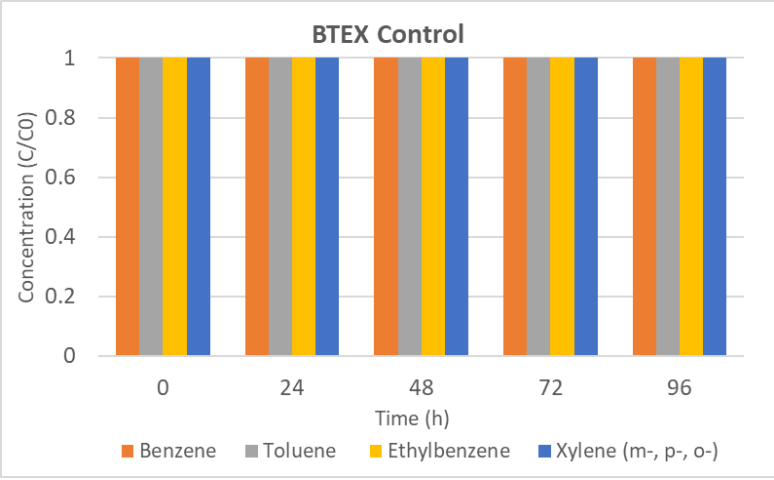


Figure C. 9. BTEX control graph for the consortium experiment

Table C. 1. List of new chemicals identified through the degradation process of consortium treatment set at T0 and T48

Chemical hit	Chemical formula
Dihydrouracil Smart Confirmation	C2HF3O2
3,3-Dimethylglutaric acid (NIST) Smart Confirmation	C4H5N2OPS
2-Methylcinnamic acid (NIST) Smart Confirmation	C3H3N2O4P

Chemical hit	Chemical formula
Uracil Smart Confirmation	C4H4N2O2
3,4-Dihydroxyacetophenone (NIST) Smart Confirmation	C2H5FN4O3
3-Hydroxy-3-methylglutaric acid (NIST) Smart Confirmation	CH9N8OP
o-Tyrosine Smart Confirmation	C7H15F2NOS
2-Isopropylmalic acid Smart Confirmation	C3H8F4N4
Guanidinopropionic acid (NIST) Smart Confirmation	C6H13NO2
Pimelic acid (NIST EL) Smart Confirmation	C7H12O4
2-Aminobenzoic acid Smart Confirmation	C7H7NO2
4-Hydroxy-3-methylbenzoic acid (NIST EL) Smart Confirmation	C8H8O3
1,3-Cyclohexanedicarboxylic acid (NIST EL) Smart Confirmation	C8H12O4
Quinolin-6-ol (NIST EL) Smart Confirmation	C9H7NO
2-Propylglutaric acid (NIST) Smart Confirmation	C8H14O4
4-Methylquinolin-2-ol (NIST) Smart Confirmation	C10H9NO
Phenylacetic acid (NIST) Smart Confirmation	C8H8O2
2-Thiopheneacrylic acid (NIST) Smart Confirmation	C9H14O2
DL-.beta.-Homoleucine (NIST) Smart Confirmation	C9H7NO
Azelaic acid Smart Confirmation	C9H16O4
Indoleacetic acid Smart Confirmation	C10H9NO2
3,4-Dihydroxybenzeneacetic acid Smart Confirmation	C9H12O3
4-Hydroxyisophthalic acid (NIST) Smart Confirmation	C12H10N2
Phenylacetic acid (NIST) Smart Confirmation	C8H8O2
Chrysin (NIST EL) Smart Confirmation	C8H23N4O3P
4-Hydroxyisophthalic acid (NIST) Smart Confirmation	C12H22O
4-Thiazolidinecarboxylic acid, 2-undecyl-, (4R)- (NIST) Smart Confirmation	C16H33NO3
Trihydroxycholestanoic acid (NIST) Smart Confirmation	C24H26F3O3P
Cholesterol sulfate Smart Confirmation	C23H46O9
Saluamine (NIST) Smart Confirmation	C15H22O3
Benzenesulfonic acid, 4-undecyl- (NIST) Smart Confirmation	C10H27FN6P2
3-tert-Butylphenol (NIST EL) Smart Confirmation	C10H14O
3-tert-Butylphenol (NIST) Smart Confirmation	C10H14O
p-Bromophenylacetic acid (NIST) Smart Confirmation	C3H3FN2O8
Decylbenzenesulfonic acid (NIST) Smart Confirmation	C14H20F2N4O
3,4-Dihydroxybenzeneacetic acid Smart Confirmation	CH4N2P4
2-Methylhippuric acid (NIST) Smart Confirmation	CH8FNOP4

Table C. 2. List of new chemicals identified through the degradation process of BTEX between the BTEX control and consortium treatment set at T48

