Water Quality Monitoring in Seven Mile Creek

By

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ABSTRACT

Seven Mile Creek is a tributary of the Minnesota River south of St. Peter, MN. The creek flows across uplands composed of unconsolidated glacial till, and cuts steeply downward on its path to the Minnesota River, forming a network of deeply incised ravines. Its watershed is primarily tile-drained agricultural land dominated by corn and soybean crops. The combination of agricultural runoff and rapid water flows poses a threat to water quality in this creek. Seven Mile Creek is considered impaired by state standards in total suspended solids, nitrate, *E. coli*, and chlorpyrifos. Nicollet County received a $1.7M grant to establish mitigation strategies in order to improve water quality; this study aims to establish methods to assess the effectiveness of these interventions.

The first objective of this study was to assist in launching a monitoring program for the creek using a team of Gustavus Adolphus College students and faculty. This team will monitor total suspended solids, nitrate, *E. coli*, and water flow in the coming years. The second objective was to use historical water quality data and flow data from the Minnesota Pollution Control Agency to analyze the relationship between precipitation and upland flow in the watershed. This information helps increase the accuracy of the monitoring by determining how soon after heavy precipitation events monitoring must be done. The result of this analysis was a four stage-monitoring plan based on agricultural practices and precipitation patterns.

ACKNOWLEDGEMENTS

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INTRODUCTION

Rivers and streams in the U.S. are becoming heavily polluted as more and more agricultural runoff makes its way into them, carrying nitrates, phosphates, insecticides, and bacteria, all of which have the potential to alter the ecosystems of the rivers and lakes that they end up in. In addition to pollutants, many streams and rivers are impaired due to high sediment loads or total suspended solids (TSS). This is an especially big problem in the Midwest, where there is an abundance of agricultural land.

In Nicollet County, Minnesota, the Seven Mile Creek watershed takes in a large amount of agricultural runoff from the surrounding tile-drained fields and has several pollutants at concentrations above the state standards (as seen in Table 1). The Seven Mile Creek (SMC) watershed is, in many ways, representative of the creeks downstream of regions of intensive agriculture. Impairment of these upland streams creates a ripple effect; Seven Mile Creek flows into the Minnesota River, which flows into the Mississippi River, which leads to the Gulf of Mexico, where a large dead zone forms every year because of these pollutants (Dodds 2006), particularly elevated nutrient levels (e.g. nitrate and phosphate). Combined with the impacts of hundreds of small upland watersheds, these relatively local decisions produce a very large impact on the ocean. If this impact on rivers, lakes, and oceans is to be minimized, upland agricultural practices must be altered. In order to assess the effectiveness of these changes, water quality monitoring is required.

	State Standard for	Historical (1996-2008) EWMC for	% Reduction Required to Achieve	% Reduction Expected Due to this
Total Suspended	**16 ppm***	**238 ppm**	**94**	**30-40%**
Nitrates	**10 ppm**	**16 ppm**	**38**	**15-25%**
E. coli	**126 col/100 ml**	**178 col/100 ml**	**29**	**20-30%**
Chlorpyrifos	**0.04 ppb**	**0.24 ppb#**	**84**	**N/A**

Table 1. Current measurements of pollutants in Seven Mile Creek and projections of remediation effectiveness in reducing them (Galles 2014)

Nicollet County has received a grant of $1.7 million for remediation of the area around the watershed in the hopes that it will reduce water quality impairment. The proposed plan includes measures such as restoring wetlands and creating buffer zones near agricultural fields. The plan targets four pollutants that have concentrations above state standards: total suspended solids (TSS), chlorpyrifos (an insecticide), nitrates, and

E. coli. Currently each of those pollutants is above the set state standards for SMC (Table 1).

Total suspended solids (TSS) include silt and fine particles, plankton, algae, and other fine debris and particulate matter (EPA 2012). TSS affects the water balance in the cells of aquatic life. In waters with high levels of TSS, organisms lose water from their cells and shrink down, causing them to reside in a depth that they may not be able to survive in. TSS also restricts the amount of light that passes through the water column to the plants below the surface, decreasing photosynthesis rates, which decreases the oxygen in the water. If oxygen levels get too low, the water can become hypoxic, meaning oxygen is too low to sustain life (EPA 2012). In Minnesota alone, more than 5,800 miles of streams are considered impaired due to high levels of suspended sediment (Ellison 2014).

