The Environmental Impacts of the Use of Potassium Acetate as an Alternative Deicer

A thesis
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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November 2022

Acknowledgements

This work would not be possible without the help of many people supporting and assisting in the research. I would like to thank everyone in the lab who braved the cold and helped me with winter field work, including: Adelle Keppers, Collin Krochalk, Isaac Overmyer, Max Brubaker, Joey Pesik, Brendon Gearheart, Liz Jaroki, Kelly Duhn, Caitlin Graeber, and Lisa DeGuire. Shawnee McMillian was a great help in running and analyzing data on instruments. Special thanks to Adelle Keppers for being my mentor in the Water Resources Science program, and for sharing her knowledge of automated sampling and lab procedures with me. Jerry Henneck was very helpful in sharing his knowledge and ideas for field sampling throughout the project.

This work was funded by the Minnesota Department of Transportation. MnDOT provided a sample of CF7 and assisted in selection of field sites and extension of the pipe on Blatnik Bridge which made bridge sampling possible. The work was done in collaboration with Iowa State University. Thanks to the ISU team of Dr. Chris Rehmann, Dr. Kaoru Ikuma, Yifei Ma, Rayla Pinto-Vilar, and Masrur Mahedi for co-sampling and sharing ideas throughout the research.

Thanks to the City of Duluth, for time and expertise in selecting field sites at the beginning of the project. Facilities workers of the city also helped install a weir after I spent many months trying to do it myself- thank you!

Thanks to Twin Cities Seed Company for supplying the switchgrass and special thanks to True Seed Supply for donating the sheep fescue. Teresa Norberg-King, Abigail Johnson, Leah Glimsdal and Dr. Dave Mount at the EPA Great Lakes Ecology and Toxicology Division conducted the aquatic toxicity tests and provided toxicological insights to on this data.

Most of all, thank you Dr. Chan Lan Chun for your role as my advisor and professor during the past few years. You taught me how to think like a scientist- and how cool microbes are! I am grateful for your guidance the past few years.

Finally, thanks to family and friends for the support and love during graduate school.

Abstract

The application of sodium chloride as a deicing agent for winter road safety has deleterious effects, from corrosion to infrastructure, impaired water quality, and toxicity to flora and fauna. The salinity of freshwater across the United States has been rising for decades, with the main driver of this being road salts. As chloride is conservative in the environment, this salinization of freshwater is expected to continue unless chloride use is significantly reduced. Due to the threat of freshwater salinization and other negative impacts of sodium chloride, there is an ongoing search for effective and environmentally friendly deicing alternatives. One such alternative, potassium acetate, is the focus of this research. While the effectiveness of potassium acetate as a deicer is established, the environmental impacts of potassium acetate are less understood. Potassium acetate is biodegradable, and the process of microbial oxidation of acetate has the potential to severely deplete dissolved oxygen in waterbodies receiving inputs of potassium acetate. Potassium and acetate may reach toxic thresholds for vegetation and aquatic life, and potassium acetate has been shown to be a more toxic compound than sodium chloride. In order to investigate the environmental impact of the application of potassium acetate as a deicer, a field evaluation with biological oxygen demand (BOD) tests, biodegradation experiments, and toxicity assays was conducted. We found that collection of stormwater and receiving water body samples find expected concentrations of potassium and acetate in receiving water bodies to be negligible in large, well-mixed systems due to dilution. Potassium acetate application resulted in a very high BOD (average near 50 mg/L in highway runoff and > 2,000 mg/L in bridge deck runoff) in stormwater and a significant demand in less mixed receiving water (up to 400 mg/L). However, sufficient (> 9 mg/L) dissolved oxygen concentrations remained even in waters with a high oxygen demand due to the slow biodegradation of acetate ($k = 0.02\ \text{day}^{-1}$ at 4°C) at low temperatures experienced in the field during the winter and spring. The acute LC_{50} to *Ceriodaphnia dubia* of CF7 as potassium is 130 mg/L, a low toxicity threshold given that the peak potassium concentration measured in the field evaluation was 560 mg/L. This acute toxicity threshold of potassium can be surpassed in smaller or less well mixed bodies of water. No adverse impacts of the deicer to roadside grasses were found at concentrations reported in the field. Potassium was found to be the agent of toxicity to freshwater zooplankton while acetate was responsible for toxicity to grass species. The findings from this research will inform salting practices as decision makers seek information on alternative deicers to keep roadways safe from ice while protecting our water resources.

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Chapter 1. The Challenge of Deicing and Anti-icing

1.1 Introduction to road salt

Every year, approximately 20 million metric tons of road salt, primarily sodium chloride (NaCl), is applied to US roads to keep drivers safe from winter ice accidents (Bolen, 2021). This salt effectively lowers the freezing point and melts or prevents ice formation but has a myriad of environmental and economic downsides. As salt is applied on roads, up to 60% can spray off the road into the soil (Blomqvist and Johansson, 1999). Salt remaining on the roadway eventually washes off into the stormwater conveyance system and enters surface waterbodies. The accumulation of both sodium and chloride has negative environmental impacts, although chloride is most often the focus of road salt concern due to its conservative nature in the environment.

The application of sodium chloride affects multiple areas, including soil, surface water, vegetation, aquatic life, human health, and infrastructure. While the deposition of NaCl from road application has been reported as far as hundreds of meters from a highway, the majority of salt that is sprayed off of the road is found within 20 meters of the roadside (Blomqvist and Johansson, 1999). As sodium builds up in the soil, it replaces other cations on negatively charged soil sites (Norrström and Bergstedt, 2001), where its accumulation degrades soil structure and mobilizes heavy metals (Amrhein, Strong and Mosher, 1992). Chloride is not absorbed in the soil and either runs off into surface waters or infiltrates to groundwater resources. In addition to the release of metals bound in the soil by ion exchange, chloride can form complexes with these metals, increasing their solubility and bioavailability to plants (Bäckström *et al.*, 2004). NaCl damage from root uptake or aerial spray is apparent on roadside plants, which includes leaf/needle browning or yellowing, impaired growth, root and leaf necrosis, and delays or lack of flowering and germination (Cain *et al.*, 2000).

The salinity of freshwater resources has been increasing globally in a trend deemed ‘freshwater salinization syndrome’ (Kaushal *et al.*, 2005). While urbanization, water softeners, and fertilizer contribute to salinity, in the northern United States road salt is the primary driver of this syndrome. Sodium chloride is retained in watersheds, resulting in release to groundwater throughout the spring and summer, and infiltration into groundwater years after application. Long term water quality monitoring has shown chloride to be increasing across rivers (Corsi *et al.*, 2010), lakes (Dugan *et al.*, 2017), and groundwater (Kelly *et al.*, 2019). High salinity induces toxicity at multiple levels of life, from altering microbial community structure and function (Tyree *et al.*, 2016; Wolf T. Pecher *et al.*, 2019), to declining macroinvertebrates and zooplankton abundance (Delaune *et al.*, 2021), to sublethal and lethal impacts to fish (Evans and Frick, 2001; Levelton Consultants Limited, 2007). Road salt contamination physically impacts lakes as well, with historically dimictic lakes experiencing increased stratification periods or completely ceasing to turnover due to the density of saline water, resulting in a permanently stratified meromictic lake (Dupuis *et al.*, 2019).

In addition to impacts to water quality and toxicity, road salt is also threatening human health through contamination of drinking water. Road salt contamination of groundwater is an increasing threat, as salt is retained in the watershed and can infiltrate into aquifers many years after application (Kelly *et al.*, 2008). While drinking water high in sodium would not be noticeable or harmful to most Americans, to the 50 million Americans with hypertension, drinking water could be an unknown and dangerous source of salt. The EPA set 20 mg/L of sodium and 250 mg/L chloride as a non-enforceable guidance level in drinking water following the American Heart Association's recommendation to protect people with hypertension or on salt restricted diets (U.S. Environmental Protection Agency Office of Water, 2003). Wells used for drinking water that were tested for sodium and chloride were found to be well over the guidance level in New York, Massachusetts, New Hampshire, Connecticut, and Minnesota (Nazari *et al.*, 2021).

The true cost of road salt is not only the price/ton; it also includes the environmental and infrastructural damages. NaCl is corrosive to steel and has resulted in billions of dollars of damage to infrastructure and vehicles. The damages to vehicles, infrastructure and ecosystems of road salt application range from an additional cost of $800-$3300/ton of salt applied (Dindorf and Fortin, 2014). In the winter of 2020-2021, the Minnesota Department of Transportation spent $116 million on salt, labor, and equipment, and used 177,164 tons of salt ('Winter Maintenance Report', 2021). Using the smaller estimate of $800/ton in damages, the damage from salting in Minnesota resulted in $142 million, greater than the cost of salting.

These widespread and expensive environmental and infrastructure impacts have created a challenge for salt applicators to protect public safety while minimizing environmental impacts. This has led to a push for best chloride practices, including use of NaCl only when weather conditions permit, use of a salt brine rather than solid NaCl that uses less NaCl, and legislation that protects salt applicators from being sued when they apply the minimum amount of salt necessary for melting. However, minimizing chloride use is not sufficient to protect water, soil, and human health. Kaushal et al. (2005) states that if chloride trends continue, groundwater would not be a viable drinking water source in the Northeastern US and surface waters would be toxic to aquatic life. A search for alternatives to chlorides that will maintain drivable roads without the serious impacts of chloride-based deicers is critically needed by salting decision-makers.

1.2 Alternative deicers and anti-icers

The deleterious effects of NaCl on the environment have led to a search for alternative chemicals and/or abrasives that would keep roads free from ice without contaminating water and corroding infrastructure. Inorganic alternatives (magnesium chloride, calcium chloride) to sodium chloride have been used for their effectiveness at lower temperatures and less toxicity to vegetation, but these still contribute chlorides to the environment and are more corrosive to infrastructure (Terry *et al.*, 2020). Organic chemicals as an alternative to chloride-based deicers have been investigated due to their biodegradable nature and interest in the sustainability of using agricultural byproducts as deicers.

Organic deicers such as calcium magnesium acetate, beet juice, urea, potassium formate, and potassium acetate are an active area of research. Agro-based chemicals such as beet juice, molasses, or corn products are often fermented or processed, then mixed with chloride deicers as research into the environmental effects found that the oxygen depletion from their degradation was too significant to be applied without mixing with an inorganic deicer. The acetate-based deicers (potassium acetate, KAc; calcium magnesium acetate, CMA; and sodium acetate, NaAc) are favored for application on bridges as the corrosivity to metals is lesser than other deicers. Formate deicers (sodium or potassium formate) and glycols (propylene and ethylene glycol) have most typically been used at airports, but glycol application is not realistic in highway settings as its discharge into the environment from aircraft runoff is collected and treated (Terry *et al.*, 2020). The cost of all alternatives is higher than the traditional NaCl, but when the benefits of alternatives such as effectiveness at a lower temperature, avoided environmental damages, and less corrosivity to infrastructure is considered, the additional up-front cost may not be inhibitive. However, the information needed to make these decisions is still being collected. While environmental impacts of NaCl are well documented, there are many questions remaining about the impacts of alternative deicers.

Potassium acetate (commercial product CF7®) is a promising deicer and anti-icer. CF7 is a liquid deicer comprised of 50% aqueous potassium acetate by weight and 1% additives such as anti-corrosion and anti-caking agents. Both CF7 and KAc will be used throughout this thesis, with CF7 generally referring to the specific deicer product applied on the roads, and KAc used as a general term for a potassium acetate deicer and the pure salt KAc. KAc is effective at much colder temperatures than other deicers or other acetates, with an effective temperature reported as low as -15/-26˚F (Akin *et al.*, 2013; Western Transportation Institute, 2017). It is both a deicer (melts ice after formation) and anti-icer (prevents ice from sticking to the road for easy removal) but will generally be referred to as a deicer hereafter. KAc has been used as a deicer in Duluth, Minnesota, and an understanding of the environmental impacts is necessary. In comparison to NaCl, very little research has been done on acetate deicers. Of KAc, NaAc, and CMA, CMA is the most studied. KAc is of growing interest due to its effectiveness at extremely low temperatures.

Acetate deicers are desirable due to the biodegradable nature of acetate, less corrosivity to metal than chlorides, and low effective temperature. However, they are more expensive than chloride deicers, are more corrosive to galvanized steel than traditional deicers (Fay *et al.*, 2008), and have been shown to damage concrete and asphalt (Shi *et al.*, 2009). The cost is much higher than chlorides, with a cost of \$30-\$100/ton for NaCl and \$600-\$1200/ton for KAc (Fay *et al.*, 2015).

The primary environmental concern with organic deicers (and KAc) is their potential for a high biological oxygen demand (BOD) which could lower dissolved oxygen in lakes and streams as bacteria consume oxygen while degrading acetate. A field study of CMA reported a much slower biodegradation of acetate at cold temperatures during which dissolved oxygen was decreased but reaeration prevented oxygen from getting too low to support aquatic life (Horner and Brenner, 1992). The BOD of a KAc deicer has not been investigated in the field, but the BOD_5 of a pure KAc deicer has been reported to be between 148,000 mg/L and 180,000 mg/L, high enough to result in oxygen depletion of receiving water bodies (Fischel, 2001; Switzenbaum *et al.*, 2001). While it is known that bacteria will have an additional carbon source from the input of acetate that could result in a high BOD, the effect of acetate on bacterial populations is unclear. In the few studies that investigated the effect of potassium acetate on bacteria, Bernhardt and Likens (2002) found that KAc inputs into a stream increased bacterial growth, while

Goldman and Lubdow (1992) found little effect of acetate from CMA on bacterial metabolism.

The additional cost of alternative deicers often inhibits agencies from widely applying them, but they are frequently selected for use in environmentally sensitive areas where a biodegradable deicer is preferable to the salinity threat posed by chloride, or on pavements with high corrosion potential from chloride deicers, such as bridge decks.

1.3 Research objectives

With the increasing threat of freshwater salinization due to chloride application, road salting decision-makers are eager for knowledge on the impacts of alternative deicers such as KAc. The Minnesota Department of Transportation (MnDOT) began using CF7 and determined it was an effective deicer through a field study (Waidley, Hirt and Wright, 2020). Following its evaluation as a potential alternative deicer to minimize chloride application, especially on bridges, the aim of our research is to determine environmental impacts of the application of CF7.

The objectives of this research were to (a) conduct an environmental field evaluation of the impacts of CF7 application to water chemistry, microbiological water quality (BOD and bacterial populations), and dissolved oxygen, (b) understand the biodegradation of CF7 and (c) determine the vegetative and aquatic toxicity of CF7 in the environment. The environmental knowledge gained from these findings will inform MnDOT and other stakeholders as they strive to keep roads safe for drivers while minimizing the cost and negative impacts of deicing agents.

Chapter 2: A field evaluation of the application of potassium acetate[1]

2.1 Field evaluation objective

The objective of this field evaluation was to determine the fate and impact of CF7 application in Duluth, Minnesota during the winters of 2019-2020 and 2020-2021. Road runoff in Duluth travels through a stormwater collection system and eventually drains into Lake Superior. The evaluation of the impact of CF7 entailed collecting stormwater samples and receiving water body samples during or following snow (and therefore salt application) events from Lake Superior, where the stormwater was being discharged. Water chemistry of samples was analyzed to understand temporal and spatial changes of KAc concentration and impacts to water quality including biochemical oxygen demand (BOD_5), major ions, and the microbiological indicator, *Escherichia coli* (*E. coli*) in runoff and receiving water bodies. BOD is the primary concern of the use of organic deicers, as the biodegradation of acetate can result in low dissolved oxygen as bacteria consume oxygen to break down the acetate.

The field evaluation was done in collaboration with Iowa State University (ISU), who assisted with study site selection, monthly fieldwork, and did additional water chemistry analysis. The field evaluation was conducted over two road salting seasons, with the first (2019-2020) focusing the methodology and logistics of sampling stormwater in the winter, and the second (2020-2021) using knowledge developed in the

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[1] Reproduced in part with permission from “Gulliver, J.; Chun, C. L.; Weiss, P., Erickson, A.; Herb, W.; Henneck, J.; and Cassidy, K. Environmental Impacts of Potassium Acetate as a Road Salt Alternative. MnDOT 2022-27A” Minnesota Department of Transportation Copyright 2022 https://www.cts.umn.edu/publications/report/environmental-impacts-of-potassium-acetate-as-a-road-salt-alternative-university-of-minnesota-evaluation

first season to sample additional KAc sites, which provided a larger dataset to better understand the impact of a KAc deicer on the environment.

2.2 Field sampling

MnDOT originally treated four roads in Duluth with CF7: Blatnik Bridge (yellow in Figure 2), Bong Bridge (purple), I-35 (green), and Central Entrance (blue). Stormwater and Lake Superior sites were visited and selected with teams from MnDOT, the City of Duluth, ISU, and the University of Duluth (UMD). A sampling plan was developed to collect runoff sample from Blatnik Bridge, I-35, and Central Entrance (Bong Bridge was not sampled due to safety concerns). Sampling was conducted from November 2019 through March 2021 after snow events. Sampling events and their relationship to snowfall and temperature during Year 1 is shown in Figure 1. A look at this figure shows that sampling dates did not always occur during or immediately post-snowfall. There was often no flow of stormwater on cold or cloudy days, and sampling was done once conditions warmed sufficiently to carry salt off the road and into the storm drain system that may have been applied days ago. Sampling was not done in April 2020 even though conditions in Figure 1 look ideal for sampling due to the Covid-19 pandemic.

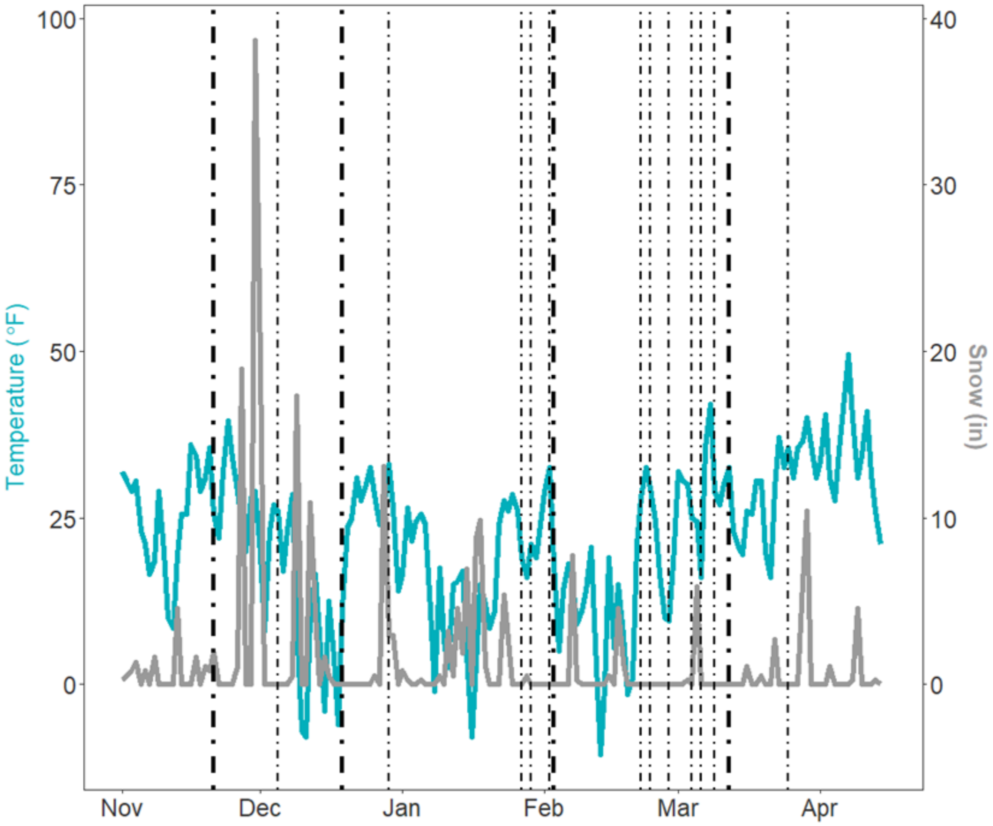

Figure 1. Sampling dates shown with dotted lines (bold dotted lines indicates ISU assisted in sampling) throughout the winter and spring of 2019-2020. Snow and temperature is overlaid across the sampling season.

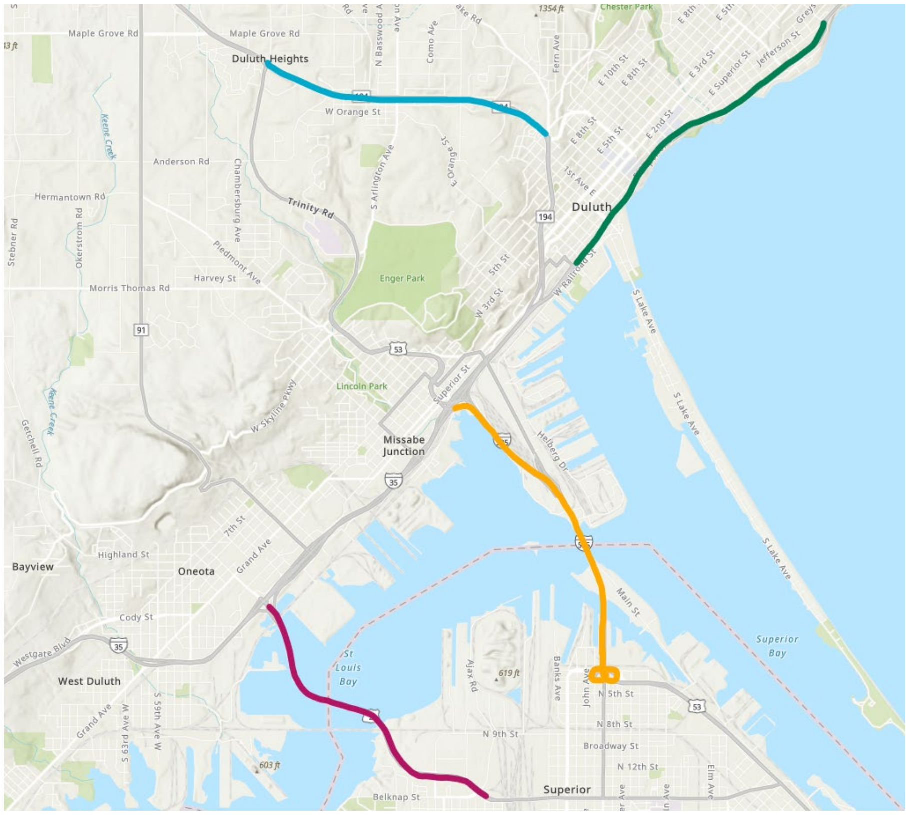

Figure 2. Roads treated with CF7 are highlighted in purple (Bong Bridge), yellow (Blatnik Bridge), blue (Central Entrance), and green (I-35).

In the first year, sample sites were targeted to collect both NaCl and KAc stormwater samples. NaCl and KAc drains were selected based on similar drainage basin sizes and land uses. The sites (Table 1) at I-35 and Central Entrance included an upstream control site where solely chloride-based deicers are applied, a treatment site which contained snow meltwater and road runoff from KAc application, and downstream sampling sites (in Lake Superior) which received runoff from both chloride-based anti-icers/deicers and KAc application. Blatnik Bridge did not have an upstream NaCl site but did have a KAc runoff collection site and downstream sampling site in Rice’s Point of Lake Superior.

During the first season, MnDOT determined that Central Entrance was not a feasible site for KAc application, and only NaCl was applied in 2019-2020. Given this alteration to the study site, both the NaCl and KAc storm drain sites were considered to be as NaCl sites for analysis purposes. In 2020-2021, the second field season, the goal was to increase KAc sample size using the lessons learned during the first field season. Chloride sites and Central Entrance were not resampled, leaving Blatnik Bridge and I-35 as the two main study sites. Each of these had an additional KAc site added, and an additional downstream sampling point was added in Lake Superior at Blatnik Bridge. Sampling site descriptions, year sampled, and IDs are displayed in Table 1.

Table 1. Study site description and ID of both field seasons.

Roadways	Anti-icer/deicer application	Site ID	Year sampled	Description
1-35	NaCl	I-35-NaCl	Year 1	A storm drain on 16th Ave above I-35
	KAc	I-35-KAc-A	Year 1 & 2	A storm drain on Lakewalk receiving runoff from I-35
	KAc	I-35-KAc-B	Year 2	A stormwater BMP on the Lakewalk; receives runoff from I-35 before being discharged into the lake
	Receiving water	I-35-LS	Year 1 & 2	Lake Superior receiving road runoff from both chloride-based anti-icer/deicers and KAc application
Central Entrance	NaCl	CE-NaCl	Year 1	A storm drain on Pecan Avenue above Central Entrance
	KAc	CE-KAc	Year 1	A storm drain receiving Central Entrance runoff
	Receiving water	CE-BC	Year 1	Brewery Creek receiving road runoff from both chloride-based

				anti-icer/deicers and KAc application
Blatnik Bridge	KAc	BB-KAc-A	Year 1 & 2	Runoff pipe from Blatnik bridge
	KAc	BB-KAc-B	Year 2	Runoff pipe from Blatnik bridge
	Receiving water	BB-RP-A	Year 1 & 2	Lake Superior at Rice’s Point where bridge runoff enters
	Receiving water	BB-RP-B	Year 2	Lake Superior at Rice’s Point; sampled by wading into the lake

2.2.1 Study sites in the winter season of 2019-2020 (Year 1)

Three sampling locations at I-35 were selected: an (NaCl treated) upstream stormwater collection system within the city of Duluth above I-35, a (KAc treated) stormwater drain of I-35, and Lake Superior water as stormwater receiving water (Figure 3). The upstream stormwater sample (green star on Figure 3 marked on 16th Avenue) I-35-NaCl was collected from the stormwater collection system at the intersection of London Road and 16th Avenue where chloride-based anti-icers/deicers (mostly NaCl) are applied above I-35. Snowmelt water from I-35 where KAc is applied was collected from the MnDOT stormwater collection system located on the eastside of the Duluth Lakewalk (pink star; I-35-KAc-A). Lake Superior water (blue star; I-35-LS) near the collection system was collected as stormwater receiving water. The Lake Superior sampling point was less than 10 ft away from where the stormwater is deposited into the lake.

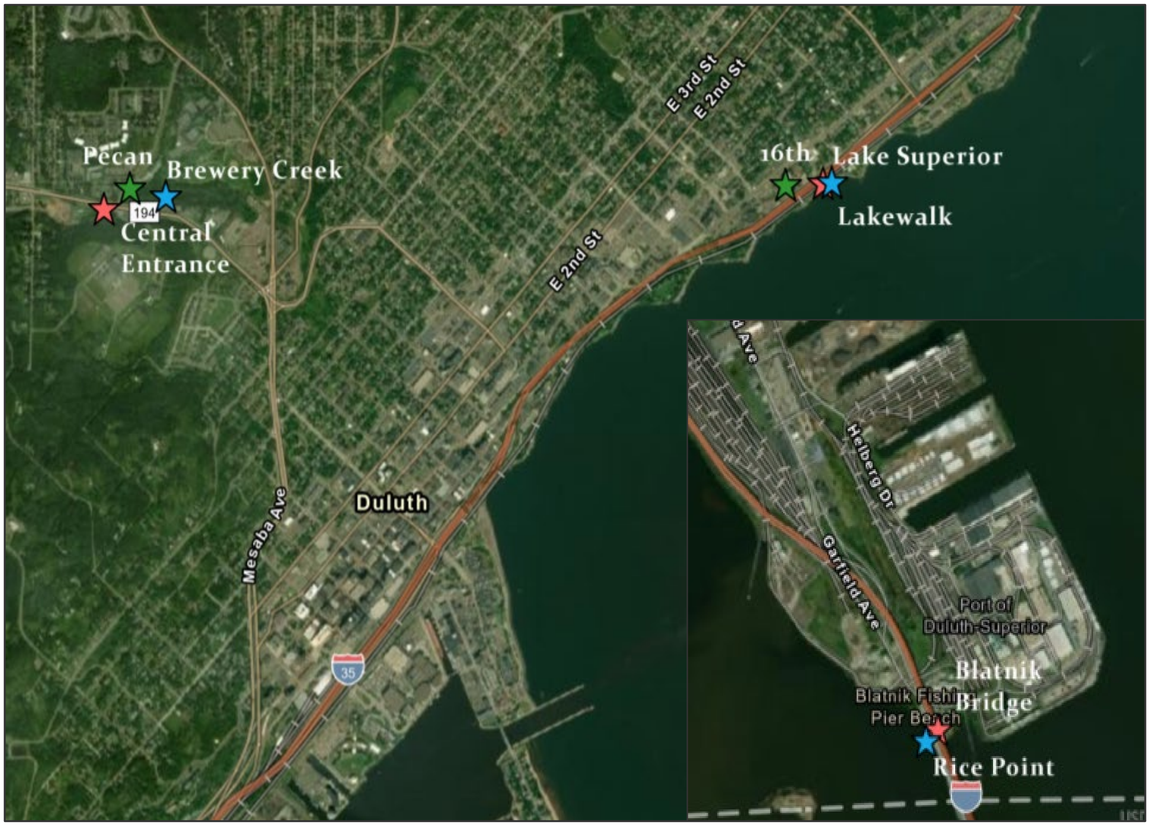

Figure 3. Study sites for Year 1 for snowpack meltwater, road runoff and receiving water body including upstream NaCl sites (green), KAc sites (pink), and waterbody receiving stormwater runoff (blue). Site IDs used throughout this thesis are CE-NaCl (Pecan), CE-KAc (Central Entrance), CE-BC (Brewery Creek), I-35-NaCl (16th), I-35-KAc-A (Lakewalk), I-35-LS (Lake Superior), BB-KAc-A (Blatnik Bridge), and BB-RP-A (Rice's Point).