Chemical hit	Chemical formula
Dihydrouracil Smart Confirmation	C2HF3O2
2-Methylcinnamic acid (NIST) Smart Confirmation	C3H3N2O4P

Chemical hit	Chemical formula
2-Deoxyribose 5-phosphate (NIST) Smart Confirmation	C3H5N2PS3
Uracil Smart Confirmation	C4H4N2O2
Hypoxanthine Smart Confirmation	C2H5FN4O2
3,4-Dihydroxyacetophenone (NIST) Smart Confirmation	C2H5FN4O3
3-Hydroxy-3-methylglutaric acid (NIST) Smart Confirmation	CH9N8OP
o-Tyrosine Smart Confirmation	C7H15F2NOS
2-Isopropylmalic acid Smart Confirmation	C3H8F4N4
Guanidinopropionic acid (NIST) Smart Confirmation	C6H13NO2
Pimelic acid (NIST EL) Smart Confirmation	C7H12O4
2-Aminobenzoic acid Smart Confirmation	C7H7NO2
4-Hydroxy-3-methylbenzoic acid (NIST EL) Smart Confirmation	C8H8O3
Suberic acid (NIST EL) Smart Confirmation	C8H14O4
1,3-Cyclohexanedicarboxylic acid (NIST EL) Smart Confirmation	C8H12O4
N-Acetyl-L-phenylalanine (NIST) Smart Confirmation	C11H13NO3
Quinolin-6-ol (NIST EL) Smart Confirmation	C9H7NO
2-Propylglutaric acid (NIST) Smart Confirmation	C8H14O4
4-Methylquinolin-2-ol (NIST) Smart Confirmation	C10H9NO
2-Thiopheneacrylic acid (NIST) Smart Confirmation	C9H14O2
Azelaic acid Smart Confirmation	C9H16O4
Indoleacetic acid Smart Confirmation	C10H9NO2
3,4-Dihydroxybenzeneacetic acid Smart Confirmation	C9H12O3
4-Hydroxyisophthalic acid (NIST) Smart Confirmation	C12H10N2
Ketoprofen (NIST) Smart Confirmation	C7H22N6O2S
Chrysin (NIST EL) Smart Confirmation	C8H23N4O3P
4-Hydroxyisophthalic acid (NIST) Smart Confirmation	C12H22O
4-Thiazolidinecarboxylic acid, 2-undecyl-, (4R)- (NIST) Smart Confirmation	C16H33NO3
Trihydroxycholestanoic acid (NIST) Smart Confirmation	C24H26F3O3P
Cholesterol sulfate Smart Confirmation	C23H46O9
Saluamine (NIST) Smart Confirmation	C15H22O3
Benzenesulfonic acid, 4-undecyl- (NIST) Smart Confirmation	C10H27FN6P2
Retinal Smart Confirmation	C18H36O2
3-tert-Butylphenol (NIST EL) Smart Confirmation	C10H14O
3-tert-Butylphenol (NIST) Smart Confirmation	C10H14O
3-Coumaric acid (NIST EL) Smart Confirmation	C12HF
Myristyl sulfate (NIST) Smart Confirmation	C13H28FN2O2P
3,3-Dimethylglutaric acid (NIST EL) Smart Confirmation	C4H5N2OPS
3,4-Dihydroxybenzeneacetic acid Smart Confirmation	CH4N2P4
2-Methylhippuric acid (NIST) Smart Confirmation	CH8FNOP4