Nitrates are compounds that contain the nitrate anion (NO_3^-). They enter water with runoff because they are in many fertilizers utilized by farmers. It has been found that the installation of tile drains can lead to the increase of nitrates in surrounding streams (Christensen et. Al 2009). In excess, they can alter the species composition of aquatic plants and algae. For example, certain types of algae, such as green algae, thrive off of nitrogen. When these excessive algal blooms die, oxygen-consuming organisms that feed off of dead algae thrive, causing hypoxia. This leads to a series of ecosystem alterations (EPA 2012).

Chlorpyrifos is an insecticide that is harmful to small animals and could potentially be harmful to people in larger quantities. It works to kill insects by ingestion and contact and inhibits the functions of neurons (Zalat, et. Al. 2014). Due to expense and time constraints, this study was not able to monitor chrlopyrifos.

E. coli bacteria are a type of coliform bacteria that live in the digestive tract of animals such as birds and cattle. When water passes over the feces of these animals, it can introduce *E. coli* and other bacteria into the runoff.

Nicollet County's remediation project does not presently have a monitoring plan in place to assess the effects of remediation efforts, and three of the four monitoring sites in the SMC watershed are no longer active (Galles 2014). This study aimed to establish a monitoring plan for total suspended solids, nitrates, and *E. coli* to be carried out by

students at Gustavus Adolphus College. If possible, chlorpyrifos should be included in future studies. This study established standard methods for each pollutant and analyzed flow and precipitation data to better understand how to effectively monitor the water quality.

Previous Work

Victoria Christensen has done several studies and articles on creeks in the Minnesota River watershed similar to Seven Mile Creek. In her article titled Assessment of Conservation Easements, Total Phosphorus, and Total Suspended Solids in West Fork Beaver Creek, Minnesota, 1999-2012, she studied the effectiveness of conservation easements at reducing transport of phosphorus and solids transport to streams (Christensen 2014). In her article titled Relations between Retired Agricultural Land, Water Quality, and Aquatic-Community Health, Minnesota River Basin, she studied eighty-two sites within the Minnesota River Basin for nutrient concentrations and measures of aquatic-community health in relation to agricultural land retirement. In Water-Quality and Biological Characteristics and Responses to Agricultural Land Retirement in Three Streams of the Minnesota River Basin, Water Years 2006-08, she studied and compiled information on biological responses to remediation efforts (Christensen 2009).

GEOGRAPHIC AND GEOLOGIC SETTING

The Seven Mile Creek watershed is located in south-central Minnesota, just south of St. Peter, MN. Of the 23,551 acres that make up the Seven Mile Creek watershed, 626 acres are in the Seven Mile Creek County Park at the mouth of the watershed (Kuehner 2001). The vast majority of the remaining area is privately owned and used for agriculture. Specifically, the agriculture within this watershed is primarily row cropping (corn/soybean rotation). The remainder of the watershed is comprised of privately owned wooded areas. The upper portion of the watershed, where most of the agricultural activity takes place, is nearly level with 0-2% slopes, but due to the 210-foot drop to the Minnesota River, the slope near the channel rises to 40-60% (Kuehner 2001).

Seven Mile Creek is 6.1 miles long and flows into the Minnesota River. It forms primarily from three county ditches, specifically CD 46, CD 13, and CD 24 (Figure 1). Two major public ditch tile systems, CD 29 and CD 58, are also contributors to the creek. These two ditches are intermittent and are only typically substantial contributors after July (Kuehner 2001). The creek itself dries up occasionally, but usually maintains flow at around 1 to 3 cubic feet per second (cfs) throughout the year because of groundwater it receives from the Jordan Sandstone Aquifer (Kuehner 2001).