Sampling locations on Central Entrance were selected in a similar manner to the I-35 sampling design: an upstream collection system above Central Entrance on Pecan Avenue (green star on Figure 3.3; CE-NaCl), a road runoff collection system from Central Entrance (pink star, CE-KAc), and Brewery Creek (blue star; CE-BC) where both runoffs enter. Brewery Creek was accessed from Marshall School and the access trail was maintained since it is a CrowdHydrology site (http://www.crowdhydrology.com/)

Runoff from Blatnik Bridge was collected from a longitudinal pipe collection system (pink star in Figure 3.3; BB-KAc-A) that drains bridge deck runoff into Rice's

Point (Lake Superior). The drainage pipes were about 60 ft off the ground, making collection difficult. The initial drainage pipe that was selected had to be moved to a pipe further down the bridge after the first sampling date as the initial site served as a MnDOT snow collection site and the sampling location was inaccessible after November 2019. In addition to collecting bridge runoff, the Lake Superior water sample at Rice's Point was collected as stormwater receiving water (blue star; BB-RP-A).

2.2.2 Study sites in the winter season of 2020-2021 (Year 2)

Central Entrance sites were removed as a study site in Year 2 given that NaCl was applied to the road rather than CF-7. The I-35-KAc-NaCl site was removed to focus on collecting KAc stormwater samples. An additional KAc site for both I-35 and Blatnik Bridge was added in Year 2 to increase KAc stormwater sample size (Figure 4). Two storm drains were sampled from I-35: I-35-KAc-A is the same site used in the first year of the study and I-35-KAc-B was added as an additional sampling point. Site I-35-KAc-B was added to have additional KAc stormwater samples and is a sediment basin for stormwater coming from I-35. Stormwater from this site is directly discharged into Lake Superior. Stormwater from I-35-KAc-A also travels into this site along with runoff from an additional stretch of I-35. As the basin was open to the air, the stormwater was completely frozen during February and March of 2021. Due to that, limited samples were taken from this site for most of February and March. The receiving water body for I-35 runoff remained the sample place in Lake Superior.

Figure 4. Sampling locations of Year 2 at (A) Blatnik bridge and (B) I-35. KAc runoff sites are shown in pink and receiving water body sites in blue.

Sites BB-KAc-A and BB-RP-A remained from the first year of sampling, but additional sites (BB-KAc-B and BB-RP-B) were added in the 2020-2021 winter sampling season (shown in Figure 4). BB-KAc-B was added to have an additional sampling point to capture bridge runoff, as bridge runoff collection was challenging in the 2019-2020 winter due to high drain point and wind. BB-RP-B was chosen as an additional receiving water site as a pipe from the bridge discharges road meltwater directly into the lake about 3 m from shore. At BB-RP-A, bridge runoff is not directly mixing with the lake, but rather traveling over the parking lot or blowing over the lake and into the water. With the more direct connection of the bridge and BB-RP-B, this site was added to examine if the runoff is detectable when bridge runoff is mixed with lake water, and if so, if the CF7 in that runoff impacts lake water chemistry. Table 1 summarizes the description of the sampling sites shown in Figure 3 and Figure 4 and gives site IDs.

2.3 Sampling methodology

Runoff collection from stormwater collection systems was conducted with a combination of grab sampling and automatic samplers (ISCO 6712 and 3700), while runoff-receiving water samples were collected through grab sampling. Sampling protocol evolved over the first field season as we developed the most efficient method to sample in the winter and made changes based on sampling challenges. Sampling methods in Year 2 were improved for safe and effective sample collection based on the first winter's experience.

Samples were collected with 1L polyethylene bottles that were washed and bleached. When grab samples were taken, bottles were rinsed in the sample water three times. Field measurements of dissolved oxygen, pH, temperature, and conductivity were taken with a YSI pro plus multimeter. Stormwater samples collected in ISCO units were open to the atmosphere before collection, which may have increased dissolved oxygen concentrations in I-35 and Central Entrance sites. Grab samples were taken in triplicate at Rice's Point, Brewery Creek, and Lake Superior.

Downstream sampling at Lake Superior and Brewery Creek was done by grab sample. Samples were taken from shore at Brewery Creek, from a dock at BB-RP-A, and by wading into the water at BB-RP-B. While the initial plan was to sample I-35-LS by reaching into the water for a grab sample as in Rice's Point, the icy rocks along shore created unsafe conditions so samples were taken from with a bucket on a rope. When ice formed at Rice's Point, an ice pick was used at BB-RP-A. Ice at BB-RP-B reached down to the bed of the lake so samples were only taken in early winter and during Spring melt.

2.3.1 Stormwater site sampling

Stormwater was collected daily in Year 1 with automated water samplers (ISCO 6712) that were deployed in the KAc sites located at Central Entrance (CE-KAc) and I-35 (I-35-KAc-A). The ISCO units were stored inside the lockbox and were powered by a solar panel that supplies continuous power to a 12V deep cycle battery (Figure 5). Samplers were programmed on an 8-hour time interval to accumulate a 0.75 L runoff sample each day. Each sample was collected in a separate ISCO bottle. A total of 24 bottles were held in a single sampler at a time but the bottles were collected weekly and after each snowstorm or snowmelt event. The weekly visit was also required for maintenance to clear snow in solar panels and ice in the tubing. These ISCO units worked well at holding charge but ice in the tubing created sampling difficulties. Tubing within the manhole rarely froze, and the section of tubing from the manhole cover to the box could be insulated with foam and hand warmers. The problematic section was the few inches going through the manhole cover. There was not enough of a gap to warm the tubing and ice would block sample from entering the unit. Visits to the sites to conduct repetitive flushing of the tube before snowfall helped clear the tubing. When ISCO units malfunctioned due to disconnection from the battery, poor suction, or ice in the line, grab samples were taken at I-35-KAc-A directly from the storm drain. Continuous autosamplers could not be installed at the I-35-NaCl and CE-NaCl sites as the NaCl storm drains were located in the street, but 3700 ISCO samplers were installed the day before each sample collection to capture composite stormwater runoff.

Figure 5. (A) In-manhole ISCO sampling at CE-NaCl and (B and C) Box set-up at I-35-KAc-A

In Year 2, the ISCO unit within a lockbox remained at I-35-KAc-A, but as Central Entrance sites were no longer in use the ISCO box was moved from CE-KAc to I-35-KAc-B. Site I-35-KAc-B (Figure 6) was added to have additional KAc stormwater samples and is a sediment basin as a best management practice for stormwater coming from I-35. Stormwater from this site is directly discharged into Lake Superior. Stormwater from I-35-KAc-A also travels into this site along with runoff from an additional stretch of I-35 so it was a good location to collect the composite stormwater runoff from I-35. As the basin was open to the air, the stormwater was completely frozen during February and March, 2021. Due to that, limited samples were taken from this site for most of February and March. Units were programmed to take a composite sample of 330 ml three times per day, for a total sample volume of ~ 1L. Bottles were collected as soon as possible after a snow event, typically within one day and a maximum of five days depending on air temperature. When the ISCO unit malfunctioned at I-35-KAc-B, samples were not able to be taken as this site is a stormwater basin with a grate preventing grab samples.

Figure 6. (A) Sampling set-up in I-35-KAc-A with adjusted weir, (B) ISCO sampling unit box used to collect stormwater from the sedimentation basin, I-35-KAc-B site, and (C) frozen stormwater in the basin during February-March, 2021

Initially, the plan was to rely on UMD’s two 3700 series and ISU’s two 6712 series ISCO automated samplers to pump composite stormwater samples into collection bottles. However, as the winter progressed, grab samples were taken at most sites due to challenges with the ISCO batteries being unable to stay charged with the below freezing temperatures and ice formation in the tubing blocked samples from being drawn. When ISCO sampling failed, grab samples were taken upon bottle retrieval. Particularly, stormwater runoff samples from NaCl storm drains were largely represented by grab samples.

It became clear after initial site inspection that flow in the storm drains was variable and not sufficient for autosampling or flow measurements. To improve stormwater sample collection and flow measurements from low flow of runoff, a volumetric weir (Thel-Mar) was installed in all storm drain sites along with an ISCO spring mount ring with a 2150 ISCO Flow Module and a weighted sample strainer (Figure 7). Flow measurements were taken at each stormwater site with a 2150 ISCO Flow Module, which uses continuous wave Doppler technology to capture flow data with an area velocity (AV) probe. The AV probe and autosampler tubing were fastened to a spring ring to hold equipment in place in the storm drain. At some locations, there were challenges with water flowing under the weir and wet cement and foam was applied to keep the weir in place without leaks. I-35-KAc-A was a particularly difficult site to sample. Due to weathering of concrete in the drain, the weir did not seal properly which caused water to flow under the weir during the Year 1 study. This was improved for Year 2 with assistance from the City of Duluth which led to the successful installation of a weir (Figure 6A) in the drain, necessary to back up flow in order for the ISCO tubing to be submerged to take a sample.

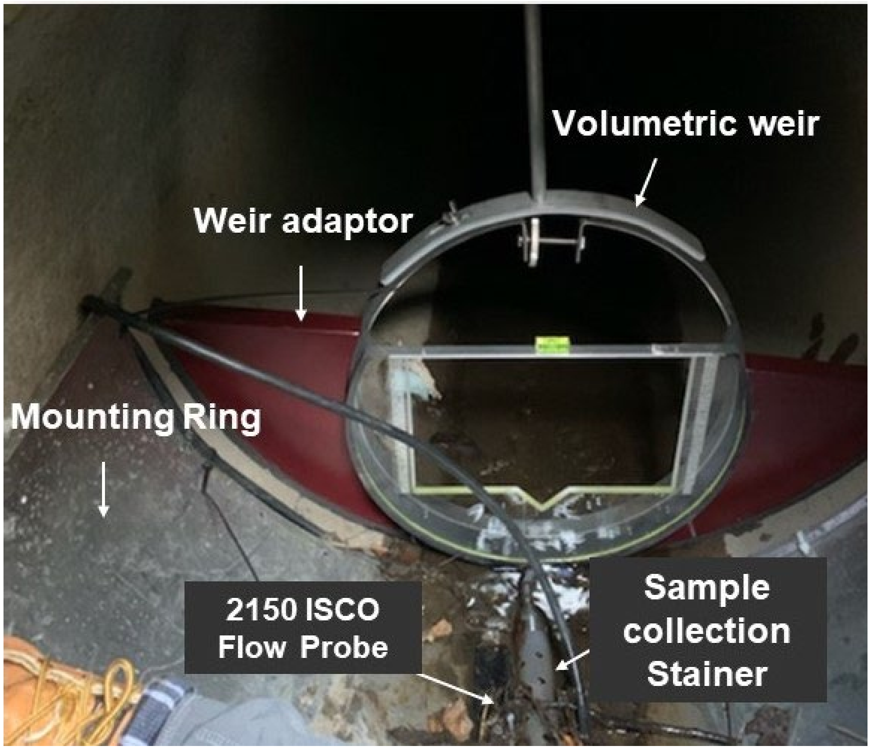

Figure 7. Runoff sampling set-up in storm drain with weir, adapter, spring mounting ring, flow probe and sample collection strainer.

Flow measurements at the storm collection systems are shown in Figure A1. CE-KAc and I-35-NaCl have long term accurate flow data. At I-35-KAc-A and CE-NaCl, obtaining accurate flow measurements was a continuous challenge due to uneven piping and flow module battery failures. I-35-NaCl and the I-35-KAc-A had continuous flow most of Year 1, while flow at CE-KAc and CE-NaCl slowed to a trickle or stopped entirely unless melt was actively occurring. During Year 2, the only storm drain sampled was I-35-KAc-A, however, much of the winter is missing due to frozen stormwater in the drain January-February and battery issues in March (total snow fall: 0.33 in and average temperature: -0.6 ~15.7˚F in February).

2.3.2 Bridge sampling

The collection of runoff from Blatnik Bridge was challenging due to the drainage pipe installed 60 ft above ground. The initial sampling location chosen with MnDOT was located at a snow removal site. Once snow began being deposited, the collection barrel was buried (Figure 8A) and the site was moved to be underneath another pipe further down the bridge (Figure 8B and C). The bridge snowmelt would fall from this pipe and travel over the parking lot into the Rice's Point sampling location. A rain chain was proposed, but due to concerns about water freezing around it and blocking the drain along with the potential hazard of the chain blowing around the parking lot in the wind, was not installed. Initially, a 55-gallon rain barrel was placed underneath the bridge deck outlet pipe to catch the runoff. The barrel was fixed with cinder bricks and fences. The barrel collected more than a sufficient amount of sample in early winter when snow was mixed with rain, but as it got colder and windy the barrel was only able to catch a little runoff from the bridge. Additionally, this posed problems with ice build-up in the bottom of the barrel blocking the nozzle, so it was modified to catch the stormwater in a 5-gallon bucket placed on top of the rain barrel with a screen cover on the top of the barrel (Figure 8B).

Figure 8. Bridge runoff collection sites at Blatnik Bridge: (A) initial runoff site, (B) current runoff site, and (C) second drainage pipe, drops water into picture B

Blatnik Bridge was visited at each sampling date, but samples were only collected four times throughout the Year 1 winter. We observed that melt was typically very gradual. On multiple occasions when melt volume was large at the other sampling locations, at Blatnik Bridge drops of meltwater were seen falling from the pipe and dissipating and scattering by wind over the parking lot. Water samples were collected from the BB-RP-A even on the dates when not enough bridge runoff was in the bucket as the runoff travels over the parking lot to enter the waterbody the Rice’s point (~50 ft away from bridge drain pipe).

In Year 2, sites BB-KAc-A and BB-RP-A remained from the first year of sampling, but additional sites (BB-KAc-B and BB-RP-B) were added in the 2020-2021 winter sampling season. BB-KAc-B (Figure 9A) was added to have an additional sampling point to capture bridge runoff, as bridge runoff collection was challenging in the 2019-2020 winter due to high drain point and wind. BB-RP-B (Figure 9A) was chosen as an additional receiving water site as a pipe from the bridge is discharging road meltwater directly into the lake about 3 m (less than 10 ft) from shore. At BB-RP-A, bridge runoff is not directly mixing with the lake, but rather traveling over the parking lot or blowing over the lake and into the water. With the more direct connection of the bridge and BB-RP-B, this site was added to examine if the runoff is detectable when bridge runoff is mixed with lake water, and if so, if the CF7 in that runoff impacts lake water chemistry.

Figure 9. (A) BB-KAc-B; with the collection bucket; (B) BB-RP-B, with an arrow pointing to the discharge pipe over the sampling location

In addition to a second KAc sampling point at BB-KAc-B, changes were made to this site in Year 2 to increase sampling success. MnDOT District 1 reconnected pipes to reach the ground at the beginning of the winter season. This assistance greatly helped to solve issues with bridge runoff dissipating in the wind, which was the major challenge to capture the runoff from the bridge in the 2019-2020 winter. During the 2020-2021 winter season, bridge meltwater at BB-KAc-A and BB-KAc-B was collected either as a grab sample when melt was actively happening, or with a collection bucket placed ahead of snow/melt events. BB-RP-A was sampled from a dock during both years, and BB-RP-B by wading into the water. During January and February, BB-RP-B and to a lesser extent BB-RP-A were frozen, in some cases down to the shore of the lake, making sampling impossible. On one such occasion, an ice/meltwater sample was taken from the surface of the ice at BB-RP-B where bridge runoff was falling on the ice and melting it. When the ice was not too thick to sample, BB-RP-A and BB-RP-B were sampled with the aid of an ice pick.

As it was no longer safe to climb down to the lake and the arm sampler typically used in these situations was not long enough, a bucket and rope was used to collect lake water. At Rice’s Point, Lake Superior, and Brewery Creek, a handheld YSI ProPlus was used for in situ measurements such as pH, DO, conductivity, and temperature. Stormwater flow was not sufficient for the probe to be submerged so YSI measurements were taken in the lab.

2.4 Water quality and chemistry analysis

Samples were processed within 24 hours of collection. Any large debris was removed from the sample by filtering the entire sample with 70 µm mesh. Water chemistry analytes are listed in Table 3.2. The sample was divided into four aliquots: non-filtered/non-acidified (frozen), non-filtered/acidified, filtered/non-acidified, and filtered/acidified for water quality analysis (Figure 10. Sample processing flow chart; analyses are in blue). Aliquots were vacuum filtered through 0.22 µm pore size membrane filters, acidified with 1% HNO_3, and stored in 50 ml centrifuge tubes at 4˚C.

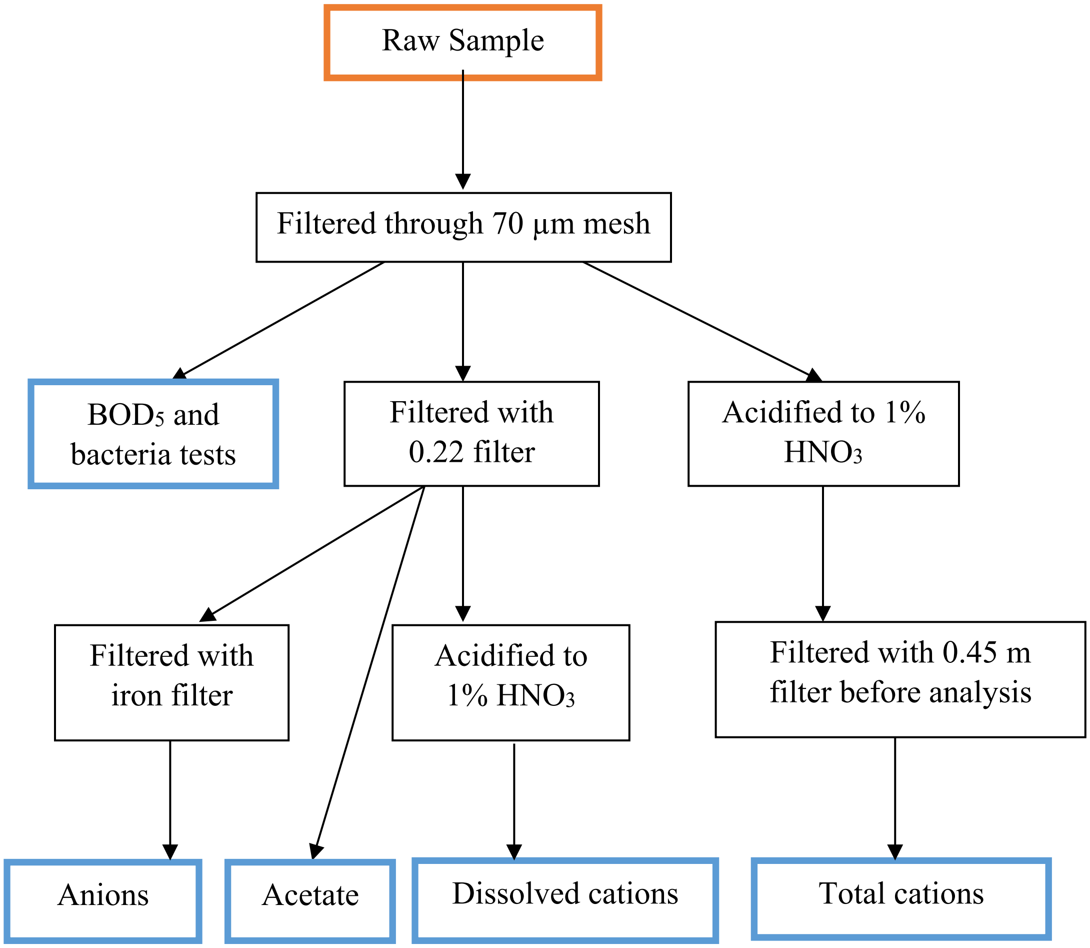

Figure 10. Sample processing flow chart; analyses are in blue

Five-day Biological Oxygen Demand (BOD_5) tests were done on the unprocessed (only mesh filtered) samples following EPA Standard Method 5210 B. The desired dilution range and dilution solutions were determined by the third sampling event. Dissolved oxygen (DO) for BOD analysis was measured with a YSI 5000 DO meter calibrated before each use. *Escherichia coli (E. coli)* enumeration (Most Probable Number; MPN) was conducted through the Colilert-18 Quanti-Tray Method (USEPA, 2003). During the University's lab hibernation due to the COVID-19 pandemic, BOD_5 and *E. coli* measurements could not be performed in late Spring 2020.

Major cation (Ca^{2+}, Mg^{2+}, Fe^{2+}, Na^{+}, and K^{+}) concentrations were determined with a Shimadzu flame atomic absorption spectrophotometer. Samples aliquoted for dissolved cation analysis were filtered and acidified, while samples aliquoted for total cation analysis were acidified and not filtered until before analysis. Anion samples were filtered and not acidified, then pretreated to remove transient metals with OnGuard II M cartridge before analysis. Anion (Fl^{-}, Cl^{-}, Br^{-}, NO_2^{-}, NO_3^{-} PO_4^{3-}, SO_4^{2-}) concentrations were determined with a Dionex 2000 Ion Chromatography System and a IonPac™ A22 column (4 × 25 mm, Dionex) with an isocratic flow rate of 1.2 ml/min. Acetate was quantified with High Performance Liquid Chromatography (HPLC) on an Dionex Ultimate 3000 with a Rezex ROA column (300 × 7.8 mm, Phenomenex) with a guard column (50 × 7.8 mm) under an isocratic flow rate of 0.8 ml/min and a wavelength of 210 nm for UV detection.

2.5 Results and Discussion

Results from the field evaluation are reported in section 2.5, including the detection of potassium and acetate from CF7 application, major ion concentrations, and the presence of CF7 additives. The effect of acetate on bacterial counts and on BOD is included in the field evaluation.

2.5.1 Potassium Acetate

The average acetate concentration (Year 1 in Figure 11 and Year 2 Figure 12) was 132 mg/L in I-35-KAc sites and 3,028 mg/L in BB-KAc sites. Acetate peaked at 940 mg/L in I-35-KAc and 13,000 mg/L in BB-KAc-A. Ac^- was not observed at CE-KAc (other than one sample at CE-NaCl which was likely due to a natural source of acetate) as MnDOT used NaCl instead of KAc on the Central Entrance site due to site conditions. While the CE-KAc site was originally intended to be a KAc site, when analyzing results, it is considered as an additional NaCl site for BOD and bacteria results given the lack of KAc applied and measured in this site. Acetate was detected in all but two samples of Blatnik Bridge meltwater and in about 70% of I-35-KAc runoff. The presence of acetate in KAc sites in most samples and lack of detection in NaCl sites at I-35 and Central Entrance indicates that when acetate is detected in runoff, it can be assumed its source is from CF7, and is not naturally occurring.

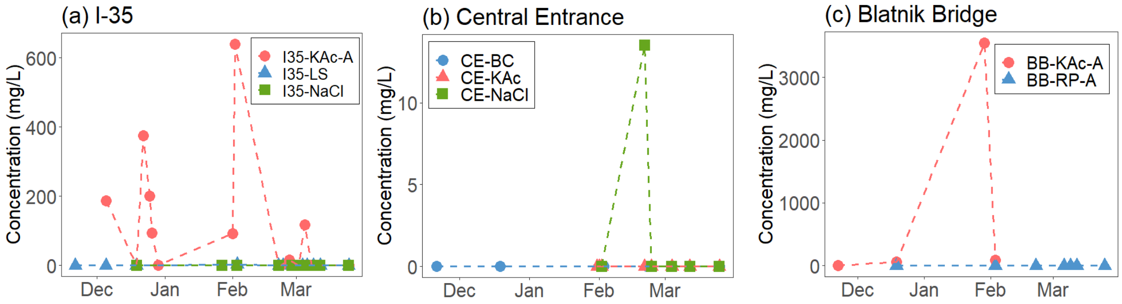

Figure 11. Acetate concentration at (a) I-35, (b) Central Entrance, and (c) Blatnik Bridge sites in 2019-2020

The variable acetate concentrations at both I-35 and Blatnik sites is typical of stormwater sampling, as concentrations vary based on dilution, precipitation amount and rate, CF7 application rate, weather, and time between application and sampling. Interestingly, acetate concentrations could be very different between BB-KAc-A and BB-KAc-B samples taken within an hour of each other. This indicates that KAc concentrations in runoff are highly variable and high concentrations likely flush out quickly to receiving water bodies.

CF7 was analyzed on the HPLC for acetate concentration, and the product has an acetate concentration of 288,000 mg/L. Using the average concentrations of acetate detected at I-35 (132 mg/L) and Blatnik Bridge (3,000 mg/L), it can be calculated that bridge runoff is extremely concentrated and carries only a 100-fold dilution of the pure liquid deicer. I-35 runoff is much more diluted, with a 2,000-fold dilution of CF7 in the runoff. Using peak concentrations detected, at most I-35 runoff reaches a 300-fold dilution and Blatnik Bridge only a 20-fold dilution from CF7.

Although Blatnik Bridge runoff was highly concentrated, no detectable acetate was found in the receiving water in BB-RP-A or I-35-LS during either year. Low concentrations of acetate in the receiving water body appear to be at background levels in freshwater (0-6 mg/L) as acetate is a common organic metabolite (Allen, 1968). However, acetate was detected up to 650 mg/L in BB-RP-B water in March 2021 (Figure 13). Acetate was found in BB-RP-B during Spring melt when the ice cover began to recede. The combination of warmer weather and the runoff carrying deicer melted a hole in the ice below the stormwater pipe, leaving a small open water area to sample. The absence of KAc in the open water sites of I-35-LS and BB-RP-A but presence in BB-RP-B indicates dilution reduces KAc concentration within well-mixed zones of the lake, but KAc concentrations can remain concentrated in smaller, less open areas, potentially impacting lake water chemistry and ecosystem functioning.

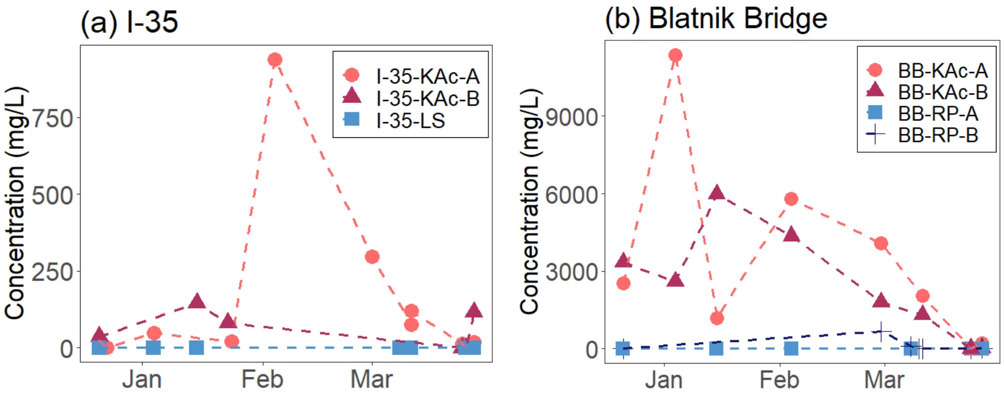

Figure 12. Acetate concentration at (a) I-35 and (b) Blatnik Bridge sites in 2020-2021.