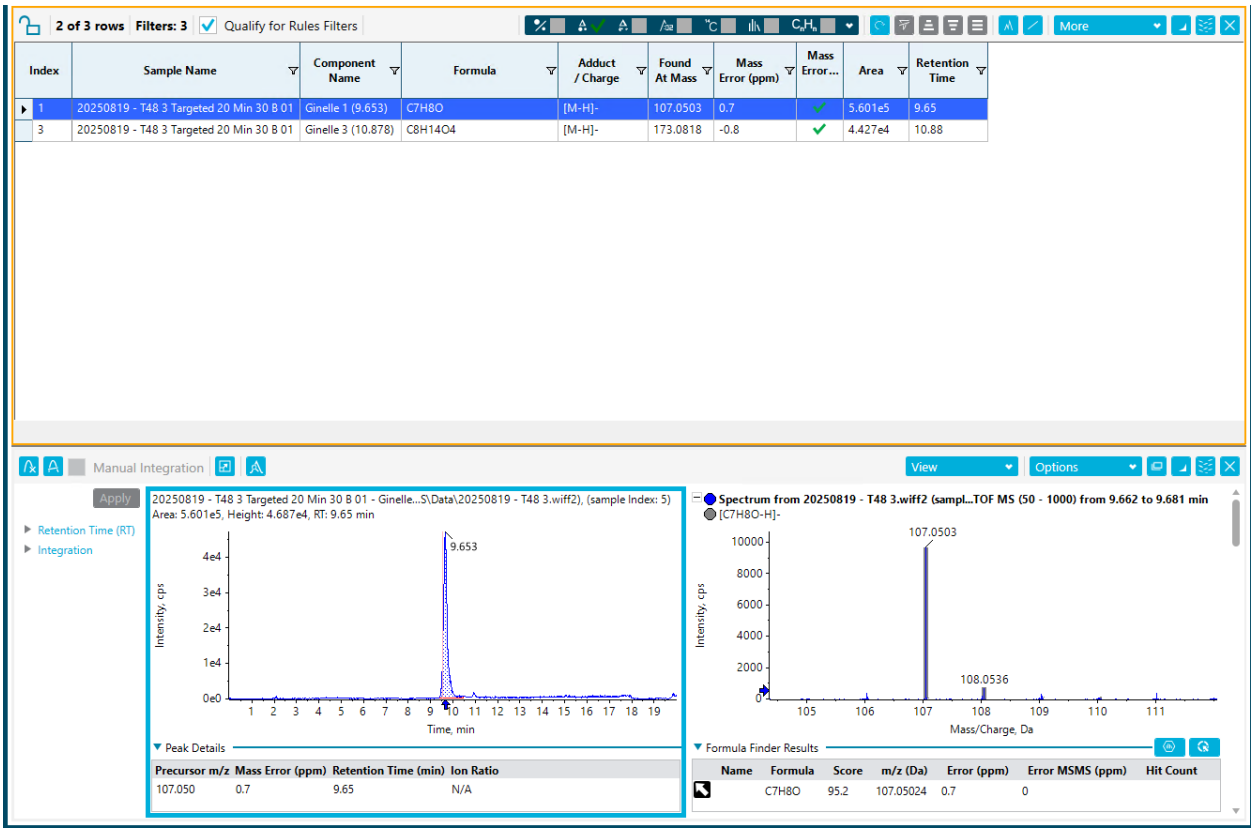


Figure C. 10. Benzyl alcohol putative ID level, m/z, and RT

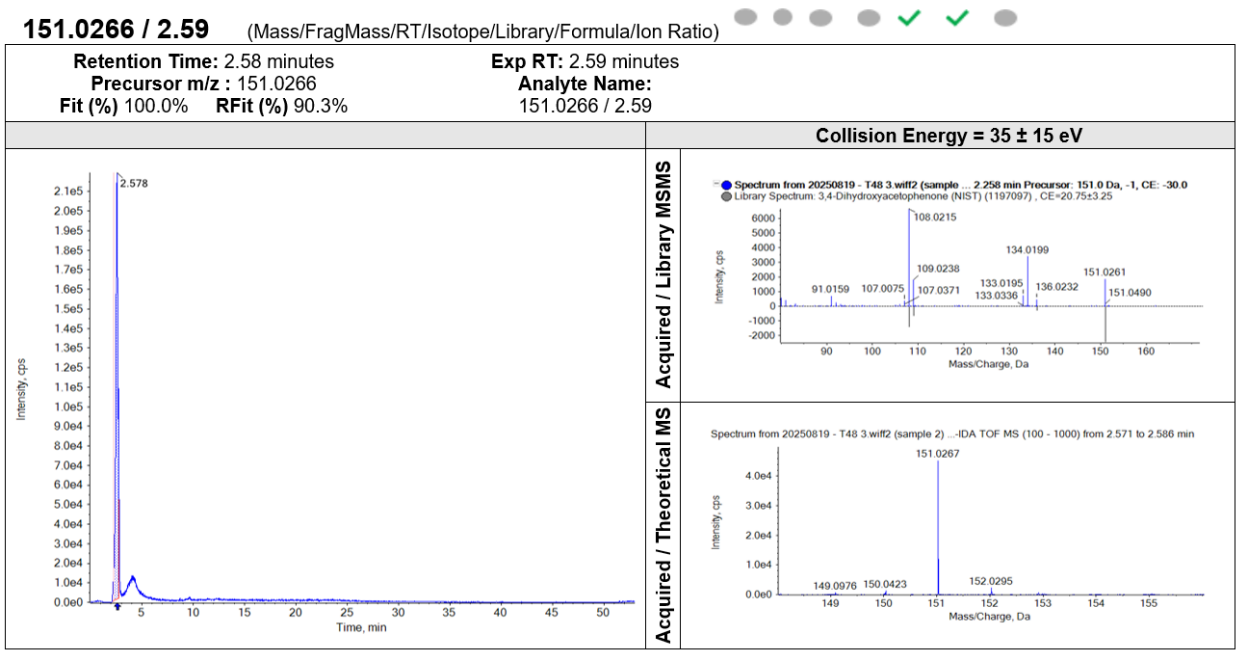


Figure C. 11. 3,4 dihydroxyacetophenone putative ID level, m/z, and RT

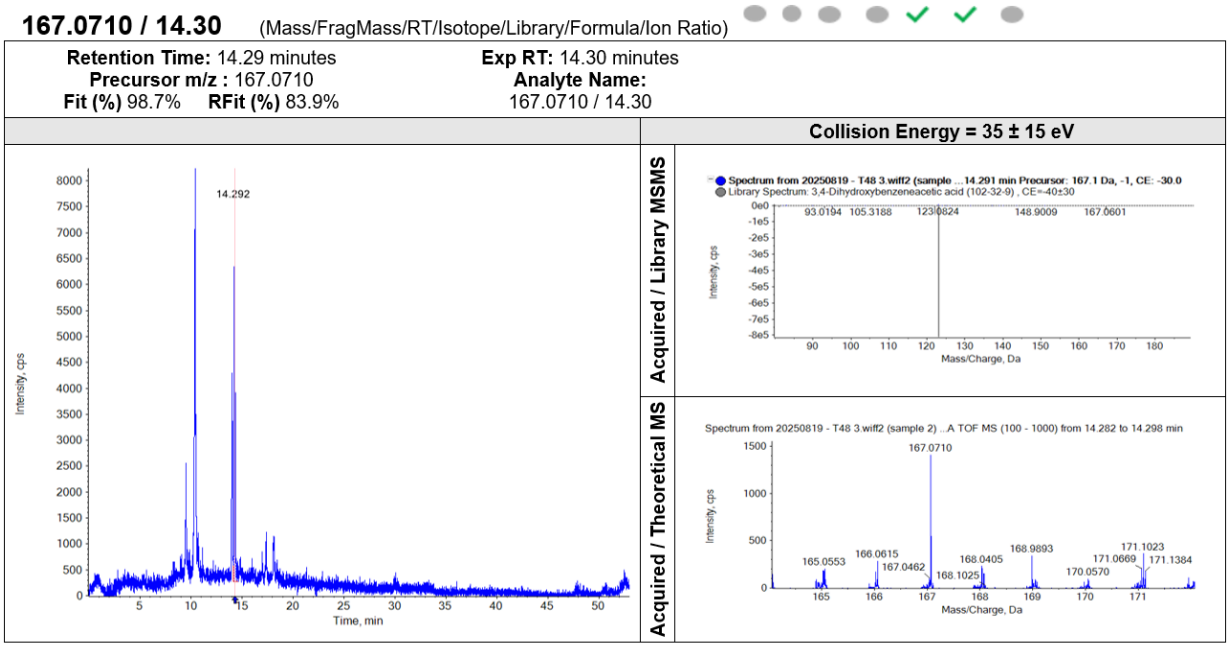


Figure C. 12. 3,4 dihydroxybenzeneacetic acid putative ID level, m/z, and RT

Appendix D : Effects of Temperature on Groundwater Properties

Calibration Curves for the anions

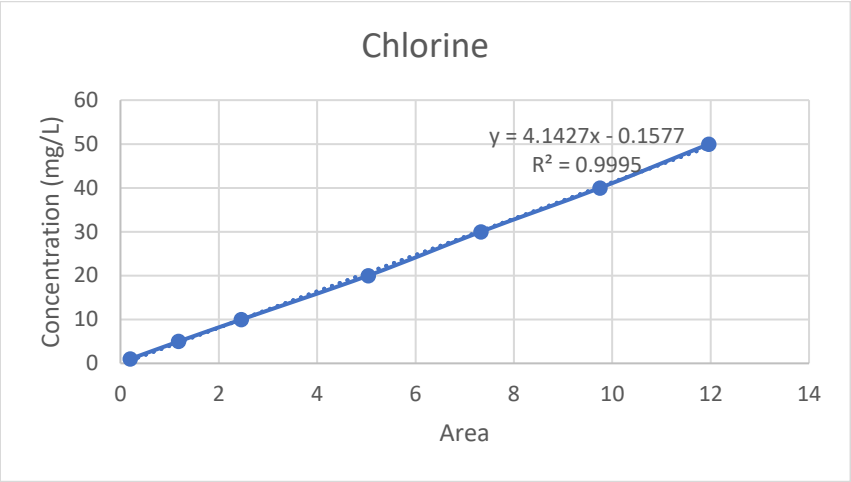


Figure D. 1. Chlorine calibration curve

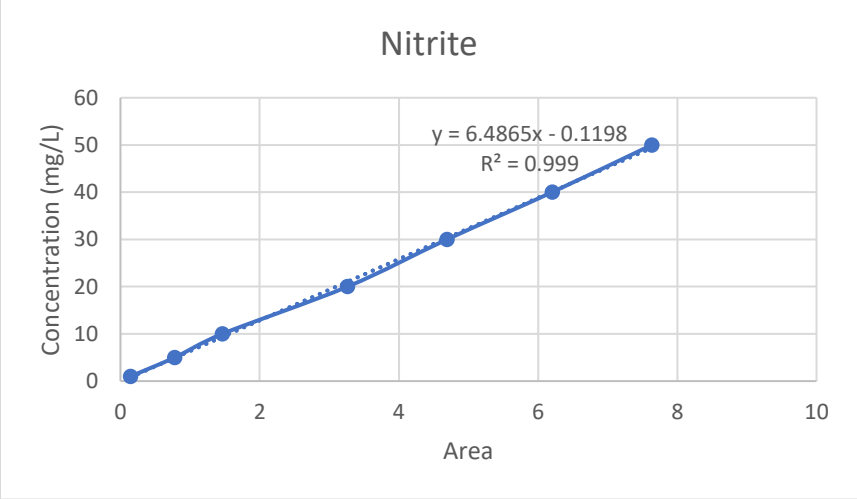


Figure D. 2. Nitrite calibration curve

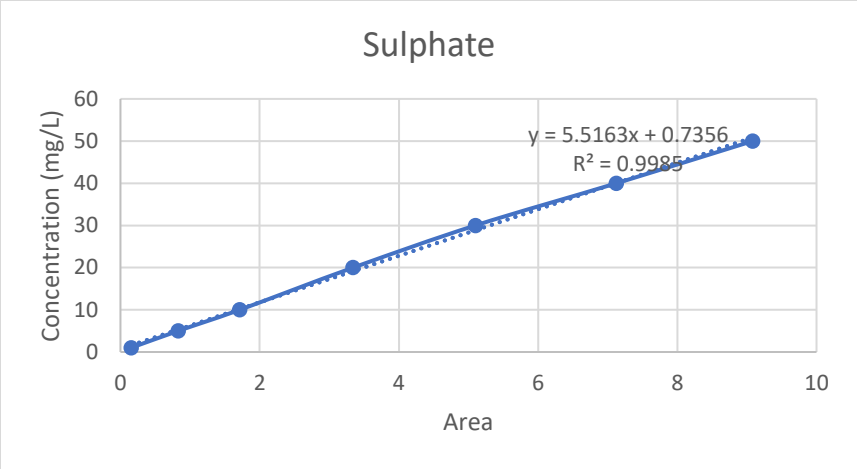


Figure D. 3. Sulphate calibration curve

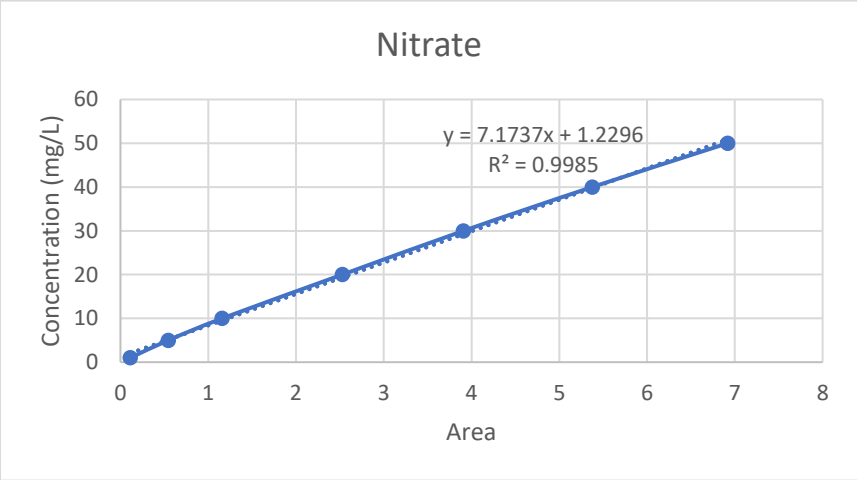