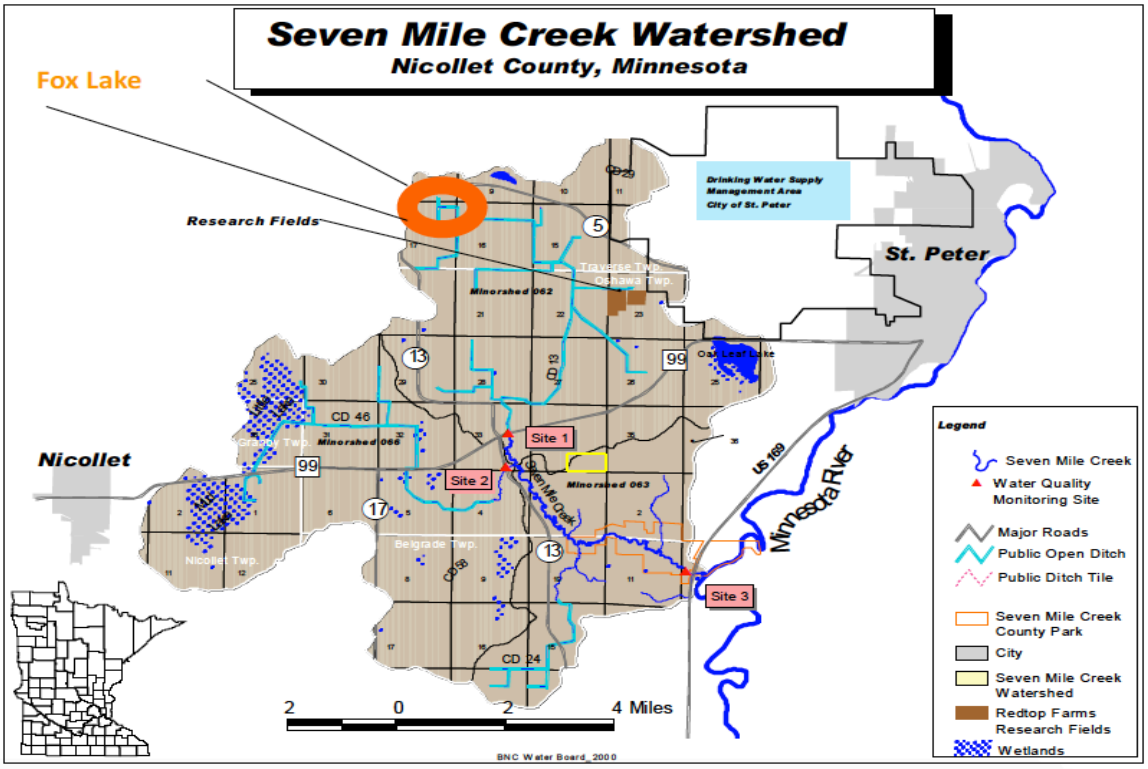

Figure 1. Seven Mile Creek watershed showing the creek, current and former water quality monitoring sites, major roads, ditches, and the boundary of SMC park (Kuehner 2001)

On level land the soils are mostly poorly drained clay loams and silty loams, but the land adjacent to the creek primarily comprises well-drained loams to poorly drained clay loams (Kuehner 2001).

In this region, the bedrock geology is sedimentary. It includes the Jordan Sandstone, and Oneota Dolomite (Runkel 1994). The Jordan Sandstone, which is Cambrian in age, underlies the Ordovician Oneota Dolomite. In Seven Mile Creek Park, the Jordan sandstone is exposed, and although the Oneota Dolomite is present across

south-central Minnesota, including exposures just north and south of the park, it is not exposed within the park itself.

Above the Jordan Sandstone in the park lies glacial till (Powell, 1935). The till was brought to the region during the last glaciation, the Late Wisconsin. The till in this particular region comes from the Des Moines lobe (Meyer et. al. 2012). The till in the Seven Mile Creek watershed is primarily a sandy textured till composed of sand, loamy sand, and gravel, as well as a loamy to coarse-grained loamy till, which is reddish and likely from the Superior provenance (Meyer, et. al. 2012).