Potassium and acetate concentrations in runoff generally trend together (visible in Figures 13 and 14, regression analysis has $r^2 = 0.34$ ($p < 0.001$) but $r^2 = 0.85$ ($p < 0.001$) when two outliers are removed. Both the lack of acetate present in NaCl sites (Figure 12) and the similar peaks in concentration between acetate and potassium (Figures 13 and 15) strongly indicates that measurements of potassium and/or acetate are capturing CF7.

Spikes of potassium or acetate in runoff or receiving waters then serves as an indicator for CF7 application. Similar to acetate in I-35-LS and BB-RP-A, potassium remains steady near or at 0 mM throughout the winter and spring in lake sites despite the peaks in I-35 and Blatnik bridge runoff that drains into these sites, indicating that dilution negates traces of CF7 in both lake sites. However, the increase in potassium along with acetate in BB-RP-B (Figure 15d) in the Spring shows that in a smaller waterbody, concentrated runoff can impact water chemistry before dilution takes effect.

KAc dissociates into K^+ and Ac^- in a 1:1 molar ratio. Variations from a 1:1 ratio can provide insight on the fate of K^+ and Ac^- in the environment. While acetate can be consumed by microorganisms (Chapter 3) and degraded into bicarbonate, carbon dioxide, and water, potassium is conservative in solution as an inorganic ion. Despite the co-occurring trends of K^+ and Ac^- in KAc application sites and receiving water, K:Ac ratios varied over time, with Ac^- higher than K^+ on occasion. In Year 1 the measured molar concentrations of K^+ were generally lower than those of Ac^- in runoff, with a K^+:Ac^- molar ratio of 0.5-1.0. In Year 2, the average K^+:Ac^- molar ratio at I-35-KAc-A was 1.3, and 1.9 at I35-KAc-B. Ratios in bridge runoff were similar, with an average K^+:Ac^- ratio of 1.8 at BB-KAc-B and 0.8 in BB-KAc-A. The variations are presumably due to different reactivity of K^+ and Ac^- with other constituents in stormwater. The degradation of Ac^- by naturally occurring bacteria or inputs of K^+ from sources other than CF7 results in a K: Ac ratio>1, while sorption affinity of K^+ to negatively-charged organic matter or clay mineral in runoff results in a K:Ac ratio <1.

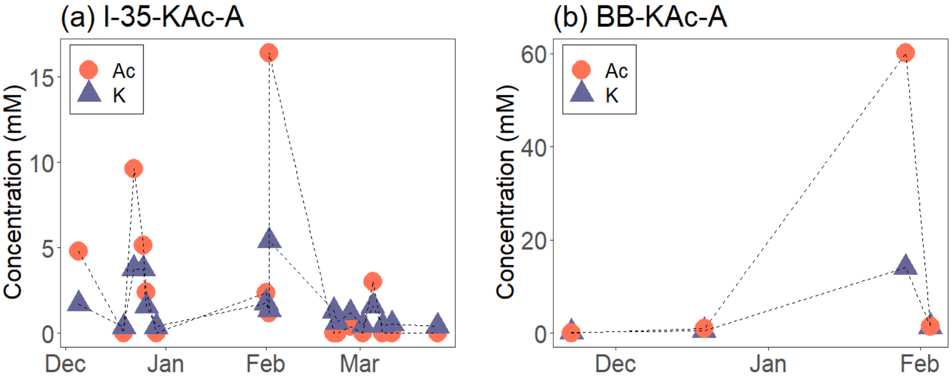

Figure 13. Molar concentration of acetate and potassium in (a) I-35-KAc-A and (b) BB-KAc-A in 2019-2020.

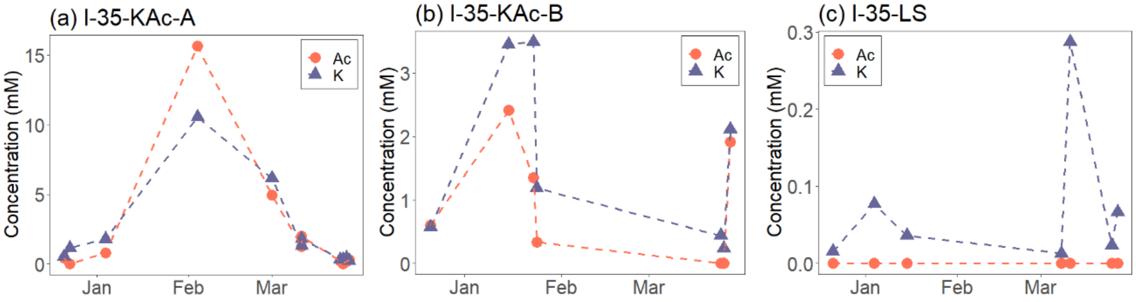

Figure 14. Molar concentration of acetate and potassium in (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS in 2020-2021

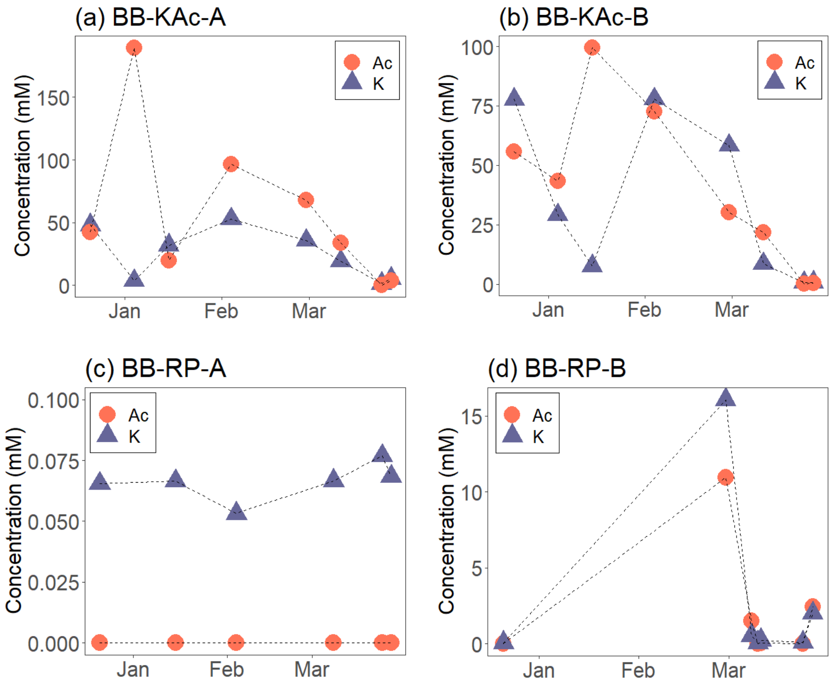

Figure 15. Molar concentration of acetate and potassium in (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B in 2020-2021.

pH was measured in field samples, as CF7 is very alkaline (pH = 11). The pH of stormwater ranged from 6.2 to 8.7, while the pH of receiving water was between 6.9 and 7.6 (Figure 16). The pH of stormwater samples high in acetate was slightly more basic, with an average pH of 7.9 at Blatnik Bridge sites, 7.8 at I-35 sites, and 7.5 at Central Entrance and I-35-NaCl sites. This seems to suggest that the pH of stormwater will be slightly increased with the presence of CF7 deicer, however, the pH of I-35-NaCl was 8, compared to the pH of I-35-KAc-A and B of 7.8. The lesser pH of the non-KAc sites may then be due to the nature of the stormwater in the Central Entrance sites rather than the increase in pH due to CF7 in KAc sites. An ANOVA test with pairwise comparisons found a significant difference in pH between stormwater sites (both KAc and NaCl) and lake sites ($p < 0.0003$), but no difference between pH of stormwater in KAc sites and stormwater of NaCl sites ($p = 0.2$). The concern with the pH of CF7 is that the high pH-runoff entering receiving water bodies may raise pH of the water- however no notable increase in pH was observed in BB-RP-B when acetate was detected the sample.

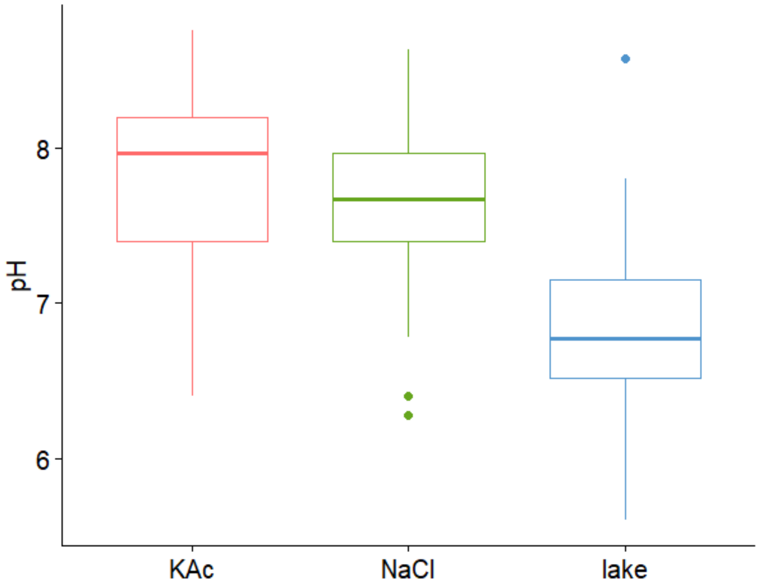

Figure 16: pH of stormwater and receiving water, grouped by site.

2.5.2 Sodium chloride and conductivity

The chloride (Cl^-) concentrations in stormwater were high enough that a log scale is required to show that values differed by orders of magnitude within sites (Figure 17Figure 18). Chloride was extremely high in runoff, with peaks of 16,035 mg/L in CE-KAc runoff and 5,672 mg/L in CE-NaCl runoff (Figure 17b). High chloride concentrations in CE-KAc were attributed from the application of chloride-based anti-icers/deicers at this site. MNDOT District 1 initially planned to apply KAc on Central Entrance during the 2019-2020 winter season, hence why CE-KAc was planned to be a third KAc application site but made a change to use chloride-based anti-icers/deicers. CE-KAc is therefore considered to be a NaCl application site for analysis of this field data. An average Cl^- level of 396 mg/L at CE-BC is unsurprising given the levels of Cl^- in runoff in that catchment. Brewery Creek chloride was regularly above the EPA's chronic limit of 230 mg/L and would be considered impaired for chloride. Chloride at the other NaCl site, I-35-NaCl, averaged about 2,100 mg/L, higher than the 1,600 mg/L at the I-35 KAc-A, where CF7 was applied (Figure 17a).

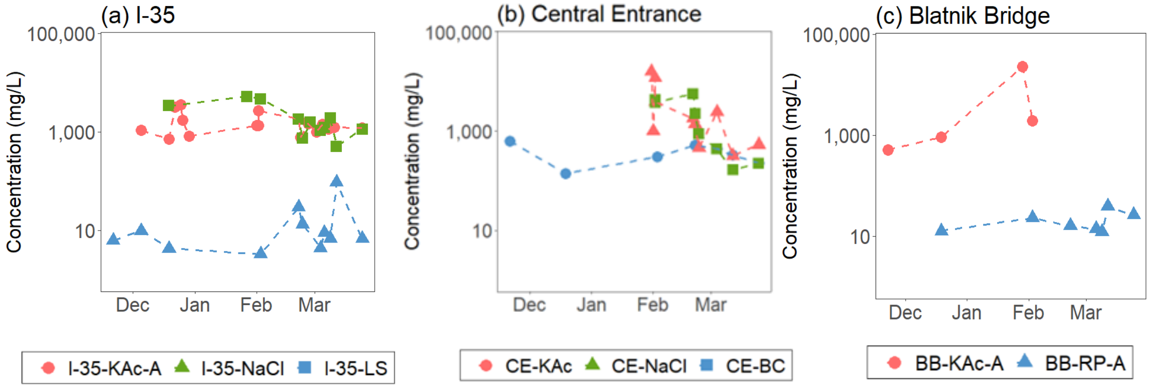

Figure 17. Chloride concentration at (a) I-35, (b) Central Entrance, and (c) Blatnik Bridge sites in 2019-2020.

While chloride is expected to be very high at NaCl application sites, given that CF7 is applied at I-35-KAc-A and B and BB-KAc-A and B, it was thought that chloride would be much lower at these sites. However, chloride was the dominant ion in I-35 stormwater and Blatnik Bridge. An ANOVA found no statistically significant difference between chloride concentrations in KAc sites versus NaCl sites (p = 0.2). As with acetate, bridge runoff was much more concentrated than I-35 stormwater. The average chloride concentration in Blatnik Bridge sites was 12,000 mg/L and in I-35-KAc sites was 1,600 mg/L. This unexpectedly high concentration of chloride at non-chloride application sites can be attributed to the occasional use of NaCl salt depending on weather conditions, and the traveling salt from spray and vehicles carried onto KAc application stretches of I-35 and Blatnik Bridge.

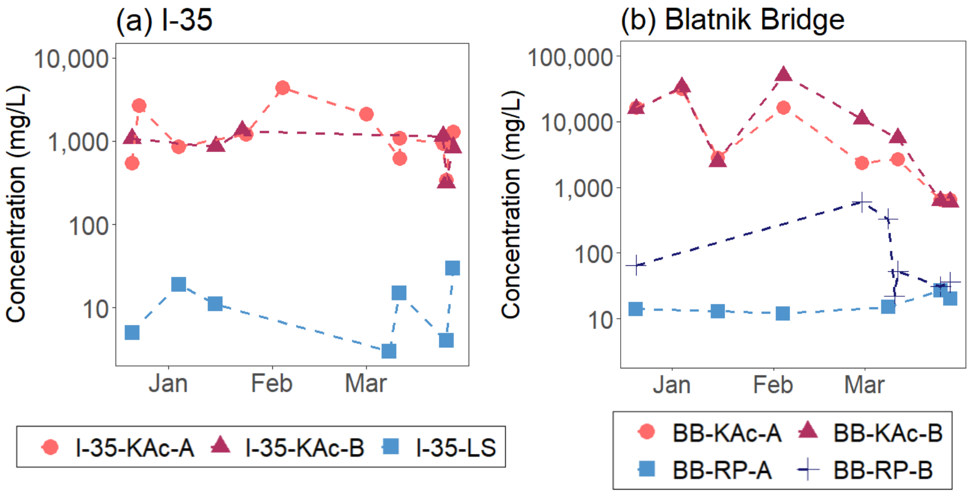

Figure 18. Chloride concentration at (a) I-35 and (b) Blatnik Bridge sites in 2020-2021.

The high concentrations of chloride in stormwater runoff did have an impact in receiving water body sites. The average chloride concentration in Lake Superior at I-35 was 15 mg/L. The spikes in concentration during spring melt are visible in Figure 17a and Figure 18a, with a peak of 95 mg/L chloride in March of 2021. Historical water chemistry datasets on Lake Superior report chloride between 0 and 4 mg/L in 1900s (Weiler, 1978). While the measured concentrations do not surpass aquatic standards, these concentrations are evidence of increasing salinity in Lake Superior and further support the need for research into alternative deicers to reduce chloride loading in sensitive waterbodies.

Chloride in BB-RP-A is similar to the concentrations seen in I-35-LS, but BB-RP-B has an average chloride concentration of 162 mg/L, with the highest measurement at 597 mg/L (Figure 18b). Considering the EPA freshwater chloride toxicity standards of 860 mg/L (acute) and 230 mg/L (chronic), chloride levels in lake water at BB-RP-B may surpass the limit depending on how long concentration were above 230 mg/L. BB-RP-A was much less impacted by Cl-, and presumably bridge runoff, than BB-RP-B. The differences are likely due to the proximity from runoff discharge as the BB-RP-B site received runoff directly from bridge in comparison to BB-RP-A in which runoff flows over a parking lot before entering the water. Acetate data from BB-RP-A in section 2.5.1 also suggests that dilution is stronger at BB-RP-A and mixing is slower in BB-RP-B.

While Cl^- is the better proxy for NaCl application due to its conservative nature, Na^+ can also be used as an indicator of NaCl. Molar concentrations of Na^+ and Cl^- in the water samples show that Na^+ and Cl^- typically trend together (Figure 19), indicating that the Na^+ and Cl^- concentrations were largely derived from road salt application. A linear regression analysis on the relationship between sodium and chloride has an r^2 of 0.74, $p < 0.001$. NaCl deicing salt has a molar ratio of 1:1, so deviations from this indicate additions of an ion from outside sources or uptake in the soil or water column. Natural sources of Cl^- and Na^+ are from rock weathering. The trends of chloride and sodium can be seen in Figure 19 for 2019-2020 winter; the NaCl molar concentration of other sites and 2020-2021 sites is included in the Appendix in Figures A2-A4. In I-35 stormwater, Cl^- concentration generally has a higher concentration than Na^+, with a molar ratio of 0.83 Na:Cl in I-35-NaCl and 0.71 in I-35-KAc-A and B. Blatnik Bridge has a lower Na:Cl ratio than I-35, with an average ratio of 0.4 in BB-KAc-A and 0.44 in BB-KAc-B. Additional Cl^- sources in stormwater could be other chloride-based anti-icers/deicers ($CaCl_2$ or $MgCl_2$).

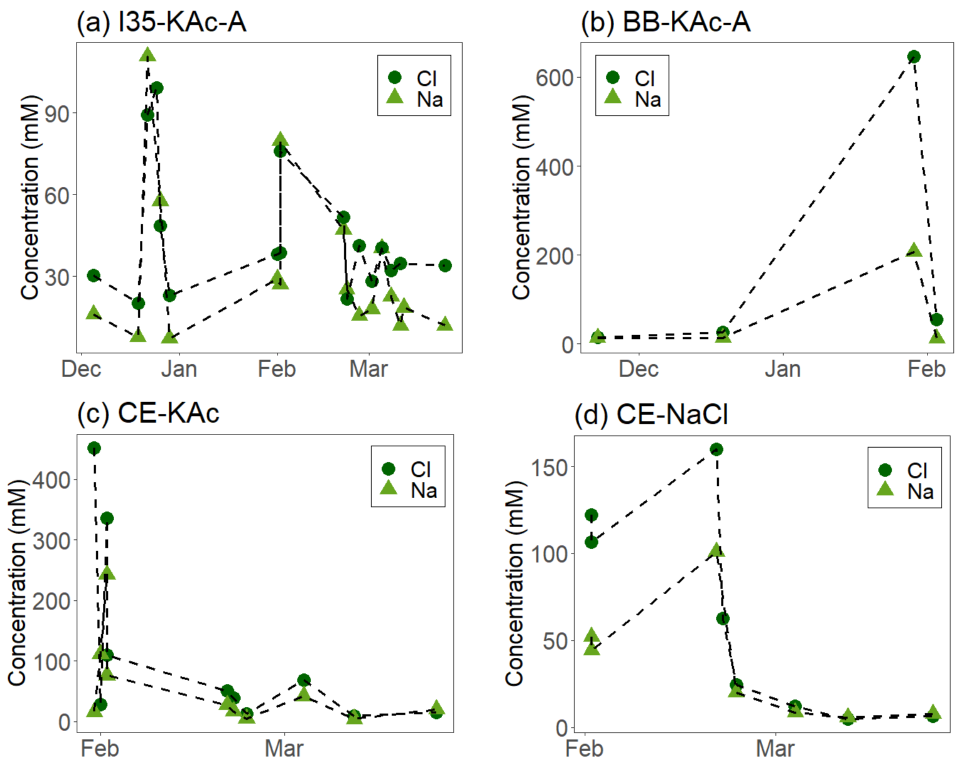

Figure 19. Molar concentration of chloride and sodium in 2019-2020 runoff sites (a) I-35-KAc-A, (b) BB-KAc-A, (c) CE-KAc, and (d) CE-NaCl.

Conductivity is a proxy measurement for the amount of ions in the water. As chloride is the dominant ion in stormwater, it was the greatest contributor to conductivity. A comparison of conductivity (Figure 20) and chloride (Figure 18) show they follow the same trends over time, and Pearson's correlation of the two variables shows they have a very strong positive relationship, with a correlation coefficient of 0.92 ($p < 0.001$) (Figure 21). Conductivity was very high in stormwater, with average concentrations of 18,000 µS/cm (BB-KAc-A) and 21,251 µS/cm (BB-KAc-B) in bridge runoff, and 3,341 µS/cm (I-35-KAc-A) and 2,061 µS/cm (I-35-KAc-B) in stormwater. Year 1 conductivity is included in Figure A5 in the Appendix. Conductivity was the lowest in I-35-LS, at 119 µS/cm, and a bit higher in Rice's Point with an average of 139 µS/cm. Higher salinity, as with chloride, is apparent at Rice's Point with BB-RP-B at 326 µS/cm.

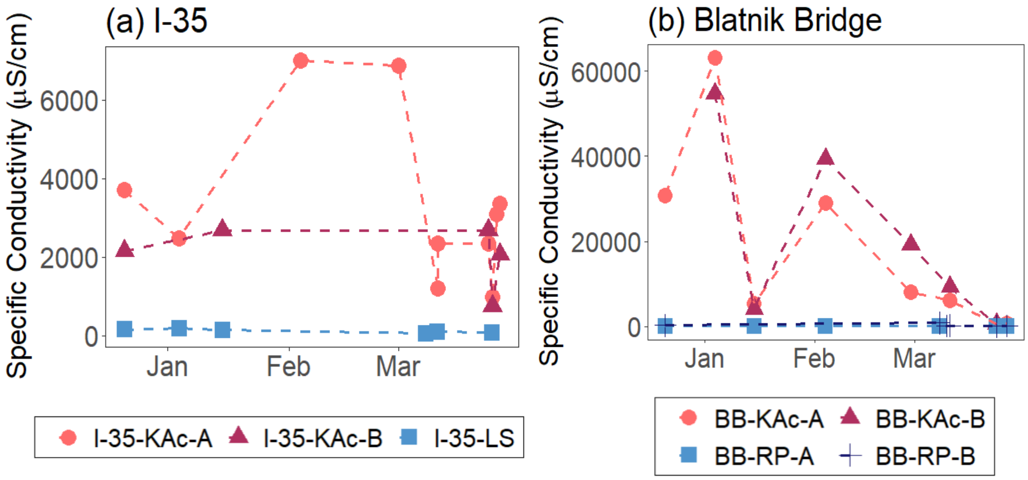

Figure 21. Conductivity of 2020-2021 sites, (a) I-35 and (b) Blatnik Bridge.

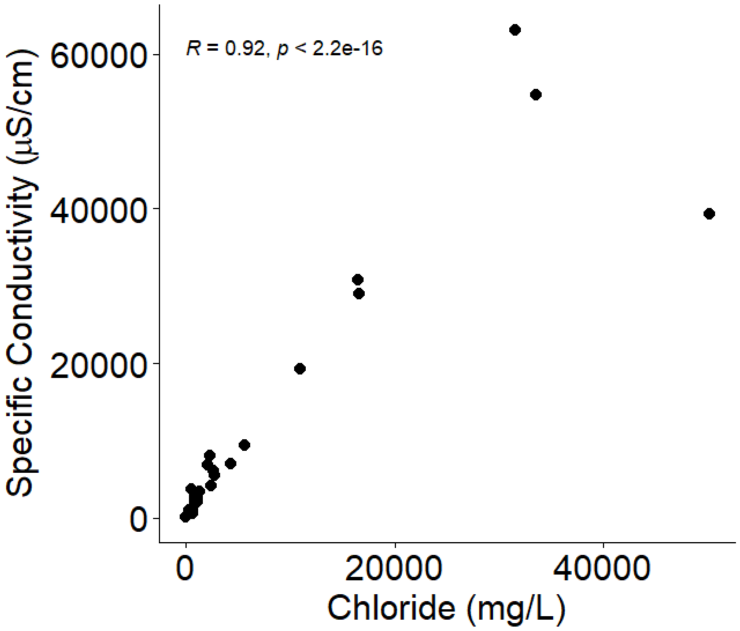

Figure 20. Linear regression of chloride and specific conductivity

2.5.3 Sulfate, Nitrates, and Phosphate

Ions other than KAc or NaCl were analyzed because deicers have been known to have additives or impurities that can contribute high concentrations in runoff. While Cl^- was the dominant anion in stormwater, nitrate (NO_3^-) and sulfate (SO_4^{2-}) were also present in most stormwater. Bromide and nitrite were detected in only a few samples. NO_3^- ranges from 0-270 mg/L in I-35 stormwater (Figure 22). Bridge runoff had more variation in NO_3^- than I-35, with some samples having no detectable NO_3^- and others with concentrations above 1,000 mg/L. Similar high variability in NO_3^- during 2019-2020 is shown in Figure A6. NO_3^- was low (<5 mg/L) in I-35-LS and BB-RP-A but reached 53 mg/L in BB-RP-B from inputs of bridge runoff. NO_3^- is a pollutant which can lead to eutrophication in receiving water bodies, given sufficient phosphorus. Generally, NO_3^- in stormwater is largely due to atmospheric deposition, fertilizer use, and nitrification during the summer season (Jani *et al.*, 2020). The concentration of nitrate in these winter stormwater samples is high in comparison to the values reported in the literature for other stormwater samples. The median concentration of $NO_3^- + NO_2^-$ is less than 1 mg/L in stormwater from freeways in the National Stormwater Quality Database (Pitt and Maestre, 2005). It is unlikely that CF7 contributed to this high nitrogen, as nitrogen is high at different times than acetate (Figure 12 and Figure 22), and CF7 was reported to have nitrite + nitrate <10 mg/L (Barr Engineering Company, 2013).

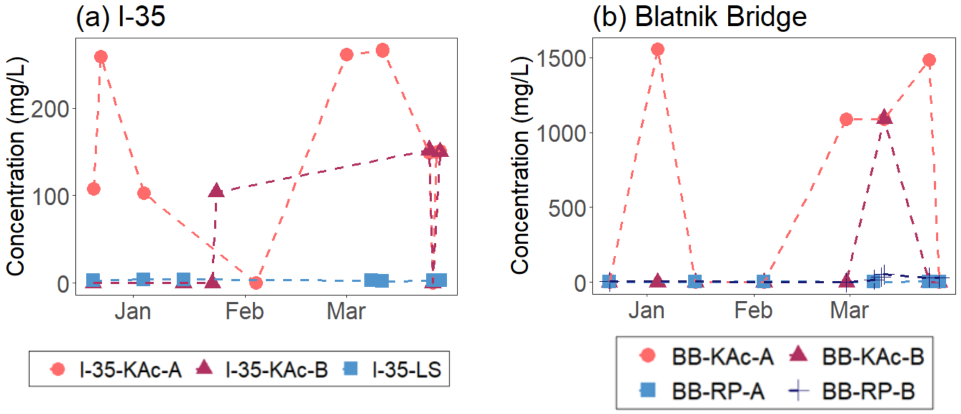

Figure 22. Nitrate in (a) I-35 and (b) Blatnik Bridge sites in 2020-2021.

SO_4^{2-} concentrations (Figure 23 for 2020-2021 and Figure A7 for 2019-2020) in I-35 stormwater during Year 2 average at 347 mg/L at I-35-KAc-A and 379 mg/L at I-35-KAc-B. As with other ions, the Blatnik Bridge water was more concentrated. There, SO_4^{2-} is very similar at each sampling date and ranges from 1,000-3,000 mg/L. SO_4^{2-} was low in I-35-LS, around 5 mg/L while it ranged from 20-60 mg/L at BB-RP-A and BB-RP-B. It is unlikely that this sulfate is from CF7, as peaks in Figure 23 do not match with trends of acetate in Figure 12. Also, 2019-2020 sulfate (Figure A7) has high sulfate at NaCl sites, where no CF7 was detected.