Figure D. 4. Nitrate calibration curve

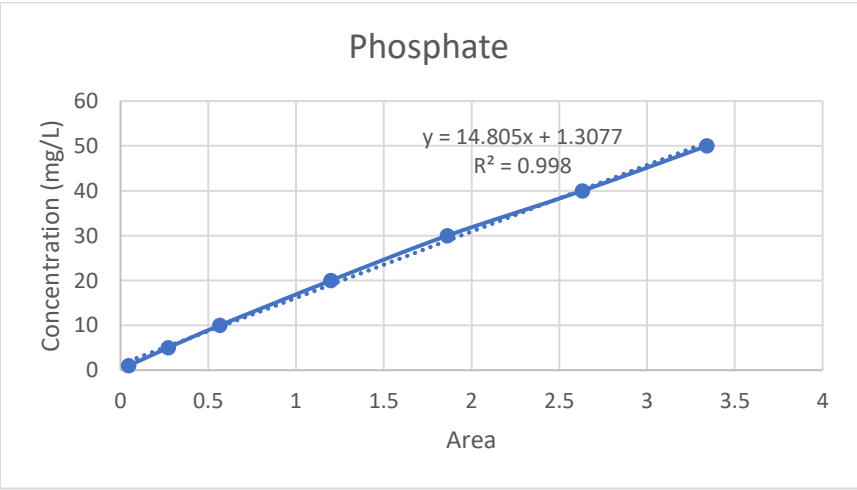


Figure D. 5. Phosphate calibration curve

Table D. 1. Mean (±SD) anion concentrations at 15 °C, 28 °C, and 40 °C on Day 1 and Day 10 under different treatments

	Cl		NO2		SO4		NO3		PO4	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Temperature	15	15	15	15	15	15	15	15	15	15
Groundwater	31± 0.11	42± 0.13	12± 0.05	20± 0.10	77± 0.36	154± 0.89	3± 0.00	3± 0.02	nd	3± 0.00
Groundwater + Consortium	17± 0.05	44± 0.00	24± 0.53	28± 0.13	94± 0.32	186± 0.28	nd	3± 0.28	nd	107± 0.05
Groundwater + BTEX (200 mg/L)	13± 0.01	33± 0.10	16± 0.04	29± 0.54	91± 0.19	174± 0.66	3± 0.01	5± 0.29	nd	105± 0.31