METHODS

Site Selection

Sites were chosen based on the location of the Minnesota Pollution Control Agency's former and current sites. Site 1 is located in the public drainage ditch off of highway 99, site 2 is located in the public drainage ditch off of highway 13, and site 3 is in the main stem of SMC. The sites can be seen in Figure 1. Sites 1 and 2 are the same. Site 3 for this study is in nearly the same location as Site 3 on Figure 1, but is slightly farther upstream within the park.

Collection

All water samples from Seven Mile Creek were collected in Nalgene bottles in the middle of the stream or ditch at approximately half the total water depth in order to get the most representative sample possible. Most of the time, this was done by wading into the stream, but occasionally a sampling rod was used. Sampling was done approximately once a week from the summer of 2015 through the fall and again from late March to through May 2016. In addition to weekly samples, extra samples were taken following periods of precipitation. Ideally sampling was done immediately following precipitation events as well as for the next day or two afterwards. All samples were refrigerated between collection and analysis.

Fecal Coliform

The procedure used for measuring *E. coli* was the Fecal Coliform Procedure from the 18th Edition of Standard Methods, 9222D. This study adhered to the sterilization, preparation, sample size selection, filtration, incubation, colony counting, and calculation

methods as described in the standard method (Eaton and Franson 1995). Samples were prepared and placed in the incubator within 24 hours of collection and removed from the incubator and evaluated for colonies within 24 +/- 2 hours of incubation in accordance with the standard procedure. The details of sample preparation are available in Appendix 1A.

Total Suspended Solids

The method used for total suspended solids (TSS) measurements was to filter the samples, dry them, and calculate the results in mg/L. The detailed procedure used was the standard method used at Gustavus, most recently updated in 2013. The time between collection and filtration is ideally 24 hours or less, but results are still viable if the sample is tested within one week of collection as long as it has remained refrigerated for the entirety of this duration. See Appendix 1B for the full detailed procedure.

Nitrate

Due to setbacks with the equipment and software, testing for nitrate was not completed within the duration of this study; however, the protocol was developed and can be used in future monitoring efforts. An ion chromatograph (IC) is used to measure nitrate concentrations in the stream. The samples should be refrigerated and tested within 48 hours of collection. No additional preparation is required before processing the samples in the IC. After shaking the sample gently to mix it, it is poured into the vial for the IC. No dilution or filtration is necessary. The rest of the procedure follows the instructions written in the lab. See Appendix 1C for more details.

Precipitation and Flow Response

Utilizing publicly available data from the Minnesota Department of Natural Resources, the response of flow to precipitation was analyzed on a month-by-month basis. Daily flow and precipitation were plotted together in order to observe the response of flow in the creek to the precipitation received in the watershed.