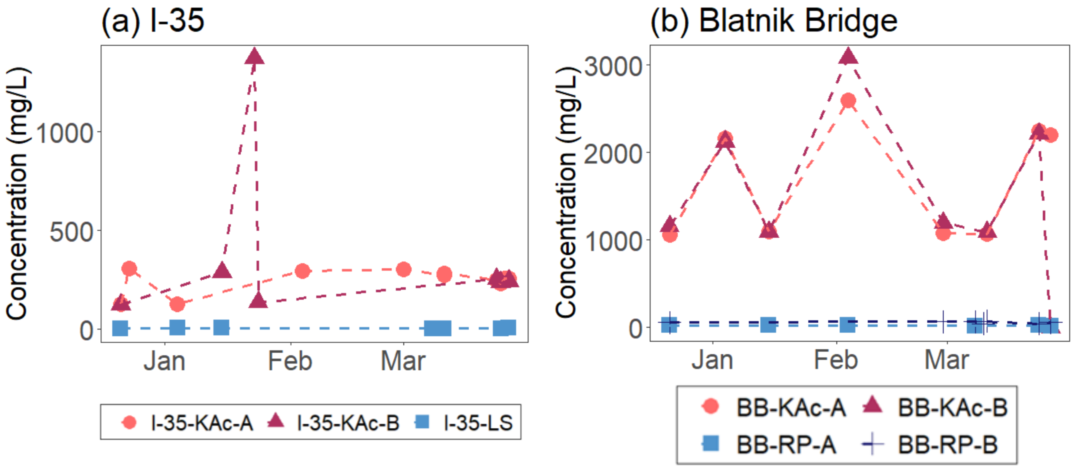

Figure 23. Sulfate in (a) I-35 and (b) Blatnik Bridge in 2020-2021

Phosphate is added to deicers as a corrosion inhibitor. Natural waters have phosphate concentrations of 10-100 µg/L, so additions of phosphate is a concern for eutrophication. CF7 was analyzed on the Ion Chromatograph for phosphate (detection limit about 100 µg/L), but had to be diluted to such an extent as to not damage the instrument with high acetate and potassium concentrations that no phosphate was detected. Levelton Consultants Limited (2007) report phosphate concentrations in CF7 of

340 mg/L. Using the average dilution of CF7 was determined in section 2.5.1, a 100-fold dilution in bridge water would result in PO_4^{3-} around 3 mg/L.

However, no PO_4^{3-} was actually detected in any Year 2 samples, and only in a few Year 1 samples (Figure 24). Phosphate as P was over 20 mg/L in 5 I-35 stormwater samples and one CE-KAc sample, which due to its site would not be sourced from CF7. Given that expected PO_4^{3-} concentrations from CF7 in I-35 would be much lower than what is seen here, and that there was not PO_4^{3-} detected in Blatnik Bridge with higher CF7 concentrations (or I-35 samples from year 2), it's likely that this phosphorus was not primarily from CF7 application. The detection of PO_4^{3-} in CE-KAc indicates sources in stormwater other than CF7. Phosphate in runoff is largely from overland runoff, which carries PO_4^{3-} from soils into the storm water runoff system.

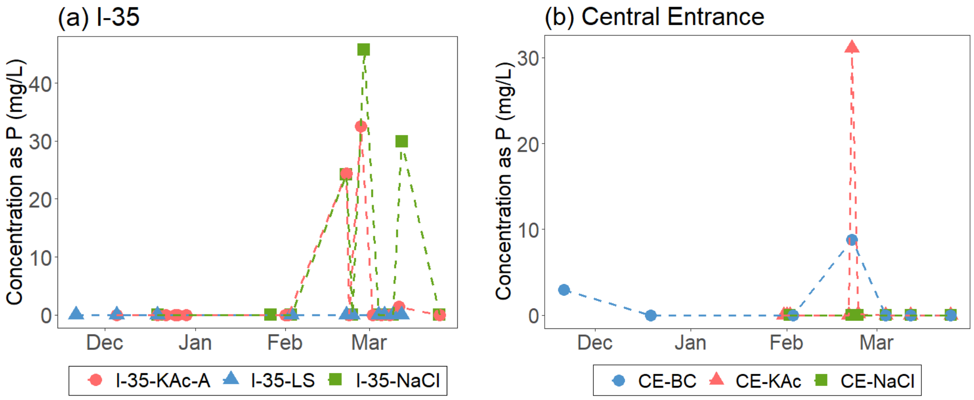

Figure 24. Phosphate in (a) I-35 and (b) Central Entrance in 2019-2020

Important to note to better understand all concentration data in the field evaluation is that 1) this data is all concentration, and not load and 2) there is a much smaller volume of water during winter months, therefore concentrations are much higher than expected for stormwater because they are less dilute. While the data in this section show very high concentrations in comparison to typical stormwater, they are winter stormwater samples which must be understood as chemically more concentrated than summer- but perhaps carrying the same load.

2.5.4 Other major cations

All 2019-2020 cation data is included in the Appendix, along with Blatnik Bridge data from 2020-2021. 2019-2020 cation concentrations were filtered, meaning the concentrations are dissolved cations. Year 2 cations were analyzed for both filtered and nonfiltered cation concentrations. This provides both dissolved (filtered) and total (nonfiltered) concentrations present in the samples. Na^+ and Fe^{2+} are often found bound to particulate matter rather than dissolved.

Just as chloride was the dominant anion due to NaCl application, sodium (Na^+) was the most abundant cation in stormwater, ranging from 200-2,400 mg/L in I-35 stormwater (Figure 25 in 2020-2021 and Figure A8 in 2019-2020), and 500-7,000 mg/L in Blatnik Bridge samples (Figure A10 and Figure A11). As with chloride and acetate, sodium concentrations in I-35 and Blatnik Bridge stormwater are higher than potassium, demonstrating the ability of chloride deicers to travel beyond the site they were applied. While sodium was lower in I-35 stormwater than Central Entrance where only NaCl was applied, it was still about 10 times higher than potassium in I-35 in 2019-2020 (Figure A8) and twice as high in 2020-2021 (Figure 26). Sodium concentrations were twice as high in 2019-2020 than 2020-2021 in I-35 stormwater, suggesting less NaCl was applied or carried into this site the second year.

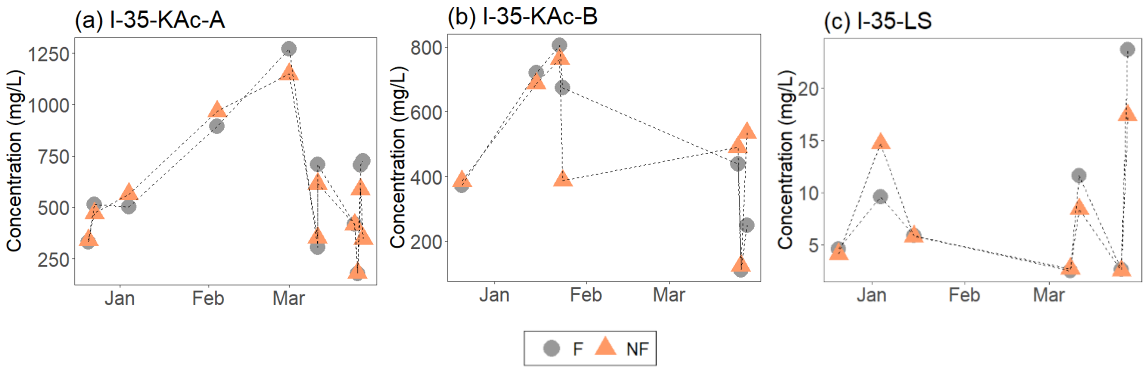

Figure 25. Filtered (F) and non-filtered (NF) sodium in (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS in 2020-2021

Potassium concentrations reach up to 600 mg/L in I-35-KAc-A (Figure 24) and 2,000 mg/L in BB-KAc-A (Figure A12). Potassium in NaCl sites in 2019-2020 (Figure Figure A8b and Figure A9b) is generally 10-70 mg/L, but reaches up to 200 mg/L, indicating some sources of K^+ that are not CF7. However, K^+ trends with Ac^- concentrations, therefore K^+ can also be used as an indicator of CF7 application even though some additions of K^+ may be from other sources such as fertilizer. Potassium concentrations in relation to acetate were discussed in section 2.5.1.

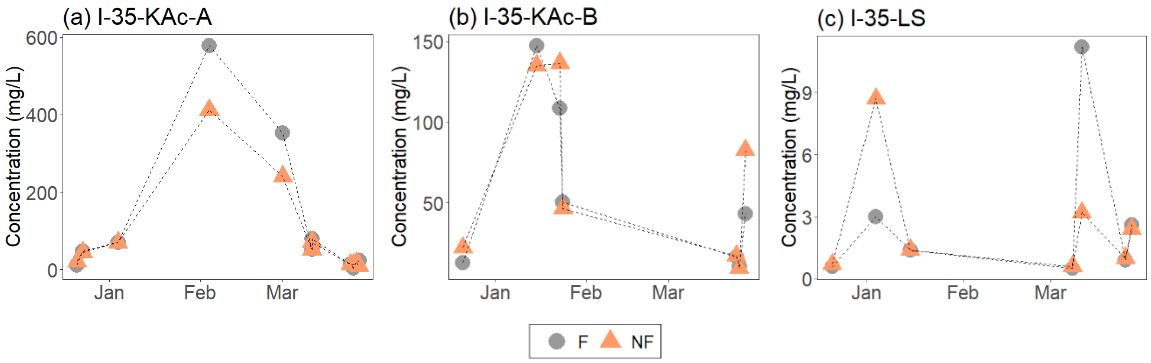

Figure 26. Filtered (F) and non-filtered (NF) potassium in (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS in 2020-2021

Na^+ and K^+ are by far the most abundant cations, with Ca^{2+}, Mg^{2+}, then $Fe^{2+/3+}$ as the next most prevalent. As with Na^+ and K^+, the other cations were variable over time (Figure 27Figure 29). Natural iron, calcium, and magnesium are dissolved in freshwater from rock weathering. Additional Ca^{2+} and Mg^{2+} could be due to the use of alternative road salts containing these species. The average total concentration of Ca^{2+} in I-35 sites is 127 mg/L, and 15 mg/L in I-35-LS. Calcium concentrations in 2019-2020 are much lower than in 2020-2021, suggesting sampling in the first year didn't capture peaks of calcium in stormwater, and perhaps additional Ca^{2+} was present in the stormwater during the second winter of sampling. Magnesium and iron in bridge runoff had a similar range as Mg^{2+} and $Fe^{2+/3+}$ in I-35, but calcium in bridge runoff was twice as high as in I-35 (Figure A14). Ca^{2+} in bridge sites BB-KAc-A and BB-KAc-B reached 570 mg/L. While for all other ions, Blatnik Bridge has been more concentrated than I-35 stormwater, Mg^{2+} is more concentrated in I-35 runoff than the bridge. The average Mg^{2+} concentration at Blatnik Bridge sites was 10 mg/L (Figure A13) while at I-35 the average was 21 mg/L.

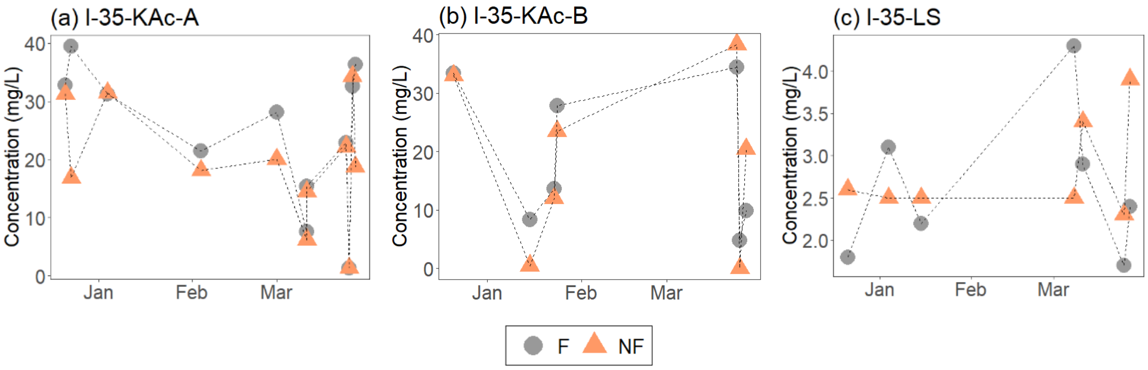

Figure 27. Filtered (F) and non-filtered (NF) magnesium in (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS in 2020-2021

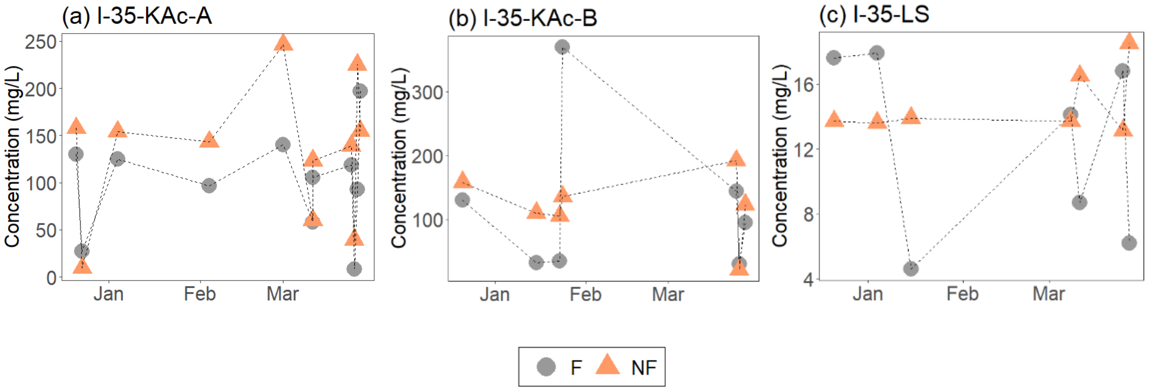

Figure 28. Filtered (F) and non-filtered (NF) calcium in (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS in 2020-2021

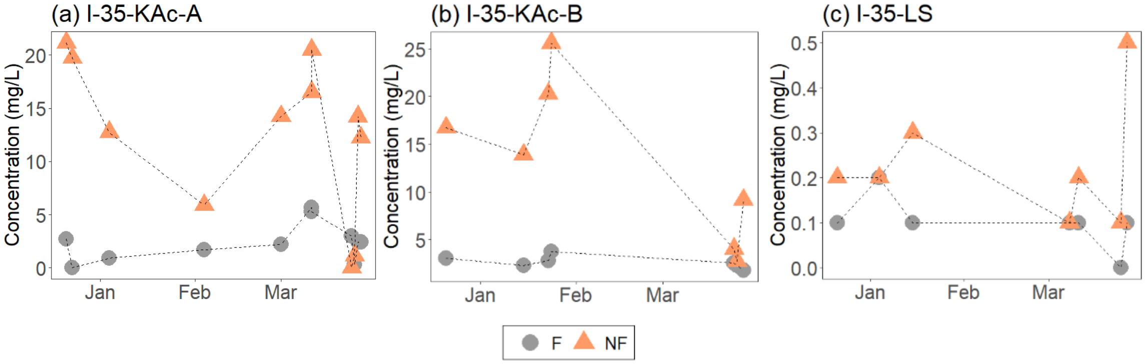

Figure 29. Filtered (F) and non-filtered (NF) iron in (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS in 2020-2021

There was not a large difference in most samples between dissolved and total concentrations of Na^+, K^+, and Mg^{2+}. $Fe^{2+/3+}$ in bridge runoff (Figure 29) was similar to the concentration in I-35 runoff, with an average of 13 mg/L, indicating that iron was originated from soil or particulates. Iron, nearly all of which is bound to solids in stormwater (Figure 29 and Figure A15), has an average of 13 mg/L in I-35 sites and very little present in I-35-LS (0.2 mg/L).

2.6 Microbiological Water quality

Coliform bacteria are measured for their utility as fecal indicator bacteria (FIB). When *E. coli* surpasses standards, it serves as an 'indicator' that human pathogens could be present in the sample. Sources of coliform bacteria in stormwater are primarily animal waste carried through overland flow into the storm system or naturalized bacteria in storm collection systems. As acetate is known as simple carbon source for microorganisms, KAc application has the potential to result in increased microorganism populations. Thus, microbiological water quality was evaluated by enumerating indicator bacteria, *E. coli*. and coliform bacteria.

Average concentrations of coliform (Figure 30) and *E. coli* (Figure 31) bacteria show the highest *E. coli* counts at bridge runoff sites, then at both Rice's Point sites. The range is very wide at CE-KAc, and Brewery Creek has a high *E. coli* count. Sites at I-35 have the lowest bacterial counts, and Lake Superior at I-35-LS is much lower than the lake at Rice's Point. Average bacterial counts in bridge runoff were 3,587 MPN *E. coli* and 4,460 MPN coliform bacteria at BB-KAc-A, and 164 MPN *E. coli* and 3,852 MPN coliform at BB-KAc-B, which are comparable to their level in wastewater. I-35-KAc-A stormwater had an average *E. coli* count of 74 *E. coli* and 830 coliform, while I-35-KAc-B had an average *E. coli* count of 93 and coliform of 1115 MPN. Central Entrance sites had an average of 11 *E. coli* and 4245 coliform at CE-NaCl, 2194 *E. coli* and 3127 coliform at CE-KAc, and 165 *E. coli* and 556 coliform at Brewery Creek. Of the lake sites, BB-RP-A had the highest bacterial populations, presumably due to the large gull population seen in the water and on the dock while sampling. Average *E. coli* counts in the lake were 162 at BB-RP-A, 53 at BB-RP-B, and 11 at I-35-LS. Average coliform counts were 881 at BB-RP-A, 1211 at BB-RP-B, and 23 at I-35-LS. While the differences between means of sites is apparent (Figure 30Figure 31), the only significant differences in total coliform were between I-35-LS and each I-35 stormwater site. There were no significant differences within I-35, Central Entrance, or Blatnik Bridge sites for *E. coli*. Interestingly, of the Central Entrance sites Brewery Creek had lower coliform than the stormwater sites, but higher E. coli count, indicating that E. coli were a larger portion of coliforms in the creek.

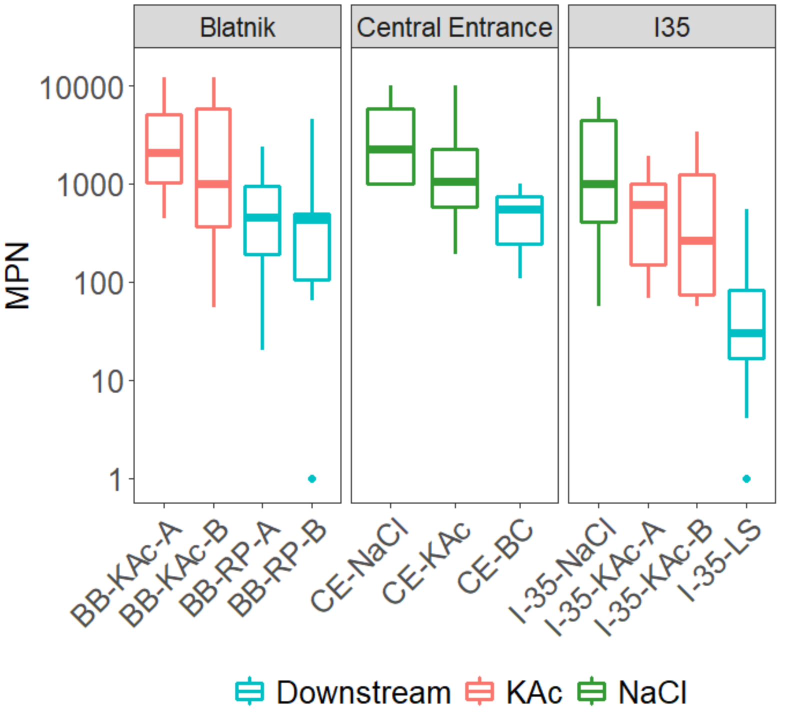

Figure 31. Coliform counts at all sites in the winter of 2020-2021.

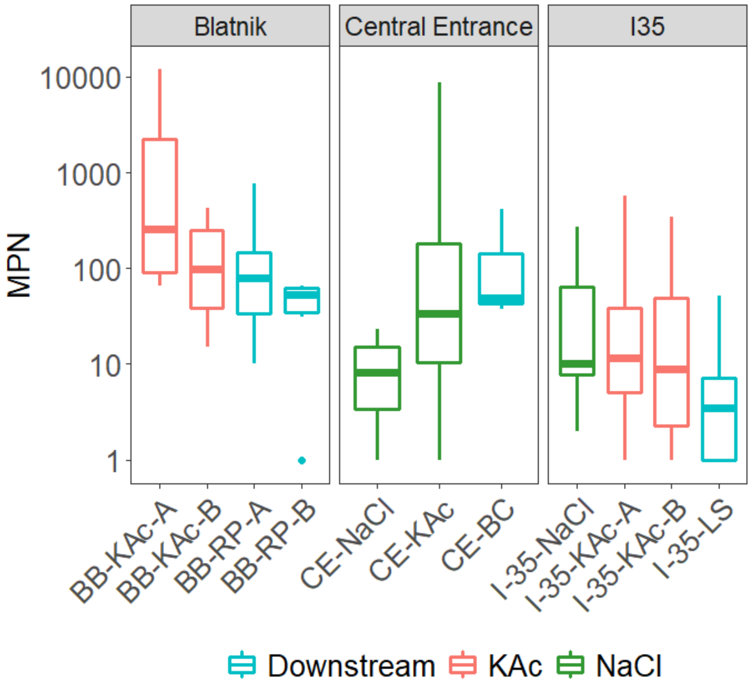

Figure 30. *E. coli* counts at all sites in the winter of 2020-2021.

All sites had very large ranges of *E. coli* and coliform, with MPNs increasing in multiple orders of magnitude on different sampling dates. The *E. coli* concentrations in freeway runoff were lower (aside from BB-KAc-A and CE-KAc) than the national average of 1,900 MPN (Pitt, Maestre and Morquecho, 2004), which is likely due to the fact that most samples included in the national database were taken during the summer. Acetate and *E. coli* have a weak positive relationship (tau = .26, $p < 0.01$), and acetate and coliform also have a weak positive relationship (tau = 0.22, $p < 0.02$) (Figure 32).

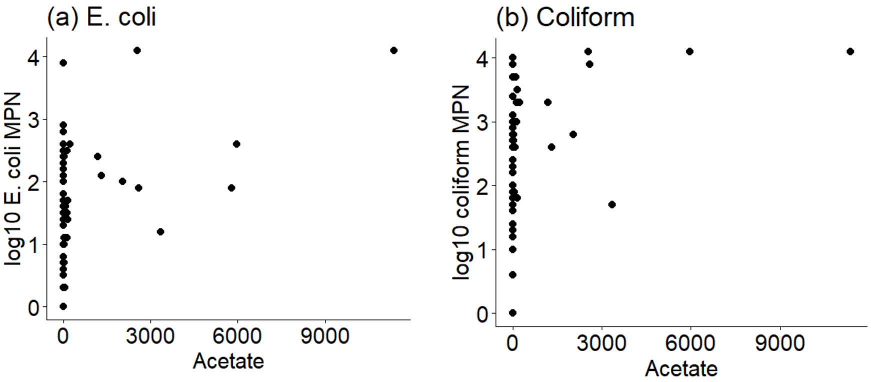

Figure 32. Relationship between (a) acetate and E. coli and (b) acetate and total coliform

Even though NaCl concentrations were as great as KAc in stormwater runoff, the influence of NaCl on FIB is unlikely as the growth of FIB and *E. coli* could have a negative relationship in a high salinity environment (Ito *et al.*, 1977; Kiaghadi and Rifai, 2019). Acetate from CF7 may slightly increase bacterial growth given the weak positive correlation from Kendall's correlation test, but the results are likely skewed towards a positive relationship given the nature of the site. Blatnik Bridge sites had higher concentrations of acetate due to higher application rates and less dilution on a bridge deck, and they generally had higher bacterial counts- possibly due to the use of acetate as a substrate but attributed to the large gull population around Rice's Point. The lack of an NaCl bridge site to evaluate bacterial counts at the bridge at a non-acetate site makes this relationship difficult to attribute to one factor.

2.7 Biological Oxygen Demand

The BOD_5 was extremely high in both bridge runoff and I-35 stormwater, and fairly high in the lake as well (Figure 33). The average BOD_5 at 20˚C was 41 mg/L in I-35-KAc-B, 66 mg/L in I-35-KAc-A, 2,102 mg/L in BB-KAc-A, and 2,371 mg/L in BB-KAc-B. These values were much higher than typical BOD_5 values reported for stormwater runoff in the literature, with the national median BOD_5 of freeway runoff reported as 8 mg/L (Pitt, Maestre and Morquecho, 2004). The impact of a high BOD_5 on oxygen levels is relative, as oxygen levels may tolerate varying BOD_5 based on the rate of reaeration, either by photosynthesis or mixing with the atmosphere. Oxygen levels below 6 mg/L can reduce salmonoid growth with an EPA acute toxicity limit of 4.5 mg/L, where salmonoids especially have a high oxygen requirement. Generally, BOD_5 concentrations under 2 mg/L indicate pristine waters, BOD_5 2-8 mg/L are mildly impacted, and BOD_5 standards for wastewater effluent fall between 20-30 mg/L. The

BOD_5 in all stormwater sites herein is greater than the acceptable BOD discharged from wastewater treatment plants.

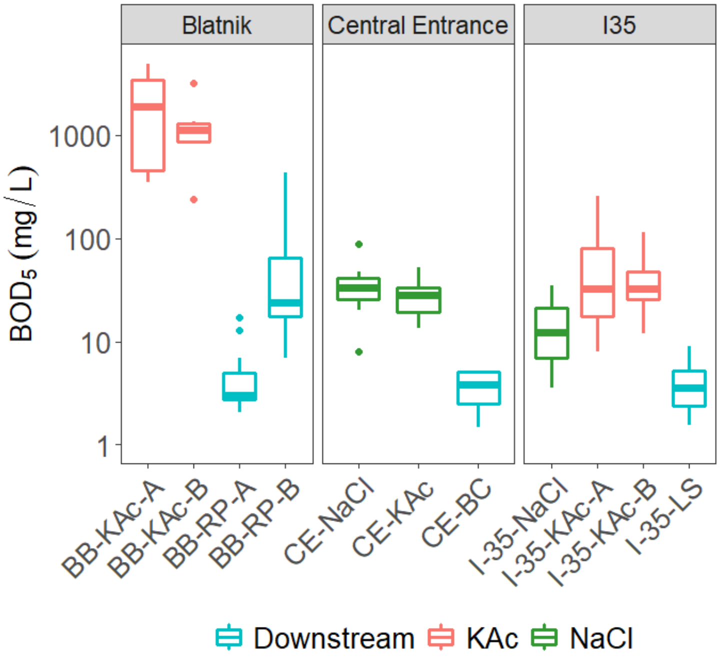

Figure 33. Biological Oxygen Demand (BOD_5) at all sites Year 1 and 2. Bold lines indicate mean values.

The levels of BOD_5 in receiving water bodies were higher than the levels in pristine waters, with an average of 4 mg/L in Brewery Creek and Lake Superior and 5 mg/L in BB-RP-A, and 99 mg/L in BB-RP-B. The trends of acetate and BOD_5 concentration in Year 2 appear to be aligned together in Figure 31. I-35-LS and BB-RP-A were excluded from the Figure as Ac^- was ~0 mg/L at these sites. The increase in BOD_5 with Ac^- concentration is particularly evident in BB-RP-B (Figure 34e). Pearson's correlation coefficient between BOD_5 and Ac^- ($r = 0.84$, $p < 0.001$) indicates a strong positive correlation between them (Figure 32).