Groundwater + BTEX (200 mg/L) + Consortium	15± 0.10	40± 0.08	23± 1.16	40± 0.22	90± 0.67	236± 0.12	nd	3± 0.02	nd	129± 0.56
Temperature	28	28	28	28	28	28	28	28	28	28
Groundwater	16± 0.15	22± 0.00	11± 0.07	18± 0.02	78± 0.59	153± 0.08	3± 0.08	3± 0.00	nd	5± 0.02
Groundwater + Consortium	15± 0.03	26± 0.07	20± 1.70	30± 0.01	92± 0.33	208± 0.26	nd	3± 0.02	53± 1.45	132± 0.37
Groundwater + BTEX (200 mg/L)	14± 0.06	27± 0.80	21± 0.51	35± 2.31	91± 0.48	175± 1.00	nd	nd	54± 0.12	124± 2.04
Groundwater + BTEX (200 mg/L) + Consortium	13± 0.04	25± 0.14	21± 1.04	40± 0.43	90± 0.12	230± 0.45	nd	4± 0.03	nd	156± 0.55

Temperature	40	40	40	40	40	40	40	40	40	40
Groundwater	12± 0.02	21± 0.07	11± 0.01	17± 0.03	78± 0.16	153± 0.34	3± 0.01	3± 0.01	nd	4± 0.05
Groundwater + Consortium	13± 0.01	28± 0.17	19± 0.72	42± 0.19	91± 0.01	253± 1.17	nd	5± 0.01	50± 1.58	172± 1.40
Groundwater + BTEX (200 mg/L)	11± 0.02	28± 0.06	34± 2.54	28± 0.08	85± 0.17	175± 1.44	nd	nd	54± 1.16	116± 0.01
Groundwater + BTEX (200 mg/L) + Consortium	15± 0.03	28± 0.09	19± 0.73	40± 0.45	91± 0.02	242± 0.44	nd	4± 0.12	51± 2.96	175± 0.56

Appendix E : Seed Germination

Table E. 1. Preliminary trials for seed selection

	Day 1	Day 8	Germination rates and notes
Wheat			-100% germination -3/5 seeds germinated in 3 days
Lettuce			-90% germination - 9/10 seeds germinated in 2 days
Oats			- 14% germination - 1/7 seed germinated after 1 week
Barley			- 71% germination rate - 5/7 seeds germinated after 1 week - 2 seeds grew white mold