RESULTS

Total Suspended Solids

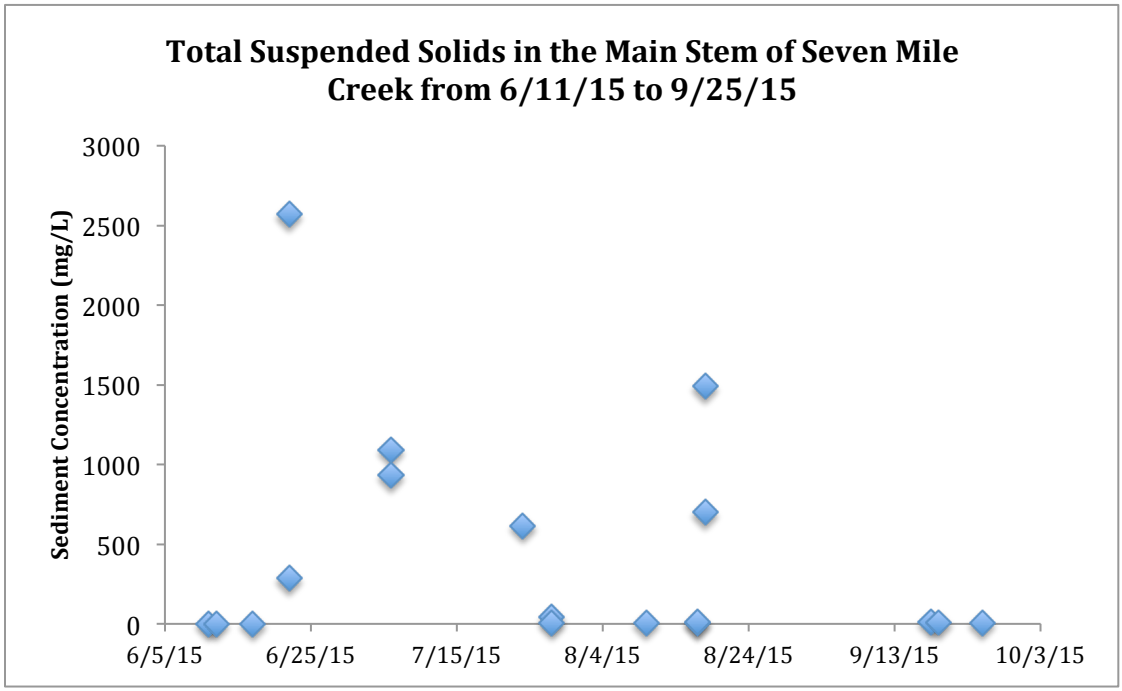

Figure 2. Sediment concentration in mg/L measured from the main stem of Seven Mile Creek from 6/11/2015 to 9/25/15

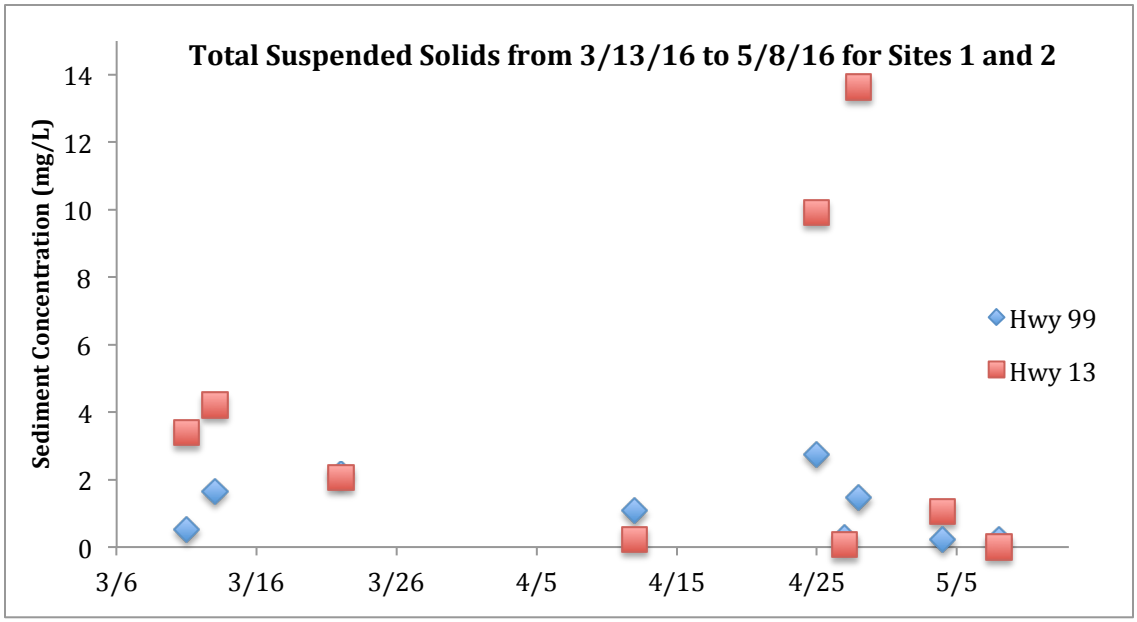

Figure 3. Sediment conentrations in mg/L from sites 1 and 2 (hwy 99 and hwy 13) from 3/13/16 to 5/8/16

The concentration of total suspended solids (TSS) in the main stem of the creek (Site 3) fluctuated dramatically from June to September. At its lowest TSS was below the detection threshold. On June 22nd the concentration was 2571 mg/L, which was the highest concentration during this period. Another large jump in TSS occurred on August 18th with a concentration of 1489 mg/L.