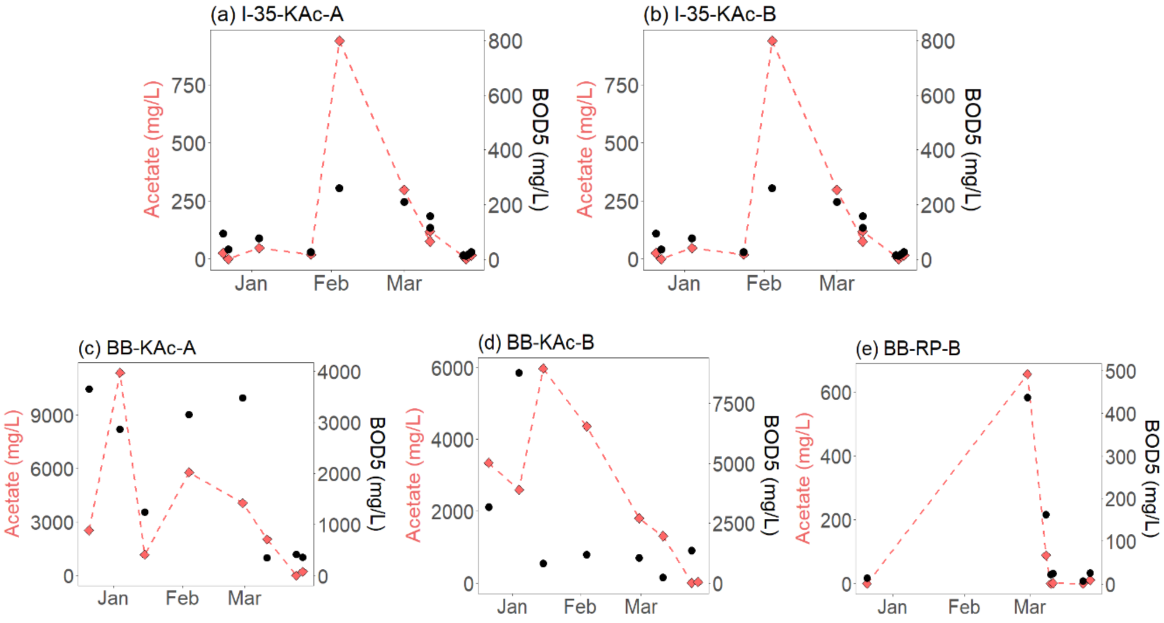

Figure 34. Acetate and BOD5 at (a) I-35-KAc-A, (b) I-35-KAc-B, (c) BB-KAc-A, (d) BB-KAc-B, and (e) BB-RP-b in Year 2

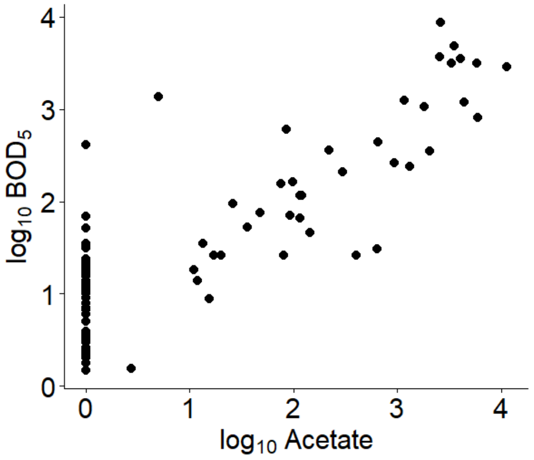

Figure 35. Relationship between acetate concentration and BOD$_5$ in both years. Pearson’s $r = 0.84$, $p < 0.001$.

While BOD$_5$ is sufficiently high in stormwater and BB-RP-B sites to deplete oxygen, dissolved concentrations remain at healthy ecological levels in the receiving water bodies (Figure 36). Dissolved oxygen was higher in lake samples than stormwater, as expected.

None of the stormwater samples had particularly low oxygen levels nor were any depletions apparent when KAc application occurred. BOD_5 is traditionally measured at 20˚C, but the BOD of KAc is exerted at much lower temperatures. The cold temperature of the water during the time of year KAc was applied significantly slows oxygen consumption. Horner and Brenner found that acetate in water takes 5 days to completely biodegrade at 20˚C, but 100 days at 2˚C (1992). Water temperatures in Lake Superior averaged between 0.5˚C and 2.5˚C during sampling. The lack of depleted dissolved oxygen in the downstream sites despite the high BOD_5 concentrations measured in runoff can be explained by both low temperatures slowing biodegradation to exert BOD over time, over which Lake Superior has sufficient aeration and mixing to prevent significant oxygen depletions. If CF7 were applied on other roadways draining into smaller waterbodies, the acetate may degrade slowly enough throughout the cold winter and spring that sufficient acetate remains in the waterbody when the water warms for biodegradation rates to speed up and deplete oxygen from the water.

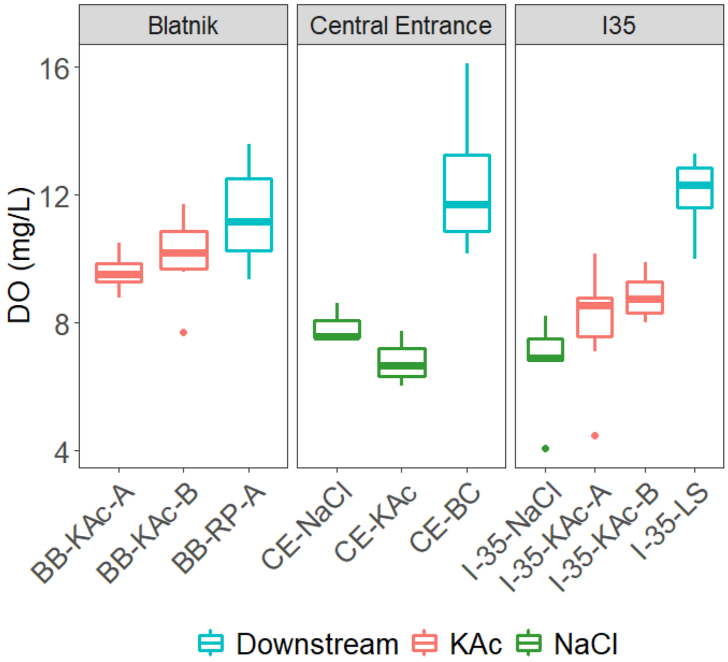

Figure 36. Dissolved oxygen (DO) concentrations in difference sites. A plus symbol indicates the mean DO.

While no oxygen depletion was detected in the receiving water bodies, with an oxygen demand so high it is likely that smaller waterbodies receiving highly concentrated storm water would see oxygen concentrations fall substantially. While there is minimal work on KAc and BOD, LaPerriere and Rea (1989) found that 50 mg/L of calcium magnesium acetate in a receiving water body (pond) is sufficient to cause oxygen depletions past concentrations safe for fish.

Chapter 3: Biodegradation of CF7

Degradation experiments of CF7 were conducted to understand the fate of CF7 in Lake Superior and to give context to its contribution of BOD levels from Chapter 2. The degradation of CF7 in the laboratory was evaluated under two temperature conditions and five concentrations. The experiment was conducted to determine 1) the degradation rate of acetate in the field, hence the experiment done at 4˚C, and 2) the stability of acetate in samples during storage before analysis, hence the filtration of some samples as was done during sample processing.

Lake Superior was used as the base matrix, as this is the system and microbial concentrations that CF7 degrades in under field conditions. Half of the lake water was filtered with a 0.22 µm filter to remove bacteria, while the other half remained unfiltered to keep biomass present. The experimental design is visualized in Figure 37. The experiment was done in both non-filtered and filtered lake water. Lake water was spiked with CF7 to reach 100, 300, 1000, 3000, or 5000 mg/L for samples kept at 4˚C and 20˚C (each in triplicate). Samples kept at 20˚C were stored at room temperature, measured from 20-22˚C, but will be referred to as 20˚C throughout this chapter. Each sample was prepped ahead of time, so rather than making one sample at a given concentration and kept at a certain temperature that is opened to withdraw a sample for each analysis, all CF7 solutions were prepared in HPLC vials at the start of the experiment, than analyzed on the HPLC for acetate concentration.

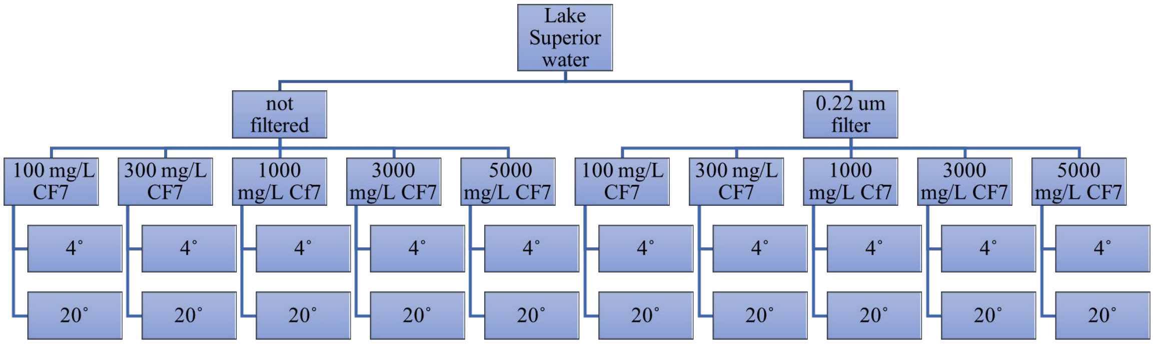

Figure 37. Design of CF7 biodegradation Experiment 1.

3.1 Results

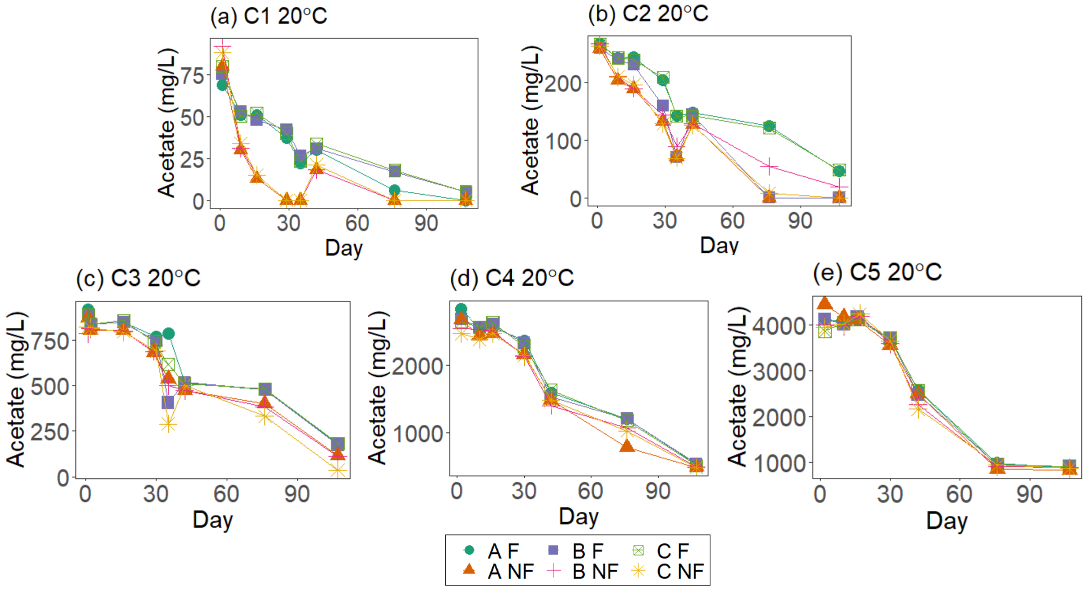

Figure 38. Degradation of acetate in filtered and non-filtered Lake Superior water spiked with CF7 as a function of concentration (100-5000 mg/L as acetate in CF7®; C1-C5) in triplicate (A, B, and C) at room temperature (20-22°C)

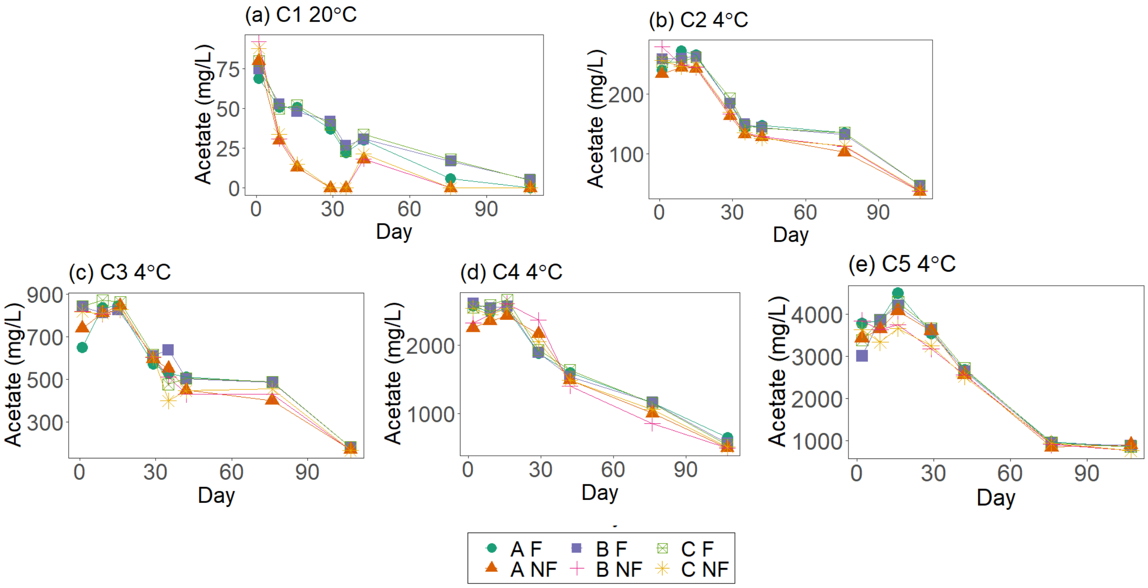

Figure 39. Degradation of acetate in filtered and non-filtered Lake Superior water spiked with CF7 as a function of concentration (100-5000 mg/L as acetate in CF7®; C1-C5) in triplicate (A, B, and C) at 4°C

Table 2. Degradation rates, lag time, and half-life of CF7 as acetate in Experiment 1.

Filtration	Temperature	Initial Acetate (mg/L)	Lag time (days)[a]	k (1/day)[b]	Average k (1/day)[c]	$t_{1/2}$ (days)[d]
Filtered (no biomass)	4˚C	100 (C1)	>9	0.02±0.003	0.02	39
		300 (C2)	>16	0.02±0.002		46
		500 (C3)	>16	0.01±0.002		53
		3000 (C4)	>28	0.01±0.001		50
		5000 (C5)	>28	0.02±0.002		41
	20˚C	100 (C1)	>9	0.02±0.003	0.02	30
		300 (C2)	>16	0.02±0.005		37
		500 (C3)	>16	0.01±0.002		50
		3000 (C4)	>28	0.02±0.002		46
		5000 (C5)	>28	0.02±0.002		41
Non-filtered (Lake Superior biomass present)	4˚C	100 (C1)	0	0.04±0.007	0.02	16
		300 (C2)	0	0.02±0.002		41
		500 (C3)	>16	0.01±0.002		50
		3000 (C4)	>16	0.02±0.001		43
		5000 (C5)	>28	0.02±0.002		41
	20˚C	100 (C1)	0	0.12±0.004	0.05	6
		300 (C2)	0	0.03±0.005		21
		500 (C3)	>16	0.02±0.003		33
		3000 (C4)	>16	0.03±0.005		28
		5000 (C5)	>28	0.03±0.006		28

[a] Lag time refers to the number of days before degradation was observed
[b] First order reaction rate ± 95% confidence interval
[c] Average of first order reaction rates for all concentrations at given temperature
[d] First order half-life was derived by $t_{1/2} = 0.693/k$

Degradation of CF7 had a lag time of 9-28 days (greater with greater acetate concentration) for all filtered samples and higher concentrations. Once degradation began, Figure 38 and Figure 39 show that it occurred more quickly at 20˚C than 4˚C for non-filtered samples. Degradation in C3-C5 (3,000-5,000 mg/L CF7) had no significant difference in rate between filtered and non-filtered samples (Table 2). Only C1 and C2 at 20˚C and C1 at 4˚C showed faster degradation in non-filtered samples. It was expected that degradation would occur more quickly in non-filtered samples in all concentrations as objective of filtering was to remove bacteria that degrade acetate. The stability of filtered samples before significant degradation indicated stormwater samples collected in the field should be analyzed for acetate within 20 days.

The degradation of CF7 at a similar rate regardless of filtration in Experiment 1 was unexpected from the typical (bio)degradation seen by bacteria. The lag time before degradation present in higher concentrations along with the lack of differentiation in reaction rate between filtered and non-filtered samples suggests there are additional degradation pathways removing acetate from the samples other than standard biodegradation. Acetate can be oxidized by reduced species in lake water or in the CF7 formula. Biodegradation of organic species in freshwater is typically undertaken in aerobic conditions, and natural bacteria present in the Lake Superior water are expected to be obligate bacteria. Therefore, the design of the experiment in which lake water (and its microbial population) are incubated in closed HPLC vials until analysis may have resulted in the death of obligate bacteria. Alternatively, the acetate and/or additives in CF7 in the higher concentrations may have resulted in toxicity to bacteria, causing mortality if not impaired oxidation. Or, perhaps the natural bacteria in Lake Superior water were not a large enough population to survive in the sample vials. Any of these scenarios may explain the lag time before degradation of acetate began, perhaps via alternative chemical reactions if natural bacteria in the sample could not survive due to anaerobic conditions or CF7 toxicity. Another experiment was designed to determine the true *bio*degradation of acetate in CF7, and to see if anaerobic degradation could explain these results.

A second biodegradation experiment was designed to answer questions raised after the first experiment. In Experiment 1, results indicated that the experiment may have begun in aerobic conditions then became anaerobic, or that bacteria were sensitive to high concentrations of CF7. A second experiment (referred to as Experiment 2) had significant differences in design from the first, although with that same goal of understanding CF7 degradation in the field. The design is visualized in Figure 40. To better control the amount of biomass in each sample, biomass was harvested and concentrated from 100 L of Lake Superior water, and 1 ml of this concentrate was added to each bottle containing carbonate buffer. The10 mM carbonate buffer, adjusted to pH 7.5, was used as the base matrix, rather than Lake Superior water. CF7 or KAc was then added to each bottle to reach 250 mM. This concentration was chosen as this is a representative concentration of acetate seen in field samples, and higher concentrations in the first biodegradation experiment may have killed the bacteria. Half of each solution was spiked with 1 ml concentrated biomass, and the other half was kept sterile. Samples of either CF7 or KAc, with or without bacteria, were then kept in aerobic vials (with lids that allow for air flow) or standard falcon tubes. A duplicate of each set of conditions was stored at either 4˚C, 15˚C, or 20˚C (replicates rather than triplicates were done for Experiment 2 simply due to time and supply constraints). These adjustments to Experiment 1 allowed for a determination if the additives in CF7 impacted biodegradation by adding KAc samples and a comparison of aerobic versus anaerobic degradation.

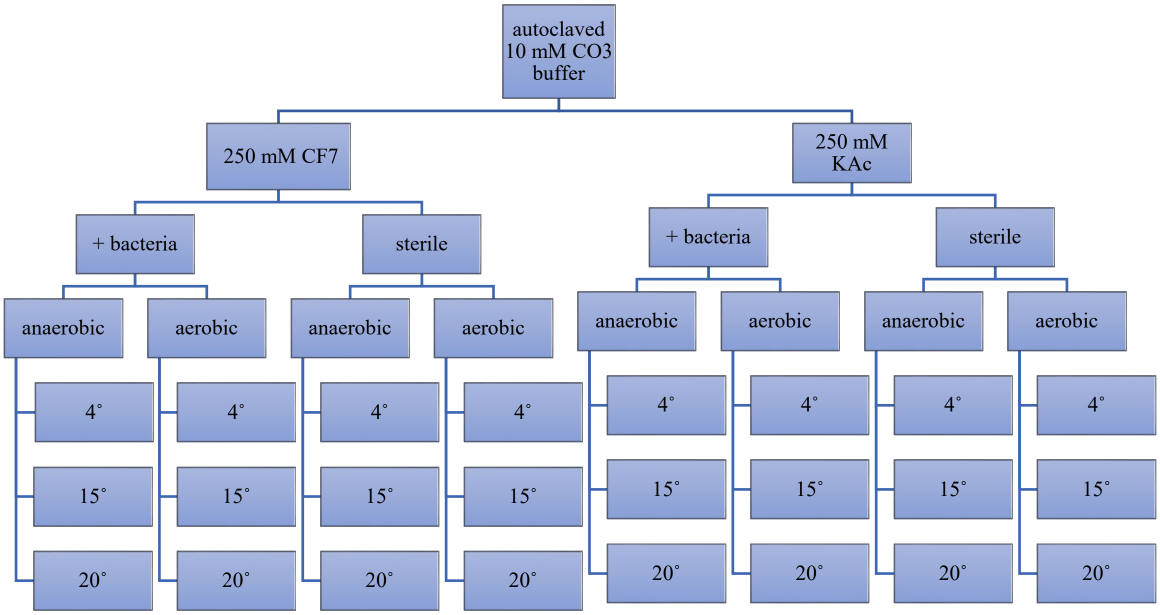

Figure 40. Design of CF7 and KAc biodegradation Experiment 2

3.2 Biodegradation rate discussion

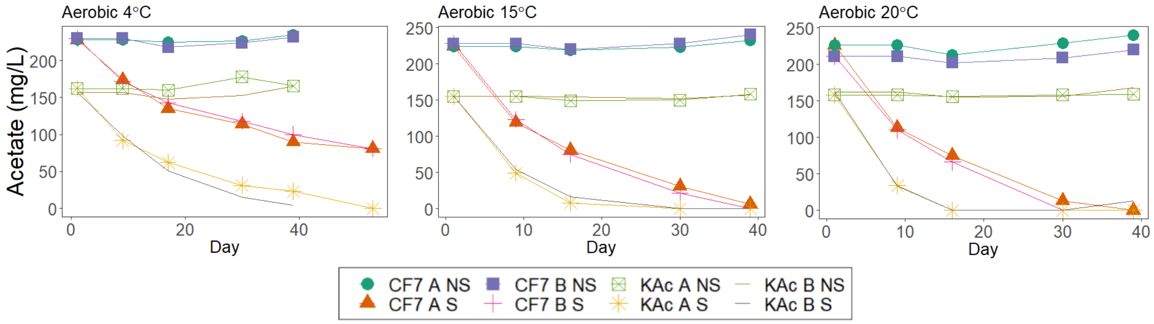

Figure 41. Aerobic degradation of CF7 and KAc at three temperatures with (S for spiked with biomass) and without (NS for not-spiked) biomass

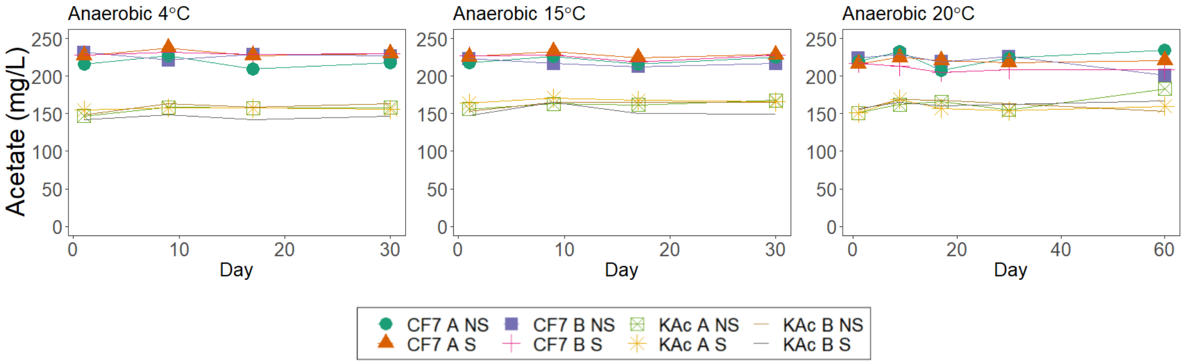

Figure 42. Anaerobic degradation of CF7 and KAc at three temperatures with (S for spiked with biomass) and without (NS for not-spiked) biomass

Figure 41 shows very clear differentiation in biodegradation of CF7 and KAc in spiked samples versus the stable concentrations of both CF7 and KAc in sterile samples. All anaerobic samples showed no degradation over 2 months, for both spiked and sterile samples (Figure 42).

Because samples in Figure 41 largely degraded in the first few days of analysis, spiked samples were redone in Experiment 3 for higher frequency analysis of acetate concentration, which would result in a more accurate degradation rate. First order reaction rates for Experiments 1 and 2 are given in Table 3 (for aerobic, spiked samples only, as the stable concentration of acetate in anaerobic and sterile conditions is visible in Figure 42). The reaction rate was a bit lower for Experiment 3 for CF7 in 20˚C, and KAC in 4˚C and 15˚C. In both experiments at all temperatures, pure KAc degraded more quickly than CF7, suggesting that additives in the deicing product may inhibit biodegradation. This can be seen in the half life in Table 3, where at 4˚C, similar to field conditions, it would take 35 days for CF7 to reach its half-life in comparison with 10-23 days for KAc. From an environmental impact perspective, the inhibition of additives on biodegradation is a positive when thinking about the BOD- a longer biodegradation exerts the BOD over a longer period of time. However, this also implies that there is some toxicity of the additives to microbes, which may have negative ecosystem impacts if microbial function that supports primary productivity in the waterbody is altered.

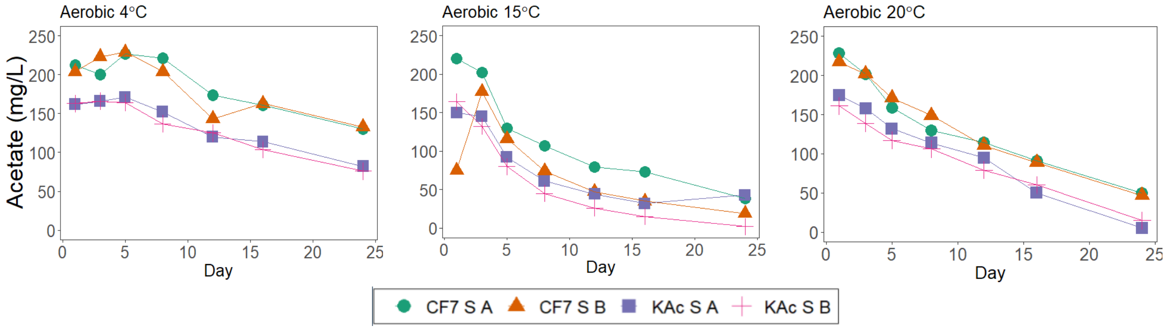

Figure 43. Aerobic degradation of CF7 and KAc in Figure 41 repeated for higher frequency analysis of CF7 and KAc biodegradation with (S for spiked) biomass (Experiment 3).

Table 3. Degradation rates and half-life of CF7 and KAc in Experiments 2 and 3.

	k (1/day)[a]				$t_{1/2}$ (days)[b]			
	Exp. 2		Exp. 3		Exp. 2		Exp. 3	
	CF7	KAc	CF7	KAc	CF7	KAc	CF7	KAc
4˚C	0.02±0.002	0.07±0.003	0.02±0.005	0.03±0.003	35	10	35	23
15˚C	0.07±0.002	0.17±0.020	0.08±0.009	0.13±0.007	10	4	9	5
20˚C	0.08±0.005	0.12±0	0.06±0.002	0.12±0.310	9	6	12	6

[a] First order reaction rate ± 95% confidence interval

[b] First order half-life was derived by $t_{1/2} = 0.693/k$

Experiment 1 reported an average degradation rate of 0.02 day^{-1} for CF7 at 4˚C, and 0.05 day^{-1} at 20˚C. Experiment 3 also found a biodegradation rate of 0.02 day^{-1} of CF7 at 4˚C, and 0.06 day^{-1} at 20˚C. However, in Experiment 1 there was identical degradation with rates of 0.01-0.03 day^{-1} for all samples, regardless of biomass present, for all but the lower concentrations of CF7, in which a separation between filtered and nonfiltered samples was seen. The nonfiltered (biomass present) degradation rate of C1 is

0.04 day^{-1} at 4˚C and 0.12 day^{-1} at 20˚C, twice as slow at 4 ˚C and twice as fast at 20 ˚C in comparison to Experiment 3.