Calibration Curves for BTEX concentrations

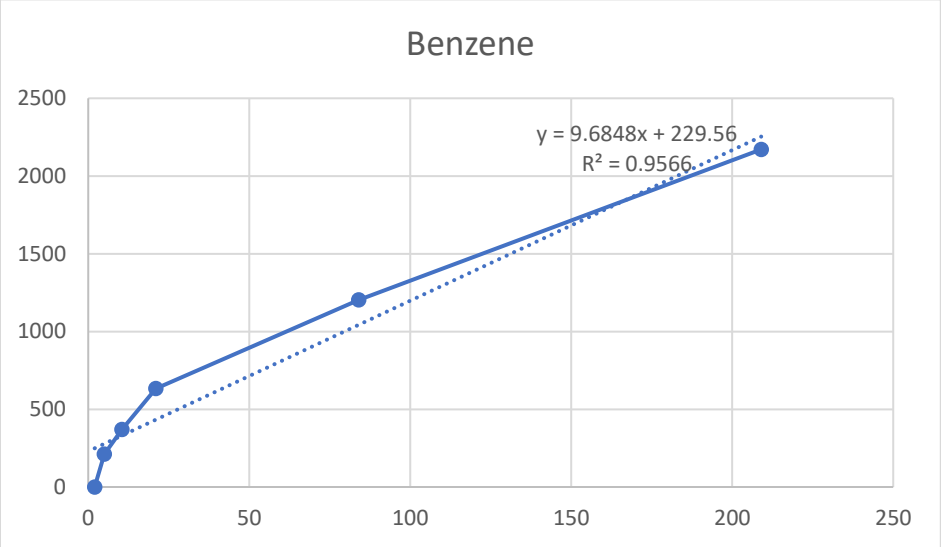


Figure E. 1. Benzene calibration curve for soil and seed analysis

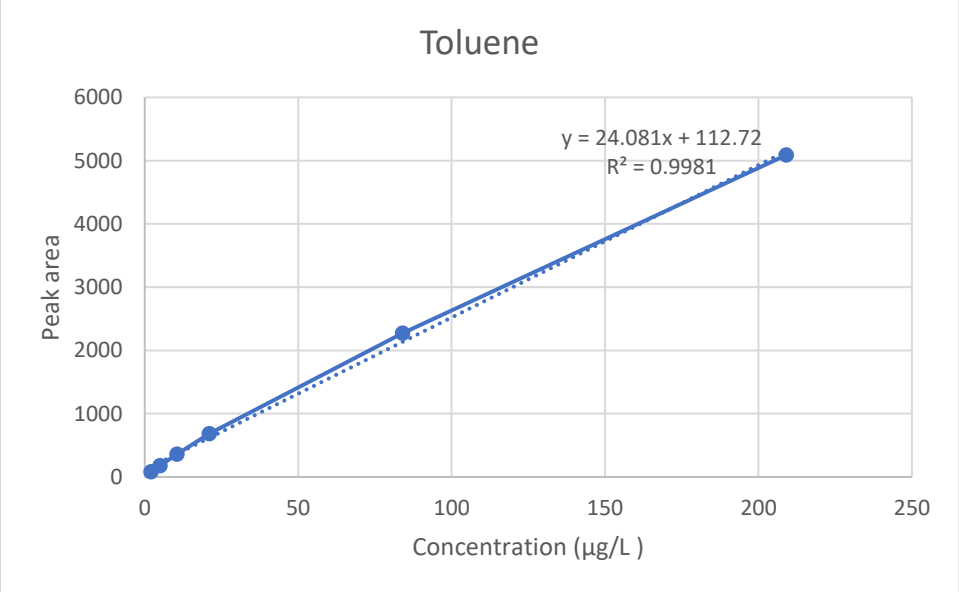


Figure E. 2. Toluene calibration curve for soil and seed analysis

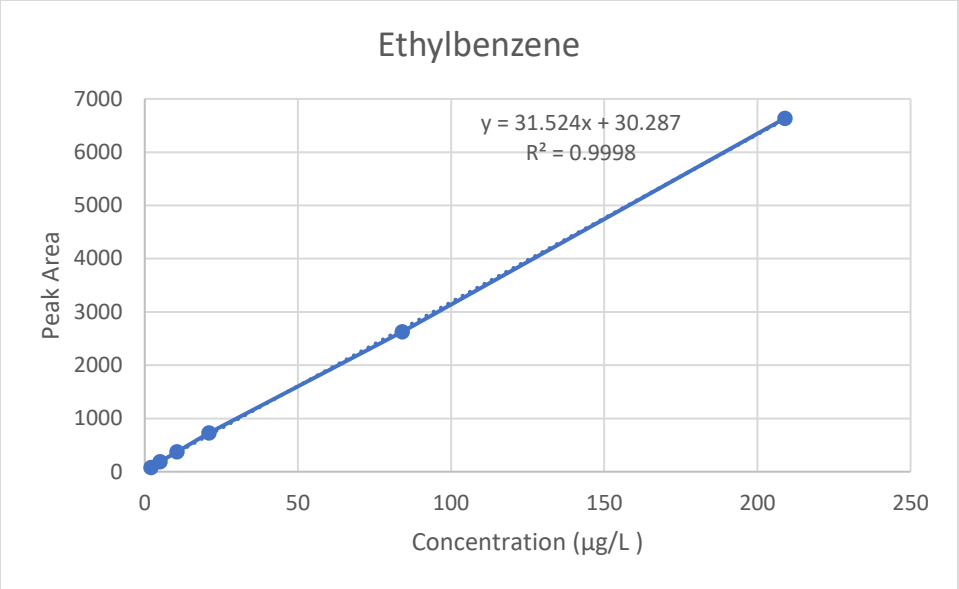


Figure E. 3. Ethylbenzene calibration curve for soil and seed analysis

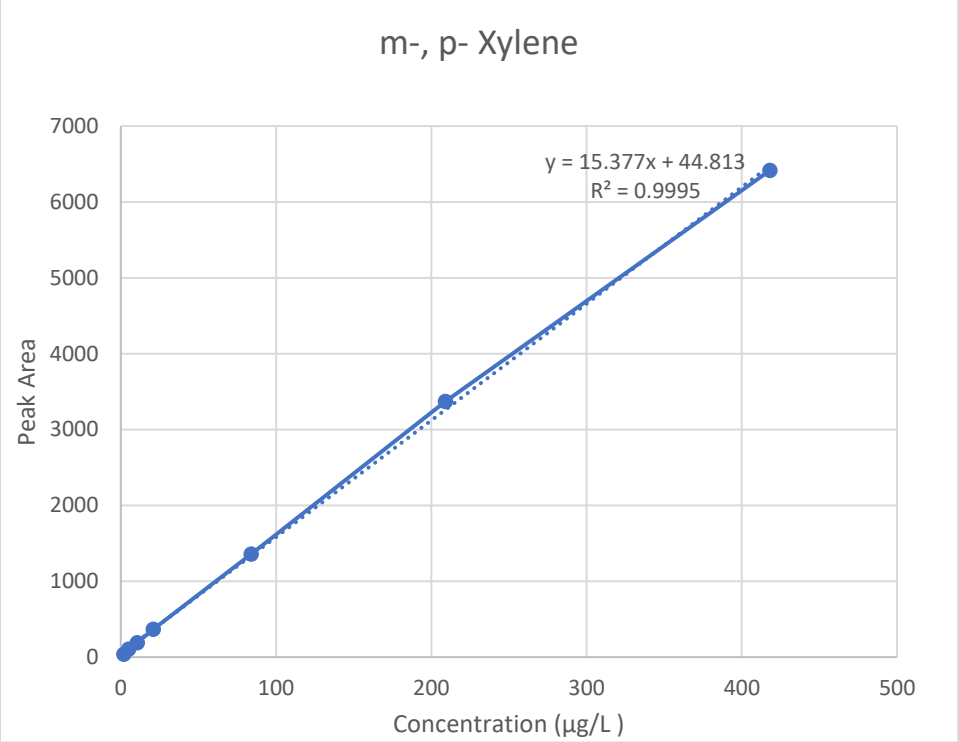