The results of Experiment 2 in which acetate remained stable in an anaerobic or sterile environment suggest that the death of the biomass in the samples, whether due to low oxygen or toxic concentrations of CF7, were not solely the explanation for the degradation. If low anaerobic conditions left only facultative bacteria present in Lake Superior to outcompete obligate aerobes, this should have occurred in Experiment 2 as well. As there was no degradation in Experiment 2's anaerobic conditions, this hypothesis can be rejected. The slower degradation of CF7 than KAc in Experiments 2 and 3 demonstrate that some component of CF7 does slow bacterial metabolism, and this likely contributes to the long lag times seen in C2-C5 in Experiment 1, as the bacterial population adjusts to its environment. As Experiment 2 demonstrated, in a sterile environment acetate is not degraded, and it is likely that some combination of chemical degradation in the natural water and microbial degradation can explain the degradation occurring in Experiment 1. Conditions in Experiment 1 are referred to as filtered and non-filtered, rather than sterile and non-sterile as in Experiment 2, because care was taken during Experiments 1 and 2 to keep those samples completely sterile by autoclaving all steps of the process. In Experiment 1, the filtered sample was exposed to natural bacteria in the air, pipettes, and containers. The lack of degradation in sterile samples in Experiment 2 suggests that slow degradation by microbes able to survive the high concentration of CF7 is likely occurring Experiment 1.

While a literature review did not find any freshwater kinetics studies of acetate degradation, acetate oxidation rate constants of 0.06-0.22 day^{-1} were reported in the ocean

(Zhuang *et al.*, 2019). The acetate degradation rate found here falls within this range, with 0.06-0.17 day^{-1} reported at 15°C and 20°C. A biodegradation study of glycol and potassium acetate deicers reported a biodegradation rate of 0.033 day^{-1} for a non-CF7 potassium acetate product at 4°C, slightly higher than the rates of CF7 found in Experiments 2 and 3 of 0.02 day^{-1} at 4°C (Revitt, Garelick and Worrall, 2002).

The slower degradation at 4˚C than at 15˚C and 20˚C is expected. Some degradation at 15˚C was faster than at 20˚C (KAc in Experiment 2 and CF7 in Experiment 3), but this is not a very large temperature gap and perhaps slightly more bacteria were in the concentrated sample these 15˚C vials were spiked with. The Q10 coefficient describes the extent to which a biological reaction is dependent on temperature. Q10 is estimated by the equation (Sherr and Sherr, 1996):

$Q10 = \left(\frac{R_2}{R_1}\right)^{10/(T2-T1)}$; where R is reaction rate 1 and 2 and T is temperature 1 and 2.

A Q10 temperature coefficient is typically near 2, indicating that for every 10 degrees increase in temperature, the reaction rate would double. Q10 for CF7 is 2.4 in Experiment 2 and 2.0 in Experiment 3, and lower for KAc at 1.4 in Experiment 2 and 1.1 in Experiment 3 (this Q10 is for the temperature dependence of reaction rate between 4°C and 20°C). A lower Q10 for the KAc experiments indicates less of a temperature dependence for acetate degradation of KAc than CF7. However, CF7 degradation is what will be occurring in the field and this temperature dependence between 2 and 2.4 is in line with what is considered standard for microbial reactions.

The 5-day biological oxygen demand tests were conducted in the field evaluation of CF7 for each sample. BOD_5 tests are done at standard temperatures (20°C) and time (5 days). Additional information about the true exerted BOD at cold temperature can be found with the biodegradation rate constants determined here. The field evaluation found that BOD_5 could be very high in stormwater samples, and when a BOD_5 of 450 mg/L was measured in Rice's Point, oxygen was not depleted. This indicates that BOD was exerted over a long period of time, which makes sense given that cold temperature biodegradation is slower than at room temperature. The relationship between the biodegradation rate of CF7 and the fraction of BOD exerted at that time can be expressed by the equation: $\frac{BOD_t}{L_u} = 1 - e^{-k*t}$; where BOD_t = remaining BOD at time in days, L_u = ultimate oxygen demand, k = biodegradation rate, and t = time in days (Revitt, Garelick and Worrall, 2002). Using a k of 0.02 as determined for CF7 at 4°C, the BOD of CF7 theoretically decreases by 2% each day. As can be seen by the fact that BOD_5 was high in BOD_5 standardized tests but no impact to dissolved oxygen (DO) was seen in Rice's Point, the BOD decreased by 2% at 4°C and by 6.8% (using the CF7 Experiment 2 and 3 average k of 0.07 day^{-1}) at 20°C.

Chapter 4. Vegetative and Aquatic Toxicity of CF7

4.1 Introduction

Freshwater salinization syndrome is a growing environmental threat characterized by an increase in dissolved ions due to anthropogenic inputs such as the salting of roads for winter deicing (Kaushal, 2016). Sodium chloride is the most commonly applied deicer, with 19.6 million metric tons applied annually in the United States (Bolen, 2021). In addition to the salinization of freshwater, other adverse effects of sodium chloride application such as corrosion of infrastructure (Fay and Shi, 2011; Kolay, Tajhya and Mondal, 2019), toxicity to aquatic life (Corsi *et al.*, 2010) and roadside vegetation (Bryson and Barker, 2002; Gałuszka *et al.*, 2011; Equiza *et al.*, 2017), and impaired soil quality (Norrström and Bergstedt, 2001) have been well studied.

The growing understanding of the cost of road salt application has led to a search for effective and biodegradable alternative deicing agents. One such alternative, potassium acetate (KAc), is evaluated for toxicity in this study. Research has shown KAc to be an effective deicer, with a higher melting capacity (Xie, Shi and Zhang, 2017) and lower effective temperature (Akin *et al.*, 2013) than sodium chloride, however, very little work has been done to investigate the environmental effects of its application. Here, we investigate the toxicity of sodium chloride and CF7, a commercial KAc product, to grass seed germination and zooplankton survival.

Spray carrying the deicer onto roadside soils induces vegetative toxicity either through altering the osmotic balance which would decrease or prevent water uptake through roots, or by the uptake of the ions into the plant, where a high concentration of one ion can be toxic to the seedling (Bewley and Black, 1994). The accumulation of excess sodium inside plant cells has been demonstrated to alter key biochemical processes in protein synthesis and metabolism (Tester and Davenport, 2003), harden the cell wall (Neumann et al., 1994), and alter permeability of the cell membrane (Cramer, Läuchli and Polito, 1985). High potassium concentrations can inhibit magnesium uptake, an important nutrient (Li *et al.*, 2018).

Aquatic organisms are exposed to deicers as the majority of the deicer applied on roads is carried off in stormwater and enters downstream waterbodies. Previous work has found KAc deicers to be more toxic to aquatic life than others (Barr Engineering Company, 2013). The only study found in the literature to investigate the toxicity of a KAc deicer to vegetation reported that duckweed and onion are very sensitive to KAc at low concentrations (EC_{50} 40-80 mg/L) (Joutti *et al.*, 2003).

4.2 Methods

This study was conducted to determine the aquatic and vegetative toxicity endpoints of CF7 to understand the environmental impact of applying CF7 as a deicer. Throughout the application of CF7, a portion of the deicer will accumulate in roadside soils while the rest will be carried off the road in stormwater and into surface waters. A seed germination experiment was performed to determine (a) the toxicological endpoints of CF7 and other salts in comparison to concentrations seen in the field and (b) the driver of toxicity in CF7 and other deicers: is toxicity driven by the cation, anion, combination or additives to commercial products? Aquatic toxicity tests of CF7 to the zooplankton daphnia was done with similar objectives as the seed germination experiment: to (a) determine toxicity endpoints of CF7 in comparison with NaCl, and (b) find the agent of toxicity in CF7 (potassium, acetate, or additives).

4.2.1 Vegetative Toxicity

Panicum virgatum (switchgrass) was selected for the germination toxicity experiment due to its presence in a MnDOT seed mix applied in Duluth. *Festuca ovina (*sheep fescue) was also chosen as there is interest in the use of modified turfgrass along roadsides. 250 mM, 100 mM, 50 mM, 20 mM, and 10 mM solutions of KAc, NaCl, sodium acetate (NaAc), potassium chloride (KCl), and CF7 as KAc were prepared with filtered Lake Superior water. Lake Superior water was chosen as the base matrix due its low concentration of ions that mimics the soil porewater environment. The control, filtered Lake Superior water, is referred to as the 0 mM concentration.

Seeds were soaked in 8% sodium hypochlorite for 15 minutes then rinsed under distilled water. Sterilized seeds were placed in 9-cm petri dishes lined with Whatman filter paper. Filter paper was wetted with 4ml of Lake Superior water for the control, or 4ml of each salt solution with the target concentration. Seeds were cold stratified in 4˚C for 6 days, then placed in an incubator at 25˚C and kept from drying out with additions of deionized water every 2-3 days.

Three petri dishes of 20 seeds each were used per treatment, for a total sample size of 60 seeds/treatment. Seeds were monitored daily for germination for 15 days, with germination considered to be the emergence of the radical. The significance of the differences between the mean germination in groups was determined with a two-way analysis of variance (ANOVA) and Tukey post-hoc test. The EC_{50} endpoints for each salt, or concentration at which germination of 50% of seeds was inhibited, were determined by regression. After the 15 days, the root and shoot length of seedlings was measured, and switchgrass root cross sections were analyzed with a Scanning Electron Microscope (SEM; Hitachi 3030 Plus with Quantax 70). Energy Dispersive X-ray (EDX) analysis was performed to observe the accumulation of ions in the roots.

4.2.2 Aquatic toxicity

Toxicity tests were done by the Great Lakes Toxicology and Ecology Division of the Environmental Protection Agency. Test organisms *Daphnia magna* and *Ceriodaphnia dubia* were used as model organisms for 48-hour acute toxicity tests, conducted at 25˚C, with an additional test at 20˚C for *D. magna*. Toxicity tests were conducted in Amended Lake Superior Water spiked with CF7, KAc, or NaAc under a 16:8-hour light/dark cycle. Five neonates less than 24 hours old were obtained from EPA culture units and placed in groups A, B, and C. Organisms in the A and B replicates were fed a mixture of yeast, cereal leaves, algae, and trout chow prior to the addition of the salt solution. The C replicate was not fed to determine if food affected the toxicological response. Survival was recorded at 24 and 48 hours and the LC_{50} (lethal concentration causing the death of 50% of a group of test organisms) was determined.

4.3 Results

4.3.1 Seed germination

Sheep fescue and switchgrass had similar responses to salinity. All data is included in Table A3, with germination of sheep fescue results shown in Figure 44 and other sheep fescue data and all switchgrass results included in Figures A16 and A19. Grass seed germination was not significantly affected until seeds were germinated in 50 mM salt solutions, and almost no seeds were able to germinate in the 250 mM solutions (Figure 44). EC_{50} endpoints are reported in Table 4, with a range from 5.1 g/L for CF7 as K to 15.8 g/L NaCl for sheep fescue. Significance was determined with a two-way analysis of variance (ANOVA) and Tukey post-hoc test (Table A1). Seeds grown in acetate salt solutions (CF7, KAc, and NaAc) were more sensitive to increases in salinity than the seeds grown in chloride salt solutions (KCl, NaCl). Generally, germination and growth switchgrass seeds were affected at a slightly lower concentration than sheep fescue.

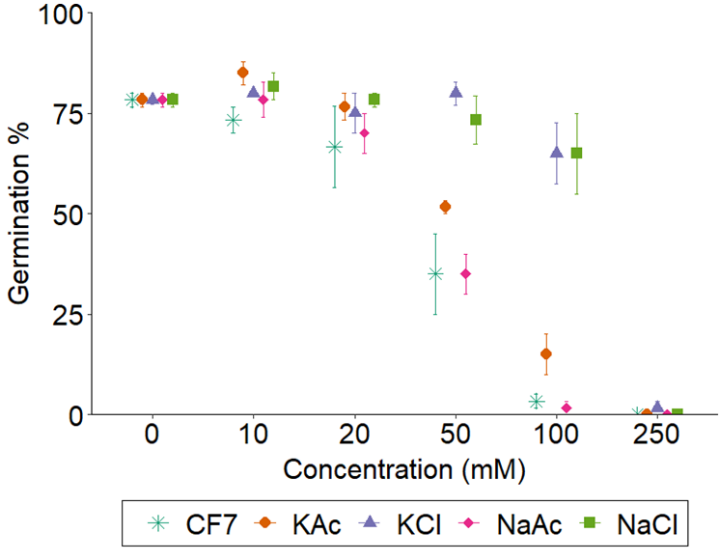

Figure 44. Germination of sheep fescue in various salt solutions and concentrations. The control is 0 mM (Lake Superior water).

Table 4. EC_{50} endpoints of sheep fescue and switchgrass germination by chloride- and acetate-based salts and CF7

	Sheep Fescue		Switchgrass	
	mM	*g/L*	*mM*	*g/L*
NaCl	112	6.6	105	7.8
KCl	149	11.1	84	4.9
KAc	67	6.6	63	6.2
NaAc	52	7.1	63	8.6
CF7*	52	5.1	51	5.0

*units for CF7 as expressed as g/L or mM of KAc in CF7

Growth parameters were evaluated as sublethal effects of salinity in addition to germination success of seeds. Both root and shoot growth were inhibited as salinity increased (Figure A17). Interestingly, most root and shoot lengths grew longer than the control at low salinities for sheep fescue, with a more subtle increase in growth for switchgrass. Root growth was more affected by salinity than shoot growth, as the root to shoot ratio was decreased with increasing salinity (Figure A18). The Vigor Index (VI = % germination * [mean root length + mean shoot length]) was calculated to serve as an overall indicator of the effect of salinity on seed germination, showing in Figure A19 that low salinity has a positive effect on grass seed germination and growth for sheep fescue grown in KCl or KAc, then as salinity increases overall growth is inhibited for both species in all salts.

EDX analysis of ion uptake in the switchgrass roots (cross section image shown in Figure A20. Cross section of sheep fescue root on the SEM germinated in 10 mM NaAc solution) showed the majority of the roots were carbon and oxygen, with the remaining <5% K, Na, Cl, Mg, and Ca. Generally, seeds did take up excess K^+, Cl^-, or Na^+ into the root when they were in solution (Figure 45). Notably, the Ca^{2+} and Mg^{2+} did not appear to be related to the degree of salinity seeds were grown in. Both the increasing concentration of ion taken into roots when in the surrounding solution and Ca^{2+}/Mg^{2+} presence indicates specific ion toxicity drives reduced germination and growth, and not solely osmotic stress.

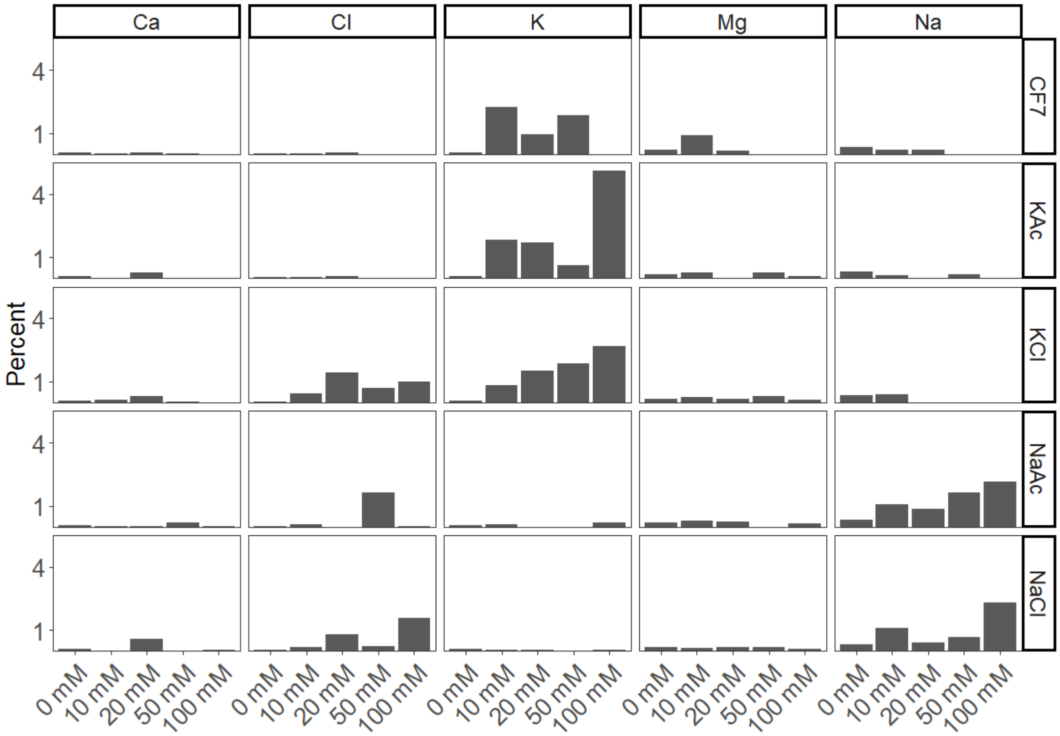

Figure 45. Atomic percent of ions in switchgrass root cross sections after germination in 0-100 mM CF7, KAc, KCl, NaAc, or NaCl solutions.

4.3.2 Aquatic Toxicity

LC_{50} endpoints shown in Table 5 are between 460 mg/L and 930 g/L for KAc and CF7, while the LC_{50} for NaAc is much higher at 840-7260 mg/L. The large discrepancy between lethal concentrations of potassium salts and an acetate salt indicate that potassium is the agent of toxicity in CF7. The lower endpoints of KAc in comparison to CF7 imply that additives in CF7 are not responsible for toxicity of CF7 to daphnia. Given that K^+ is the driver of toxicity, LC_{50} concentrations expressed as the concentration of the cation are shown in Table 5. There is no meaningful difference in the toxicity of any salt at different temperatures to daphnia magna.

Table 5. LC_{50} endpoints in mg/L of daphnia in CF7, KAc, and NaAc solutions.

	C. dubia 25˚C	*D. magna* 25˚C	*D. magna* 20˚C
CF7 (as K^+)	730 (130)	870 (150)	930 (160)
KAc (as K^+)	460 (180)	460 (180)	460 (180)
NaAc (as Na^+)	7260 (1230)	8340 (1410)	8400 (1420)

4.4 Discussion

In addition to determining vegetative toxicity endpoints of CF7, we were also interested in understanding the driver of toxicity- is the cation, the anion, or additives in the deicer responsible? There was a clear difference in the tolerance of seed germination between acetate salts and chloride salts and no statistical difference in germination between seeds germinated in KAc or CF7 solutions, identifying acetate as the agent of toxicity in CF7, not potassium or deicer additives. The decreased root:shoot ratio at high salinities indicates that seeds that are able to germinate under salt stress are likely to be impaired as smaller roots have less ability to allocate and transport nutrients and resources (Maskova and Herben, 2018).

With an EC_{50} of NaCl five times higher than CF7 for sheep fescue and twice as high for switchgrass, switching from a chloride deicer to a potassium acetate deicer would result in much lower concentrations of CF7 impacting grass seed germination and growth than NaCl. However, the EC50 of CF7 is 5.0-5.1 g/L, while soil porewater has been reported to reach concentrations of 1.5 g/L of NaCl (25 mM) from deicer application (Pedersen, Randrup and Ingerslev, 2000). The concentrations greater than 20 mM seen in all of the studied salt solutions before grass germination was affected indicate that use of CF7 deicer is not likely to cause toxicity to grass seeds.

The 2-day acute LC_{50} of CF7 to *C. dubia* reported in table 5 is 730 mg/L. This is on the low end of toxicity to *C. dubia* reported in the literature, with Mussato and Guthrie reporting a 7-day LC_{50} of 660 mg/L (Fischel, 2001), an acute LC_{50} of 1,290 mg/L (Barr Engineering Company, 2013), a chronic LC_{50} of 1,994 mg/L (for KAc, not CF7) (Levelton Consultants Limited, 2007), and an acute LC_{50} of 1,398 mg/L (for a KAc deicer, CF7 not specified) (Corsi *et al.*, 2009). The finding that additives to the commercial deicer product did not add to toxicity supports findings by Corsi et al. (2009) but contradicts other findings (Barr Engineering Company, 2013). More work should be done to investigate the role of additives in the toxicity of deicers.

As the concentration of K^+ determines the toxicity of CF7, potassium concentrations measured or modeled in the field can be used to contextualize the laboratory findings into real-world effects. Using potassium measured in Rice’s Point (BB-RP-B) as an example of a potential small lake or pond receiving stormwater carrying CF7, concentrations of K^+ were detected up to 660 mg/L (section 2.5.1), higher than the 130 mg/L LC_{50} of CF7 as K^+.

The finding of the high potential for CF7 application to result in daphnia mortality and low likelihood of affecting roadside grasses fills a gap in our knowledge of the toxicity of alternative deicers. We report the first toxicity endpoints of CF7 to grass species, to our knowledge. Toxicity endpoints determined here can be used by road salt decision makers to make informed decisions to minimize chloride application.

Chapter 5. Conclusions and Implications

5.1 Summary of research findings

Efforts to better understand the environmental impact of the application of a potassium acetate deicer, CF7, involved a field evaluation, biological oxygen demand tests, biodegradation experiments, and toxicity testing. The extremely high concentration of sodium and chloride found in stormwater in the field investigation calls attention to the necessity for research into alternative deicers. Not only the results of the field evaluation, but its methods are useful information. Few studies have attempted a winter field investigation of stormwater, as ice makes sampling very challenging. The methods used to sample stormwater in the winter can provide a blueprint for others who are interested in winter sampling.

While the focus of this research is on the environmental impact of KAc, an additional output of this work is knowledge of winter stormwater sampling. Most stormwater sampling takes place during the summer; field work in the ice and snow greatly complicates sample collection. Stormwater sampling had to plan for three main barriers to sampling: ice, battery life, and low flow. Ice was a major challenge to sampling, as ice would form in the tubing, preventing samples from being taken into the ISCO units, ice would seal the manhole covers shut, and during the coldest weeks of the year stormwater was completely frozen through. Manholes were opened by use of a sledgehammer to break the ice seal. Ice was prevented from forming in ISCO tubing by programming it to rinse the sample tubing three times after each sampling uptake. A field torch was used to melt ice that sealed ISCO parts together when repairs were needed. Battery life is a major barrier to sampling in cold weather, this was addressed through the set up of ISCO units in boxes outside the manhole within a box fitted with a solar panel that continually charged a 12v battery. Sites without this setup had ISCOs placed in the storm drains (where it is warmer) and handwarmer packets were taped around the battery the day before sampling. In the winter, there was often merely a trickly of flow in the storm drains, making automatic collection impossible with tubing. This was managed by installing weirs, which would back up flow behind plexiglass in order to have sufficient water depth to pull a sample from. A drawback to this was that the weir blocked debris passing through the storm drain, and this had to regularly removed. While weirs would most likely function as intended in a newer storm drain, the City of Duluth's stormwater conveyance system is over 100 years old and when weirs were outfitted it was discovered pipes were not completely circular, causing water to flow under the weir. Sealing weirs against the storm drain in a wet environment proved challenging. Another key lesson learned from winter sampling includes the importance of selecting sample sites with ice and snow in mind- one storm drain became covered when the road width decreased from snow, and sites on Central Entrance were buried each time the plow went by. Ultimately, winter sampling demands more time (at sites and visiting sites) to maintain sites for sampling.

The field evaluation provided insights into the fate of KAc once it is applied on the roadway. Concentrations of K^+ and Ac^- in stormwater runoff reached 940 mg/L in freeway runoff and 13,000 mg/L in bridge runoff, giving an estimate of concentrations expected to be found in the field based on KAc application rates. CF7 was diluted by an average of 2,000-fold in I-35 and 100-fold at Blatnik Bridge. The lack of acetate detected in NaCl sites indicates acetate can be used as a proxy for CF7. Aside from contributing to the general high conductivity of runoff, CF7 did not have any adverse effects on water chemistry in comparison to NaCl. Although phosphate from CF7 is a potential concern with this deicer in the literature, phosphate was largely not detected in KAc samples.

The main environmental concern of the application of organic deicers is the potential for oxygen depletion in waterbodies receiving the deicer due to the BOD of the organic material. Here, we found a very high BOD in stormwater carrying CF7, no trace of the deicer or its oxygen demand in the main waterbody of Lake Superior, and a BOD_5 up to 430 mg/l in Rice's Point site BB-RP-B. While the results from the field evaluation inform Minnesotans about the direct impact of CF7 application to Lake Superior, the findings from this research can be extrapolated to environments beyond this one site. The field evaluation does not only inform of how certain sites in Lake Superior dilute stormwater inputs but shows how waterbodies of various sizes and mixing may be affected by CF7. The impacts of CF7 in terms of concentrations of potassium found above toxicity thresholds and BOD greater than 400 mg/L at BB-RP-B can be assumed to be how CF7 would impact a smaller, less mixed lake. Figure 46 outlines the potential impacts (these are theoretical based on the possibilities found in this research and in the literature) of KAc deicer loading in a freshwater lake. A smaller lake can warm quickly in the summer- since the BOD in this study was found to be exerted slowly due to the cold temperature, this puts forward the possibility of significant oxygen demand of acetate remaining in the lake as water warms, quickening biodegradation and potentially depleting oxygen. The addition of a high concentration of KAc may increase stratification and slow or stop turnover (as demonstrated with NaCl), combining with the BOD to deplete oxygen in the hypolimnion. If the hypolimnion is anoxic, a major environmental concern is internal phosphorus release from the sediment. The addition of KAc would impact the microbial community, potentially causing cascading effects across trophic levels. This is discussed further in Section 5.2, but it is likely that the addition of salt transforms bacterial communities to halophiles. If the functional role of bacterial community is altered by a shift in community structure, or if anaerobic respiration takes place in an anoxic environment, this could alter nutrient cycling and the base of the trophic level. Hintz *et al.* (2017) demonstrated that road salt inputs can affect an ecosystem due to cascading impacts across trophic levels.

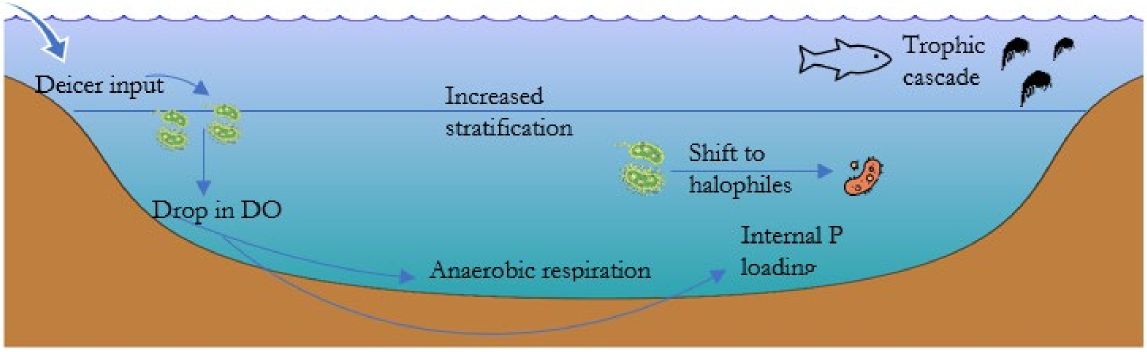

Figure 46. Diagram of potential impacts of a KAc deicer to a lake

A key finding of the field evaluation is that simply having a high BOD is not sufficient to assume the deicer should not be used in the field. The way that BOD is exerted is essential to if oxygen depletion occurs. BOD_5 in Rice’s Point was very high for a lake, yet dissolved oxygen was not impacted. The lack of oxygen depletion was attributed to the cold temperature of water slowing microbial activity, resulting in a BOD exerted over a longer period of time during which reaeration and mixing put more oxygen into the water column than bacterial oxidation removed. This was confirmed by biodegradation testing in Chapter 3, where CF7 was found to have a Q10 of 2-2.4 and a reaction rate 3-4 times lower at 4˚C than at 20˚C.

While the biggest concern with CF7 was expected to be its BOD, the aquatic toxicity is what will limit recommendations to use CF7 rather than NaCl. The LC_{50} of CF7 to *C. dubia* is 730 mg/L (130 mg/L of K^+) and 870 mg/L to *D. magna* (150 mg/L as K^+). K^+ was measured at over 560 mg/L in BB-RP-B, surpassing these acute toxicity endpoints. Aquatic LC_{50} endpoints were surpassed in the field, but EC_{50} endpoints for grass seed germination were well above concentrations expected to be found in roadside soils. However, many studies have reported the toxicity of NaCl deicers to vegetation and based on our findings CF7 would affect vegetation at even lower concentrations than NaCl. Therefore, while the vegetation toxicity assays done in Chapter 4 show no toxicity of grass seeds to CF7 application, additional toxicity testing of more species found near roadsides should be investigated.