Figure E. 4. m-, p- xylene calibration curve for soil and seed analysis

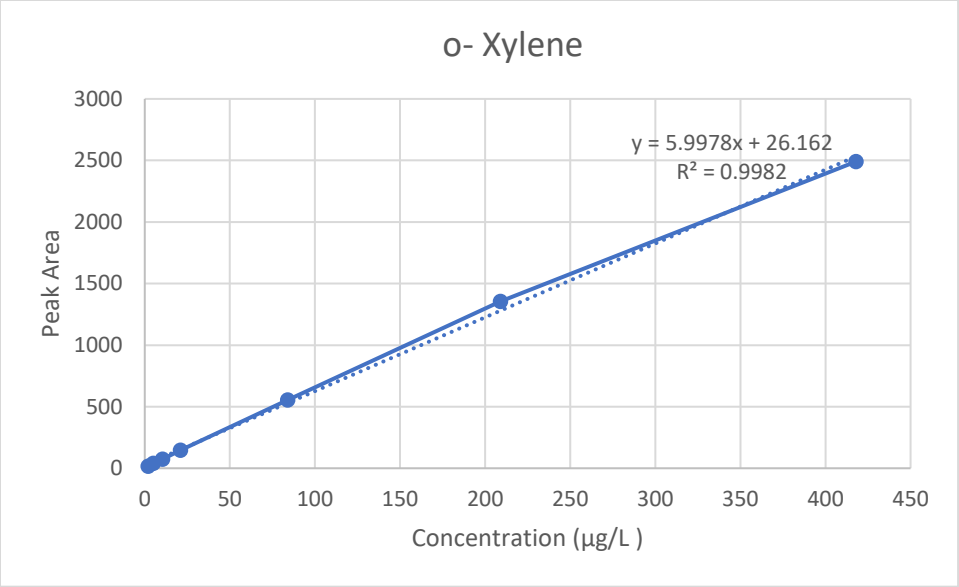


Figure E. 5. o- xylene calibration curve for soil and seed analysis

Table E. 2. Log $K_o w$ Ranges for Individual BTEX Compounds (at 20–25 °C) (Environment and Climate Change Canada (ECCC), 2024)

	log $K_o w$
Benzene	1.56–2.69
Toluene	2.69–2.82
Ethylbenzene	3.03–3.53
Xylenes	2.73–3.48