K^+ was found to be the driver of toxicity of CF7 to daphnia, and Ac^- to grasses. Interestingly, neither toxicity assay found significant difference in KAc versus CF7 toxicity, suggesting that additives were not meaningfully contributing to toxicity. However, in biodegradation experiments, the reaction rate was lower for CF7 than KAc, meaning that the additive seems to have some inhibitory effect on bacteria.

The results of this investigation into the environmental impacts of the application of CF7 fill in gaps in the literature on alternative deicers. Field and lab data reported here can be used in models to predict the impact of CF7 application in other locations, and have already been used in a lake BOD model by ISU (Rehmann, Ikuma and Perez, 2021). A more robust understanding of the environmental impact of applying a KAc deicer is valuable to salting decision makers concerned about the use of chlorides and looking to make informed decisions on how to keep roads safe and minimize chloride applications in the environment.

5.2 Future work

While this work has provided needed knowledge on the fate and toxicity of KAc in the environment, there are still many avenues of research to explore. Future work should expand research into the fate of KAc in additional environments and the toxicity of KAc to other species. The range of concentration of potassium and acetate found in highway and bridge runoff from CF7 application, BOD_5 concentrations, biodegradation rates, and toxicity endpoints can be used in models to expand the findings of this study beyond the Duluth area.

As mentioned in section 1.1, one of the many problems with NaCl is the accumulation of sodium in the soil, where it displaces other cations from soil exchange sites and can result in the leaching of heavy metals through the soil and into groundwater. The high concentration of any cation in a deicer, whether sodium, calcium, magnesium, or potassium has the potential to do this, but potassium has stronger affinity for these sites than sodium. This indicates it would take a smaller concentration of potassium to have the same effect. Laboratory or field experiments should investigate the role of potassium in the soil. The only study found that reports the impact of excess potassium from deicers in the soil investigated the fate of a potassium formate deicer in the soil, where the potassium was reported to exchange with soil cations (Hellstén *et al.*, 2005).

While this work determined the toxicity of KAc to grass seed germination and daphnia, there are many more organisms that should be evaluated for sensitivity to KAc.

The impacts on vegetation other than grass could be studied, and toxicity assays could expand beyond germination of grasses and lab studies and into growth in field settings. While daphnia are a common test organisms, fish, macroinvertebrates, and algae have been studied for toxicity to NaCl.

The impact of KAc (and any deicer) on bacteria is an area that is understudied given that salinity is one of the strongest environmental factors determining bacterial community structure (Wu *et al.*, 2006). This work found that KAc may have a slight positive impact on bacterial counts, but little work has examined the potential for road salt applications to result in bacterial communities being replaced by halotolerant species. Cochero (2016) reported that press and pulse inputs of NaCl reduced biofilm density, and Pecher (2019) studied the effects of road salt on soil bacteria and found the community shifted to be composed of halophiles. It is likely that a shift in aquatic bacterial community structure occurs because of road salt application, and the resulting question is if there is also a change in the function of the bacteria. Ecosystem studies of NaCl found cascading effects throughout trophic levels, and if bacterial function is changed by road salt application it could change the primary production and nutrient cycling of the ecosystem and affect aquatic life on higher trophic levels.

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Appendix

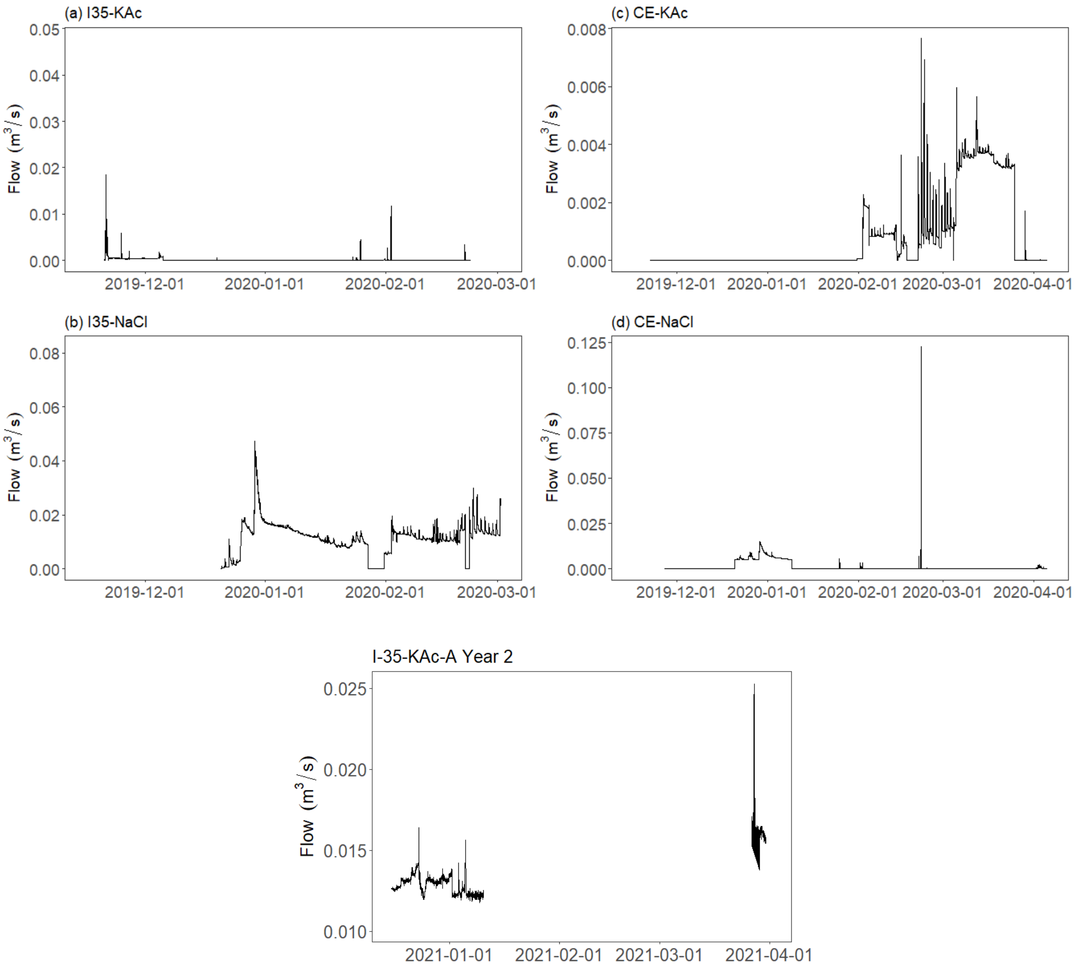

Figure A1. (a) Flow at the I-35-KA-Ac site Year 1. Only peak melt events are captured as the weir was not backing up enough flow for the AV probe to be submerged most of the season. The flow dataset ends early due to equipment failure (b) Flow at I-35-NaCl site Year 1. (c) Flow at CE-KAc site Year 1. (d) Flow at CE-NaCl site Year 1. While flow was often nonexistent or a trickle at this site, the data is low quality as there was an ill-fitting weir installed much of the season. (e) Flow at I-35-KAc-A site Year 2.

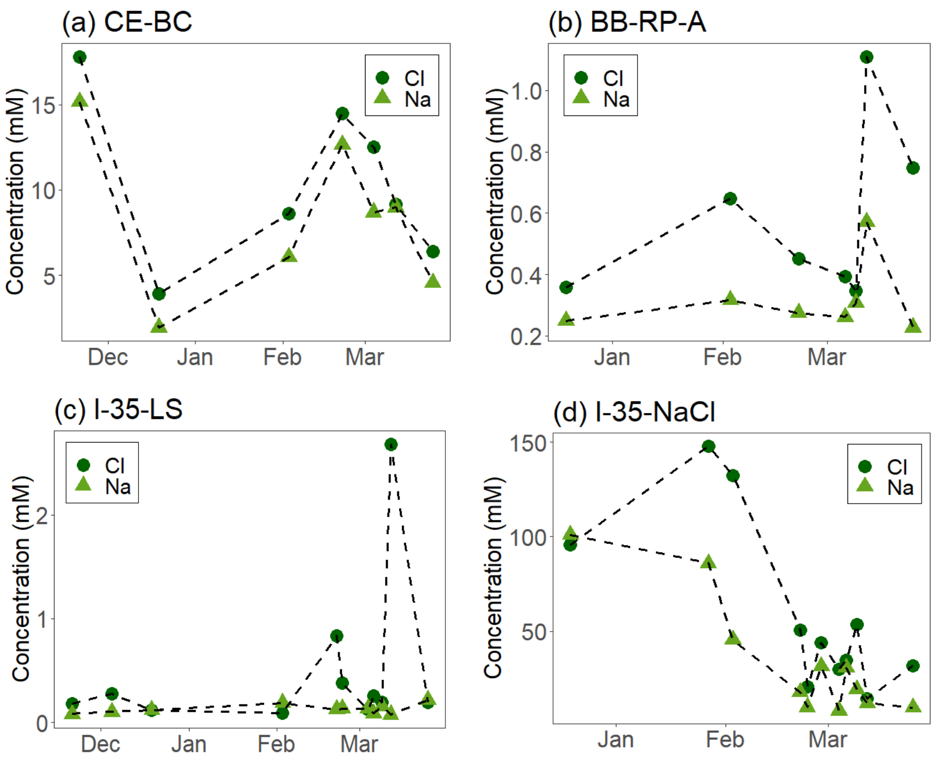

Figure A2. Molar concentration of chloride and sodium in 2029-2020 sites (a) CE-BC, (b) BB-RP-A, (c) I-35-LS, and (d) I-35-NaCl.

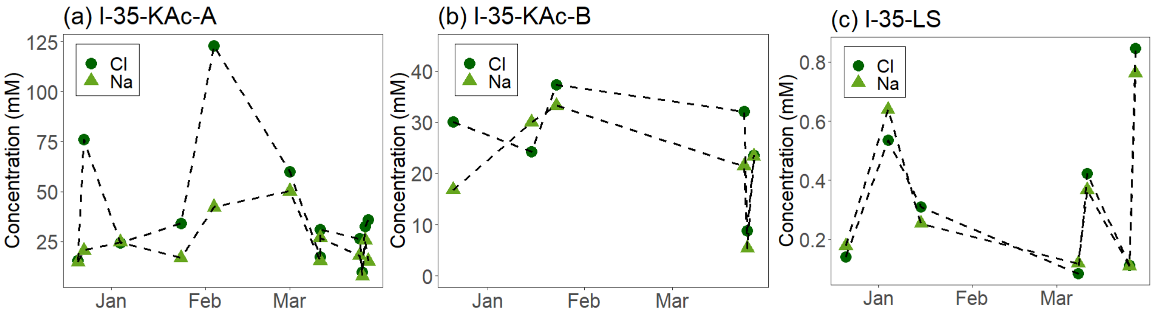

Figure A3. Molar concentration of chloride and sodium in 2020-2021 sites (a) I-35-KAc-A, (b) I-35-KAc-B, and (c) I-35-LS.

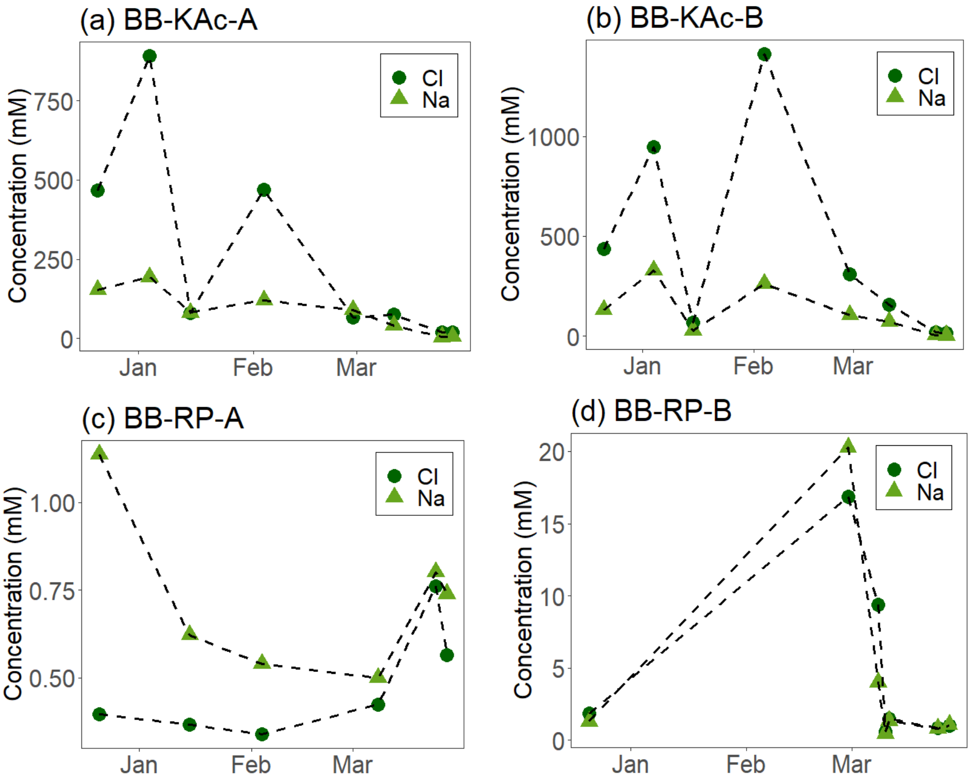

Figure A4. Molar concentration of chloride and sodium in 2020-2021 at (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B.

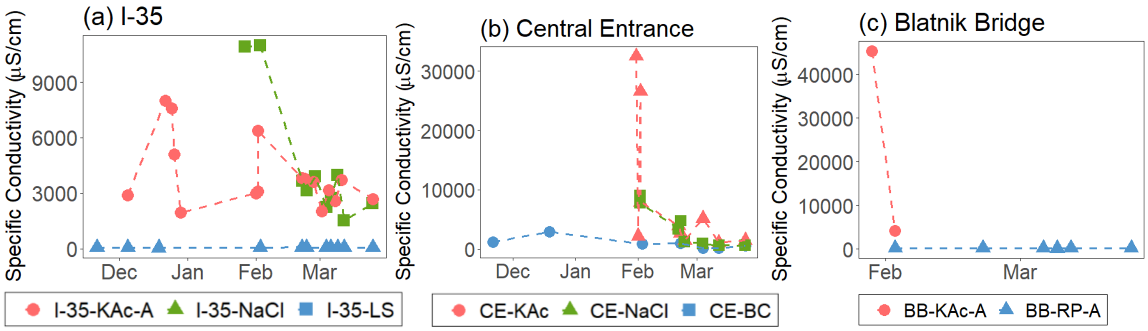

Figure A5. Conductivity at (a) I-35, (b) Central Entrance, and (c) Blatnik Bridge in 2019-2020.

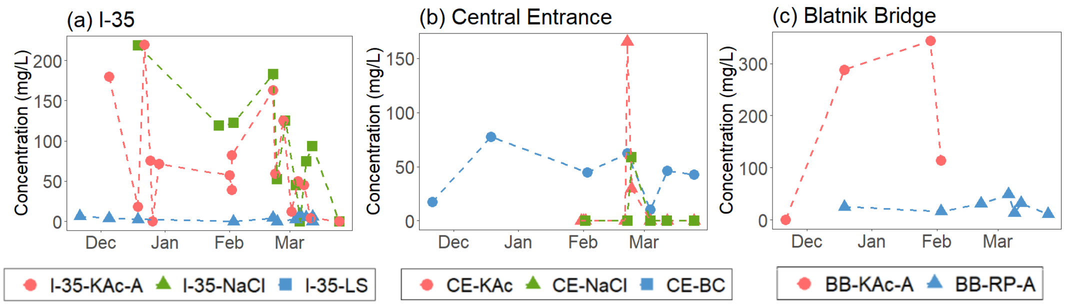

Figure A6. Nitrate in (1) I-35, (b) Central Entrance, and (c) Blatnik Bridge in 2019-2020

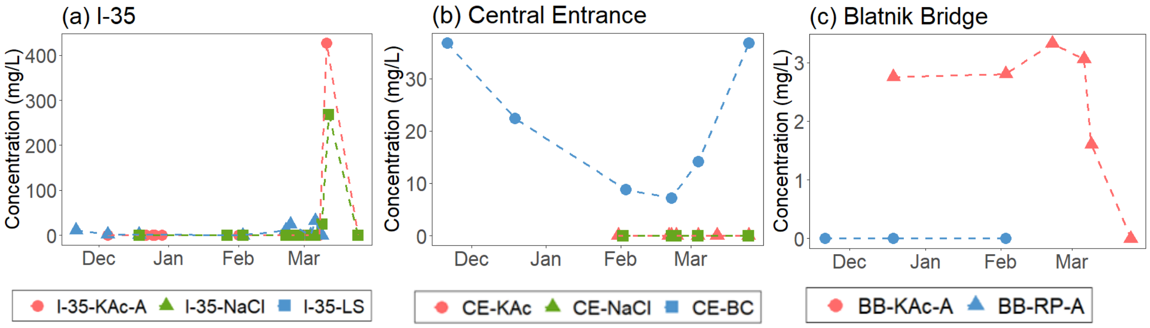

Figure A7. Sulfate in (1) I-35, (b) Central Entrance, and (c) Blatnik Bridge in 2019-2020

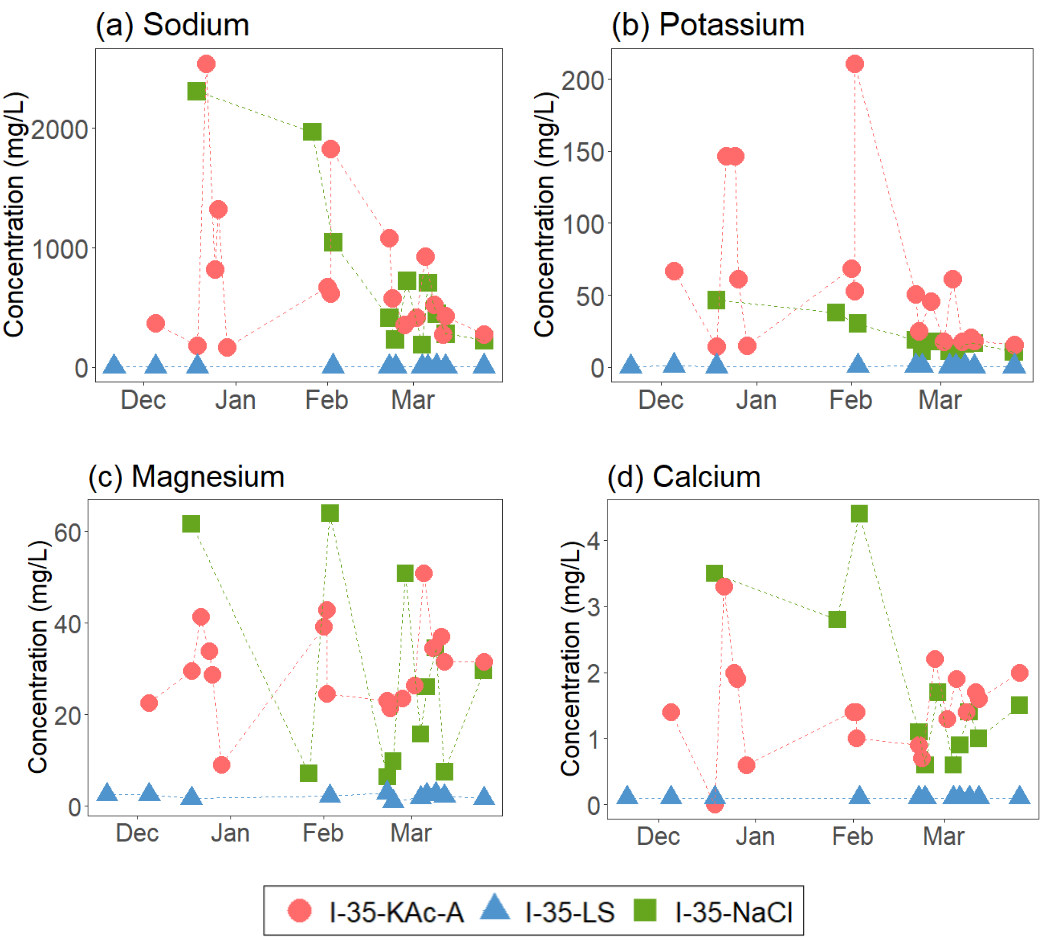

Figure A8. (a) Sodium, (b) potassium, (c) magnesium, and (d) calcium in I-35 sites in 2019-2020

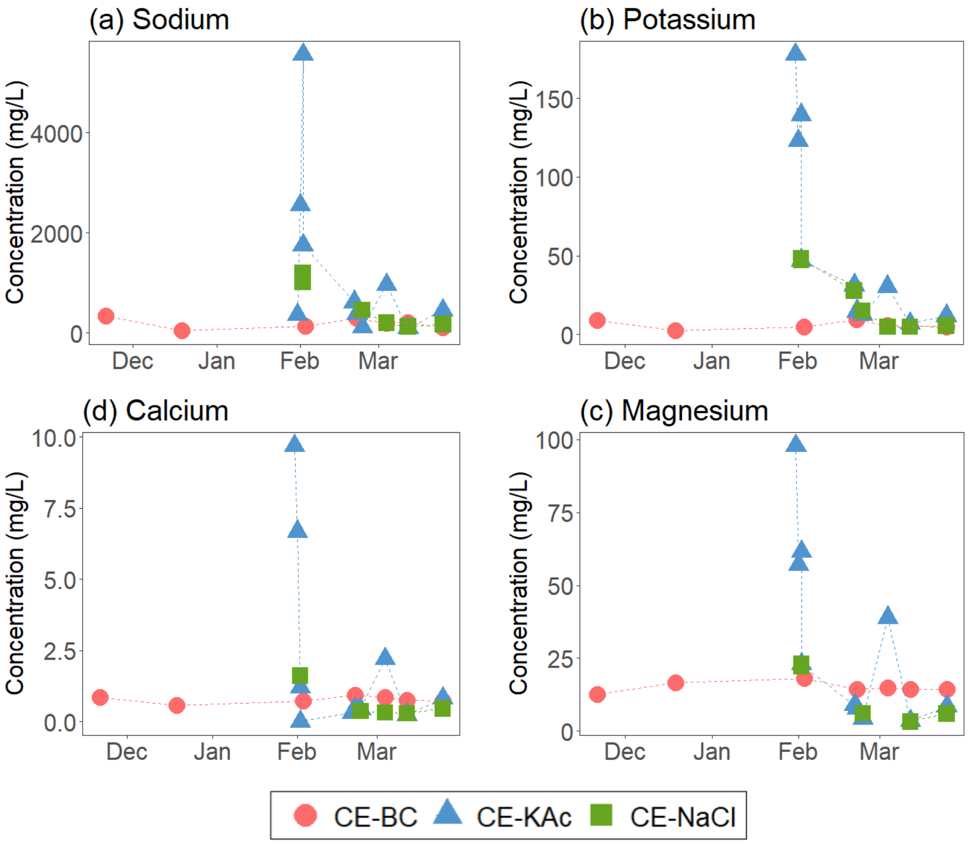

Figure A9. (a) Sodium, (b) potassium, (c) magnesium, and (d) calcium in Central Entrance sites in 2019-2020

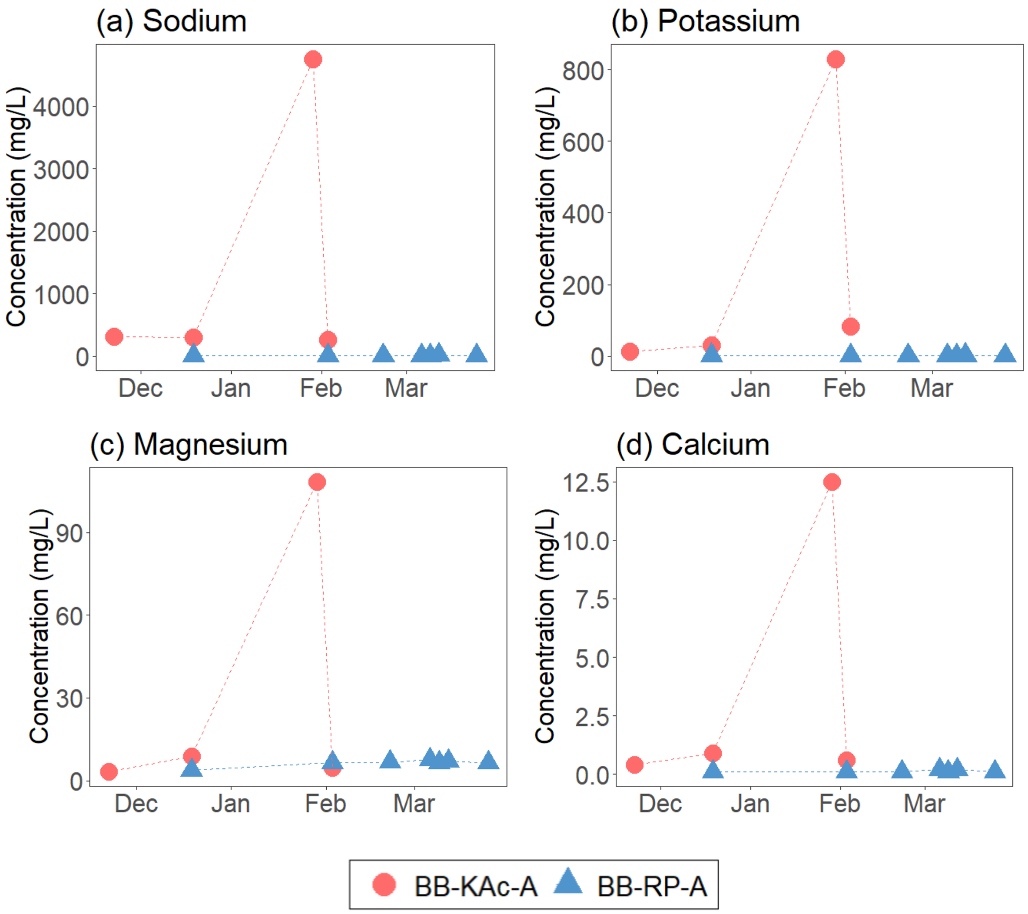

Figure A10. (a) Sodium, (b) potassium, (c) magnesium, and (d) calcium in Blatnik Bridge sites in 2019-2020

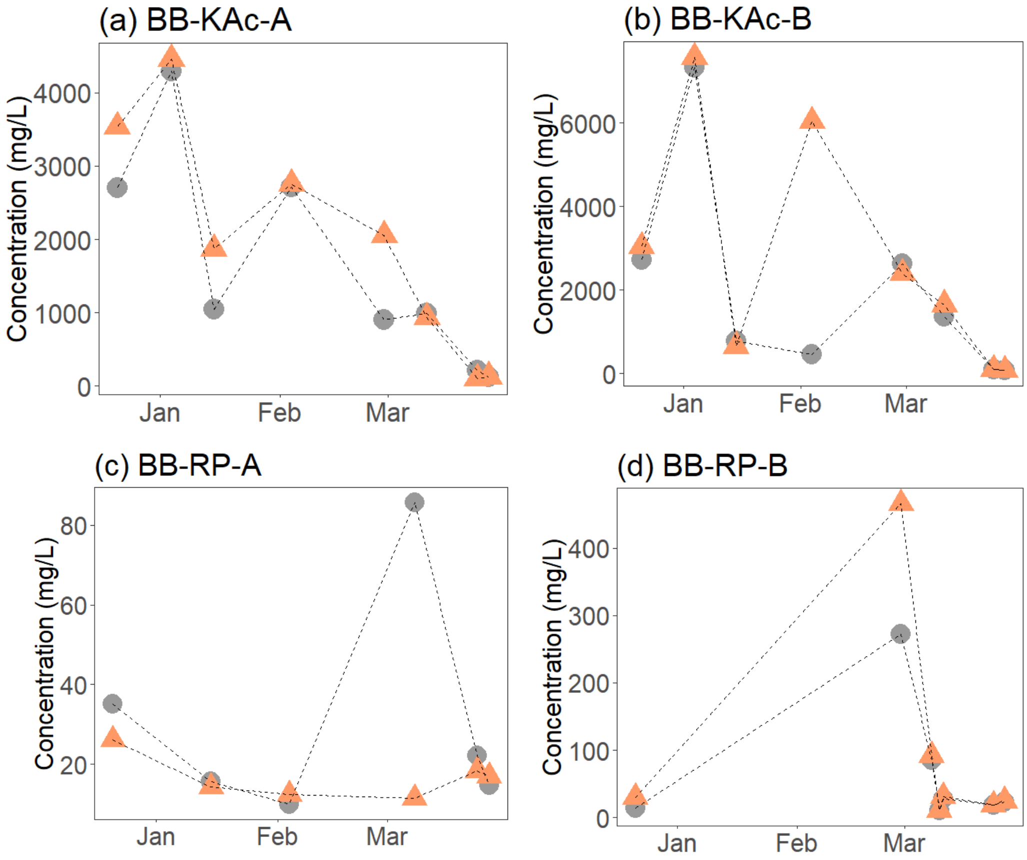

Figure A11. Filtered (F) and non-filtered (NF) sodium in (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B in Blatnik Bridge sites in 2020-2021

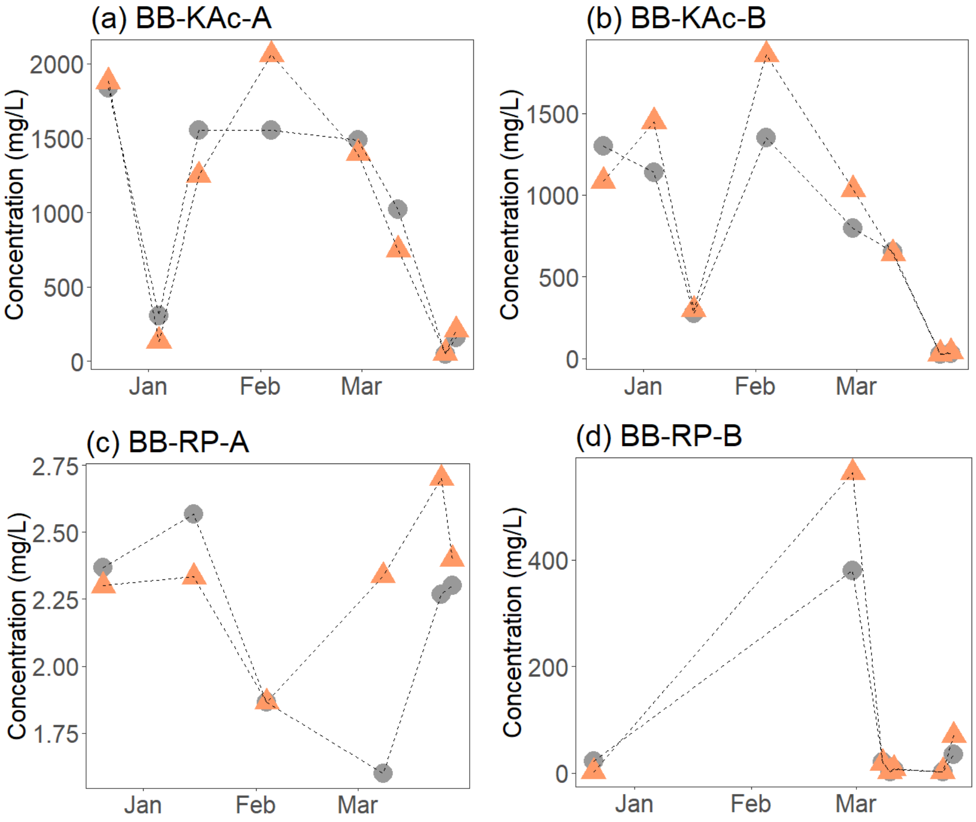

Figure A12. Filtered (F) and non-filtered (NF) potassium in (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B in 2020-2021

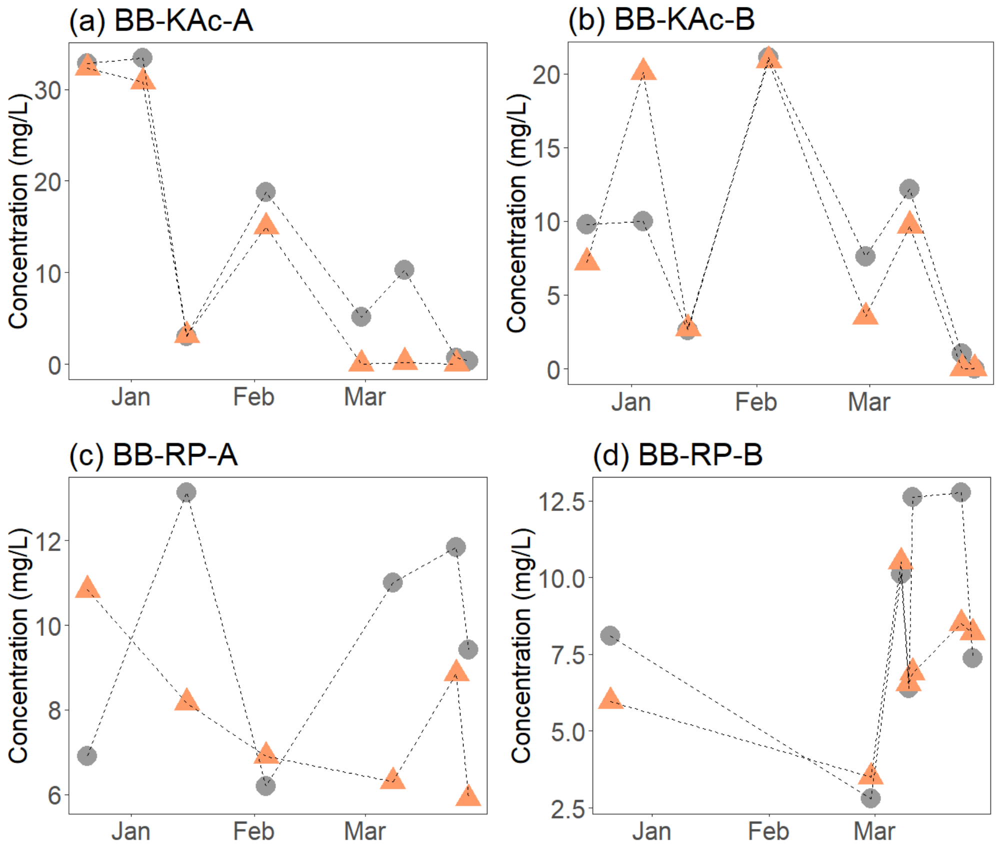

Figure A13. Filtered (F) and non-filtered (NF) magnesium in (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B in 2020-2021

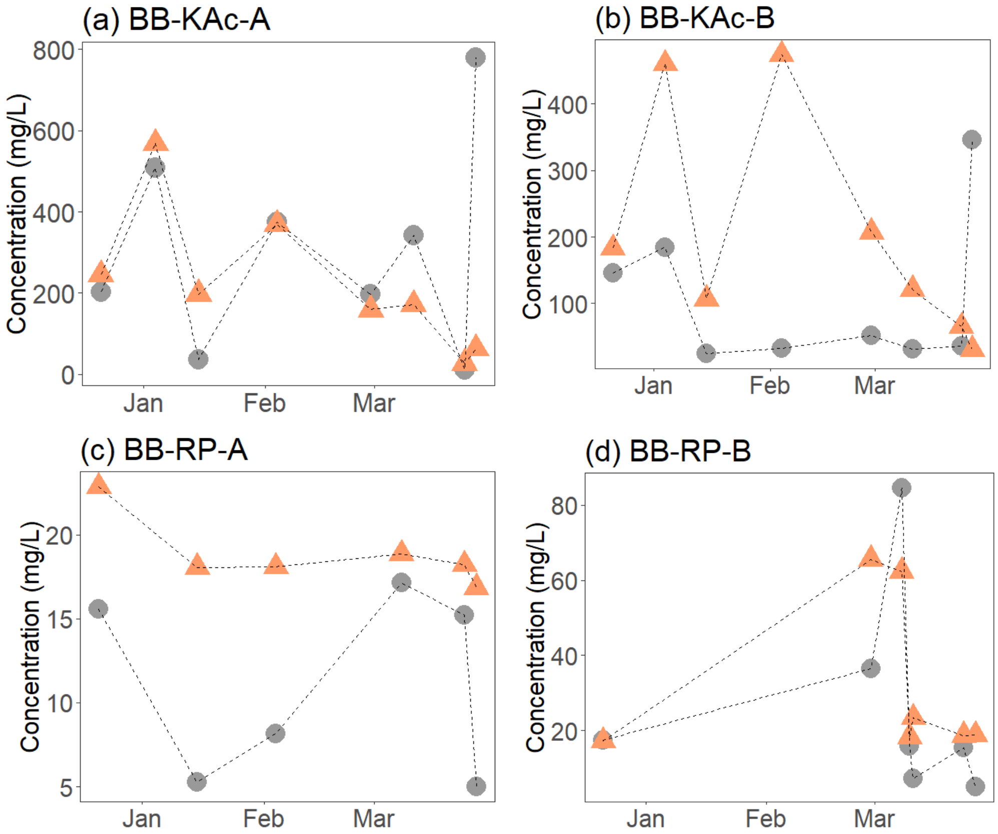

Figure A14. Filtered (F) and non-filtered (NF) calcium in (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B in 2020-2021

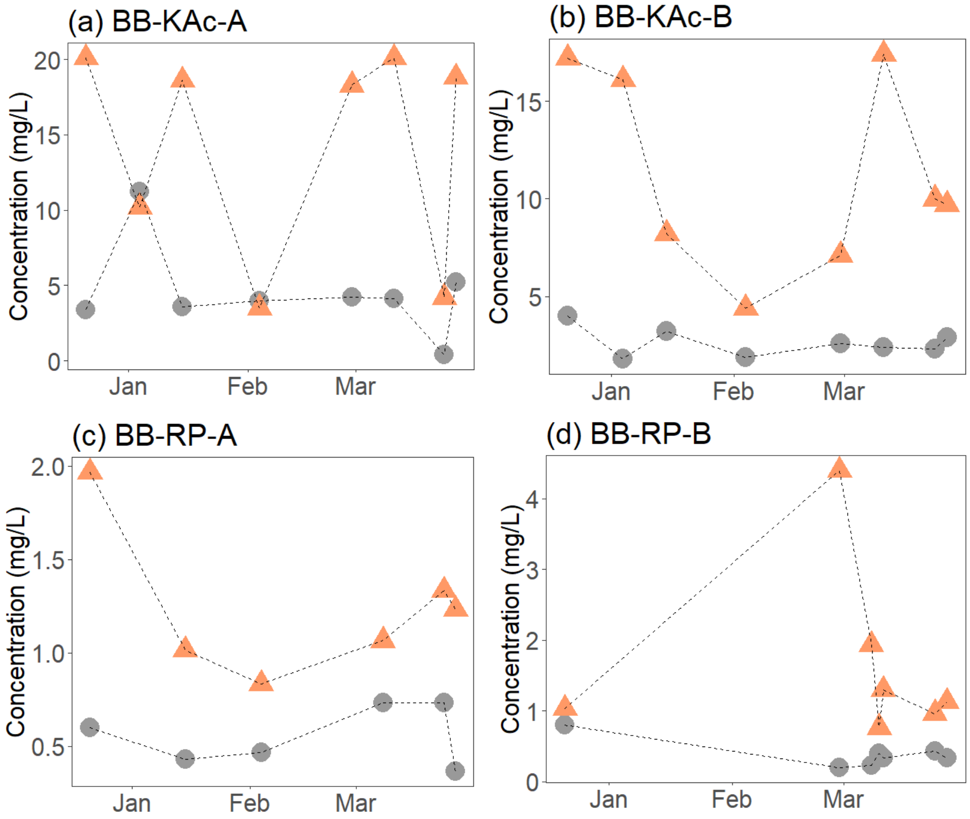

Figure A15. Filtered (F) and non-filtered (NF) iron in (a) BB-KAc-A, (b) BB-KAc-B, (c) BB-RP-A, and (d) BB-RP-B in 2020-2021

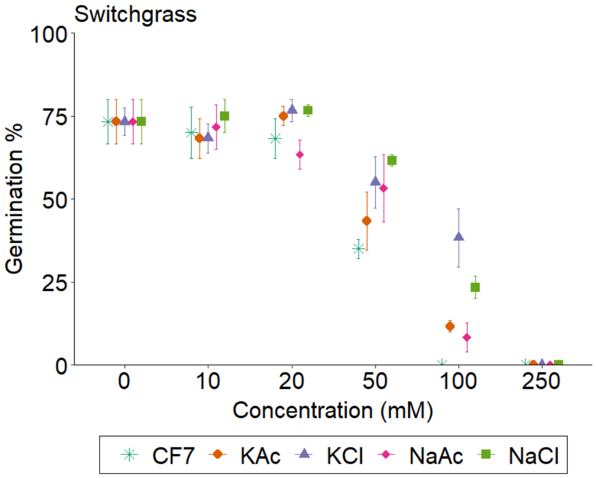

Figure A16. Germination of switchgrass in 10-250 mM salt solutions. The control is 0 mM (Lake Superior water).

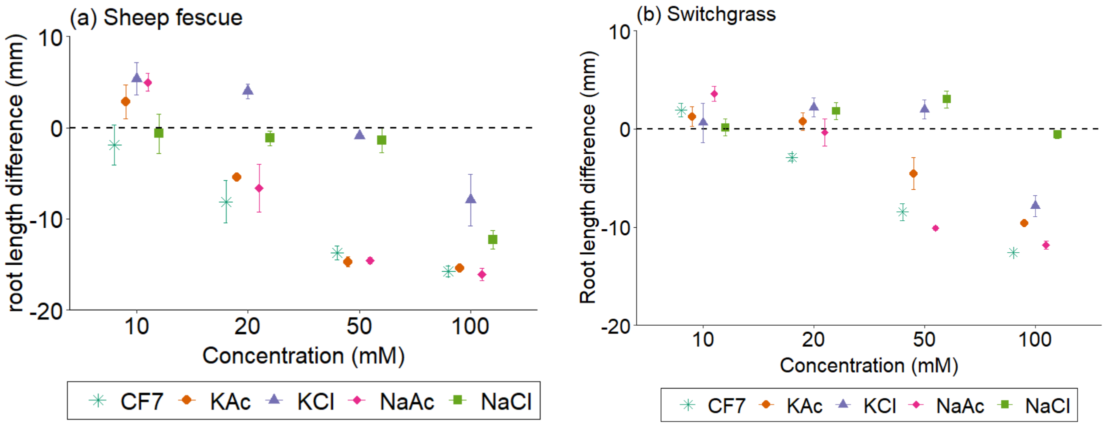

Figure A17. Root length of (a) Sheep Fescue and (b) Switchgrass germinated in 10-250 mM salt solutions. The control is 0 mM (Lake Superior water).

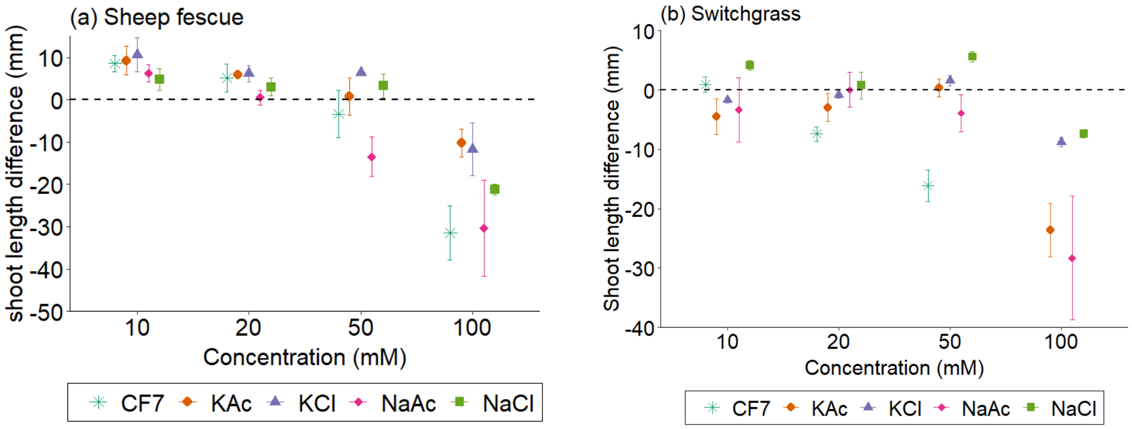

Figure A18. Shoot length of (a) Sheep Fescue and (b) Switchgrass germinated in 10-250 mM salt solutions. The control is 0 mM (Lake Superior water).

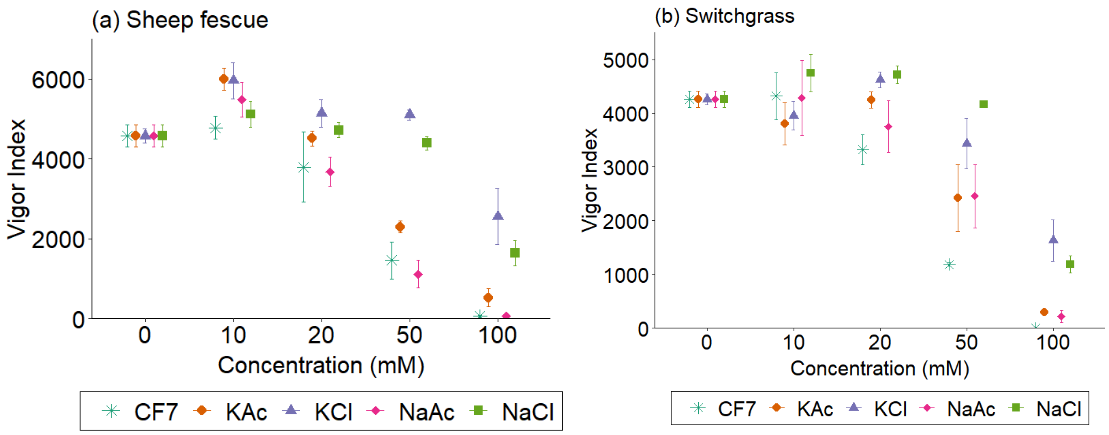

Figure A19. Vigor Index of (a) Sheep Fescue and (b) Switchgrass germinated in 10-250 mM salt solutions. The control is 0 mM (Lake Superior water).

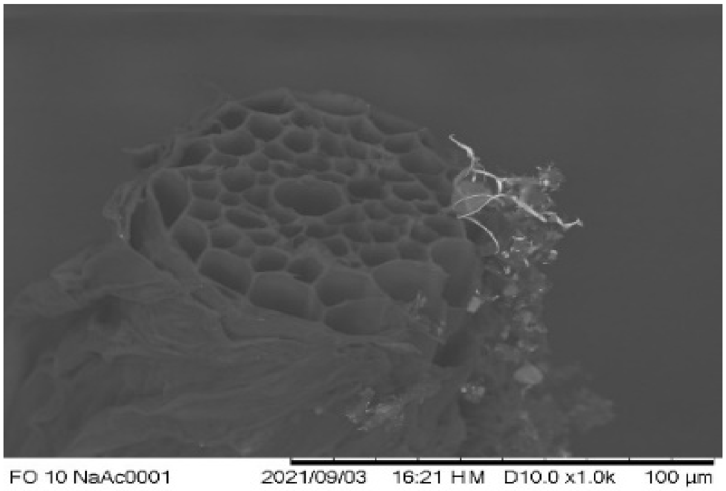

Figure A20. Cross section of sheep fescue root on the SEM germinated in 10 mM NaAc solution

Table A1. Significance of differences in germination within salt groups for sheep fescue according to Tukey post-hoc test

	NaCl	KCl	NaAc	KAc	CF7
control vs 10 mM	ns	ns	ns	ns	ns
control vs 20 mM	ns	ns	ns	ns	ns
control vs 50 mM	ns	ns	***	*	***
control vs 100 mM	ns	ns	***	***	***
control vs 250 mM	***	***	***	***	***
10 mM vs 20 mM	ns	ns	ns	ns	ns
10 mM vs 50 mM	ns	ns	***	***	***
10 mM vs 100 mM	ns	ns	***	***	***
10 mM vs 250 mM	***	***	***	***	***
20 mM vs 50 mM	ns	ns	***	*	***
20 mM vs 100 mM	ns	ns	***	ns	***
20 mM vs 250 mM	***	***	***	***	***
50 mM vs 100 mM	ns	ns	***	***	**
50 mM vs 250 mM	***	***	***	***	***
100 mM vs 250 mM	***	***	ns	***	ns

ns: not significant, p: 0.05, * $p < 0.05$, ** $p < 0.005$, * $p < 0.0005$

Table A2. Significance of differences in germination within salt groups for switchgrass according to Tukey post-hoc test

	NaCl	KCl	NaAc	KAc	CF7
control vs 10 mM	ns	ns	ns	ns	ns
control vs 20 mM	ns	ns	ns	ns	ns
control vs 50 mM	ns	ns	ns	*	**
control vs 100 mM	***	**	***	***	***
control vs 250 mM	***	***	***	***	***
10 mM vs 20 mM	ns	ns	ns	ns	ns
10 mM vs 50 mM	ns	ns	ns	ns	***
10 mM vs 100 mM	***	*	***	***	***
10 mM vs 250 mM	***	***	***	***	***
20 mM vs 50 mM	ns	ns	ns	*	**
20 mM vs 100 mM	***	**	***	**	ns
20 mM vs 250 mM	***	***	***	***	***
50 mM vs 100 mM	**	ns	***	*	**
50 mM vs 250 mM	***	***	***	***	**
100 mM vs 250 mM	ns	**	ns	ns	ns

ns: not significant, p: 0.05, * $p < 0.05$, ** $p < 0.005$, * $p < 0.0005$

Table A3. All sheep fescue and switchgrass germination data, including germination %, root and shoot growth, and vigor index.

		Sheep fescue					Switchgrass				
		Germination (%)	Root length (mm)	Shoot length (mm)	Root:shoot	Vigor Index	Germination (%)	Root length (mm)	Shoot length (mm)	Root:shoot	Vigor Index
Control		78.3 ± 1.7	15.7 ± 1.7	41.8 ± 3.0	0.4 ± 0.0	4573.9 ± 277.5	78.3 ± 1.4	16.8 ± 1.7	41.8 ± 3.0	0.4 ± 0.0	4574 ± 277
10 mM	NaCl	81.7 ± 3.3	16.1 ± 2.2	46.5 ± 2.5	0.4 ± 0.1	5122.0 ± 327.4	81.7 ± 2.7	16.1 ± 2.2	46.5 ± 2.5	0.4 ± 0.1	5122 ± 327
	KCl	80.0 ± 0.0	21.1 ± 1.8	52.4 ± 4.1	0.4 ± 0.0	5962.7 ± 452.3	80.0 ± 0.0	22.1 ± 1.8	52.4 ± 4.1	0.4 ± 0.0	5963 ± 452
	NaAc	78.3 ± 4.4	19.8 ± 1.0	48.1 ± 2.0	0.5 ± 0.0	5478.8 ± 432.5	78.3 ± 3.6	21.7 ± 1.0	48.1 ± 2.0	0.5 ± 0.0	5479 ± 433
	KAc	85.0 ± 2.9	23.3 ± 1.9	51.0 ± 3.4	0.4 ± 0.1	6004.8 ± 276.4	85.0 ± 2.4	19.6 ± 1.9	51.0 ± 3.4	0.4 ± 0.1	6005 ± 276
	CF7	73.3 ± 3.3	13.0 ± 2.2	50.3 ± 1.9	0.3 ± 0.1	4782.0 ± 288.0	73.3 ± 2.7	14.8 ± 2.2	50.3 ± 1.9	0.3 ± 0.1	4782 ± 288
20 mM	NaCl	78.3 ± 1.7	15.3 ± 0.8	44.8 ± 2.1	0.3 ± 0.0	4725.2 ± 190.3	87.3 ± 1.4	15.6 ± 0.8	44.8 ± 2.1	0.3 ± 0.0	4725 ± 190
	KCl	75.0 ± 5.0	20.2 ± 0.8	47.9 ± 1.9	0.4 ± 0.0	5140.5 ± 339.9	75.0 ± 4.1	20.7 ± 0.8	47.9 ± 1.9	0.4 ± 0.0	5140 ± 340
	NaAc	70.0 ± 5.0	13.9 ± 2.6	42.3 ± 1.8	0.2 ± 0.1	3680.7 ± 357.3	70.0 ± 4.1	10.1 ± 2.6	42.3 ± 1.8	0.2 ± 0.1	3681 ± 357
	KAc	76.7 ± 3.3	11.1 ± 0.1	47.6 ± 0.3	0.2 ± 0.0	4516.6 ± 188.1	76.7 ± 2.7	11.3 ± 0.1	47.6 ± 0.3	0.2 ± 0.0	4517 ± 188
	CF7	66.7 ± 10.1	13.1 ± 2.3	46.9 ± 3.4	0.2 ± 0.0	3795.3 ± 879.6	66.7 ± 8.3	8.6 ± 2.3	46.9 ± 3.4	0.2 ± 0.0	3795 ± 880
50 mM	NaCl	73.3 ± 6.0	16.3 ± 1.3	45.0 ± 2.9	0.3 ± 0.0	4398.0 ± 164.6	73.3 ± 4.9	15.4 ± 1.3	45.0 ± 2.9	0.3 ± 0.0	4398 ± 165
	KCl	80.0 ± 2.9	15.5 ± 0.2	48.1 ± 0.7	0.3 ± 0.0	5110.5 ± 128.4	80.0 ± 2.4	15.8 ± 0.2	48.1 ± 0.7	0.3 ± 0.0	5111 ± 128
	NaAc	35.0 ± 5.0	2.8 ± 0.4	28.3 ± 4.7	0.1 ± 0.0	1114.3 ± 345.4	35.0 ± 4.1	2.2 ± 0.4	28.3 ± 4.7	0.1 ± 0.0	1114 ± 345
	KAc	51.7 ± 1.7	3.1 ± 0.5	42.6 ± 4.4	0.1 ± 0.0	2293.3 ± 147.5	51.7 ± 1.4	2.0 ± 0.5	42.6 ± 4.4	0.1 ± 0.0	2293 ± 147
	CF7	35.0 ± 10.0	1.7 ± 0.8	38.4 ± 5.6	0.1 ± 0.0	1456.8 ± 459.9	35.0 ± 8.2	3.0 ± 0.8	38.4 ± 5.6	0.1 ± 0.0	1457 ± 460
100 mM	NaCl	65.0 ± 10.0	6.5 ± 1.0	20.6 ± 1.3	0.2 ± 0.1	1644.4 ± 311.8	65.0 ± 8.2	4.5 ± 1.0	20.6 ± 1.3	0.2 ± 0.1	1644 ± 312
	KCl	65.0 ± 7.6	5.0 ± 2.8	30.1 ± 6.2	0.3 ± 0.1	2551.0 ± 701.0	65.0 ± 6.2	8.8 ± 2.8	30.1 ± 6.2	0.3 ± 0.1	2551 ± 701
	NaAc	1.7 ± 1.7	NA	NA	NA	60.0 ± 60.0	1.7 ± 1.4	NA	NA	NA	60 ± 60
	KAc	15.0 ± 5.0	2.0 ± 0.3	31.5 ± 3.2	0.0 ± 0.0	523.7 ± 225.7	15.0 ± 4.1	1.3 ± 0.3	31.5 ± 3.2	0.0 ± 0.0	524 ± 226
	CF7	3.3 ± 1.7	0.0 ± 0.4	15.5 ± 5.3	NA	56.7 ± 33.2	3.3 ± 1.4	1.5 ± 0.4	15.5 ± 5.3	#DIV/0!	57 ± 33
250 mM	NaCl	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0
	KCl	1.7 ± 1.7	NA	NA	NA	0.0 ± 0.0	1.7 ± 1.4	NA	NA	NA	0.0 ± 0.0
	NaAc	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0
	KAc	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0
	CF7	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0	0.0 ± 0.0	NA	NA	NA	0.0 ± 0.0