In the upper two sites at hwy 99 and hwy 13, the TSS was measured during the spring of 2016. At their lowest, the concentrations at both sites were below the detection threshold. The Peak concentration for hwy 99 (site 1) was 2.75 mg/L on 4/25/16. For hwy 13 (site 2) the peak concentration was 13.63 mg/L on 4/28/16.

E. coli

A standard method for measuring *E. coli* was adopted and tested in the Gustavus laboratories. Preliminary results of method testing were inconsistent (Table 2). Because there are inconsistencies in results, it is unclear whether these results are accurate. Filtering 10 mL and 50 mL of the same sample from Hwy 99 on April 12th yielded different results. The 10 mL produced 20 col/100mL, whereas the 50 mL produced a result of 6 col/100mL. The overall results are low, with nearly all of them being between 0 and 3 col/100mL.

Date Sampled	**Site**	**Water filtered(mL)**	**Colonies**	**Col/100mL**
3/22/16	Hwy 13	1	0	0
3/22/16	Hwy 13	10	1	10
3/22/16	Hwy 13	50	0	0
3/22/16	Hwy 99	1	0	0
3/22/16	Hwy 99	10	1	10
3/22/16	Hwy 99	50	0	0
4/12/16	Hwy 13	10	2	20
4/12/16	Hwy 13	50	0	0
4/12/16	Hwy 99	10	2	20
4/12/16	Hwy 99	50	3	6

Table 2. *E. coli* results including the date the sample was taken, the site at which it was taken, the amount of water that was filtered, the number of colonies assessed, and the final concentration of *E. coli* in col/100mL

Precipitation and Flow Response

The relationship between flow and precipitation is not consistent. In some months, such as May and June, there appears to be an increase in flow in the creek following precipitation, with a lag of a day or so (Figures 4a. and 4b.). However, in July there appears to be almost no correlation whatsoever (Figure 4c.). Precipitation increases and decreases several times within the month while the flow in the creek steadily decreases.

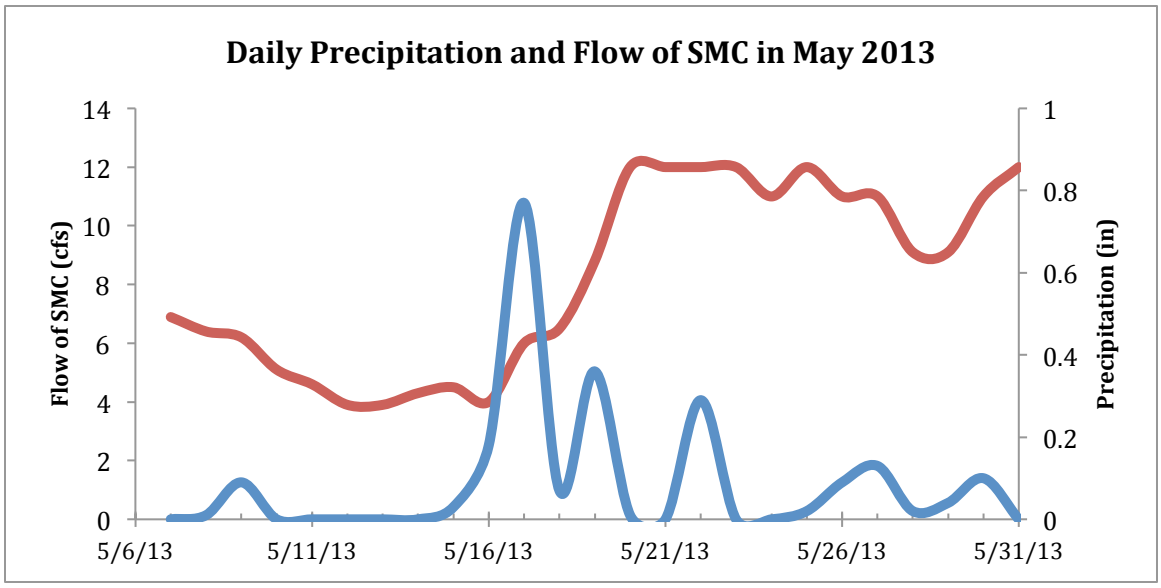

Figure 4a.

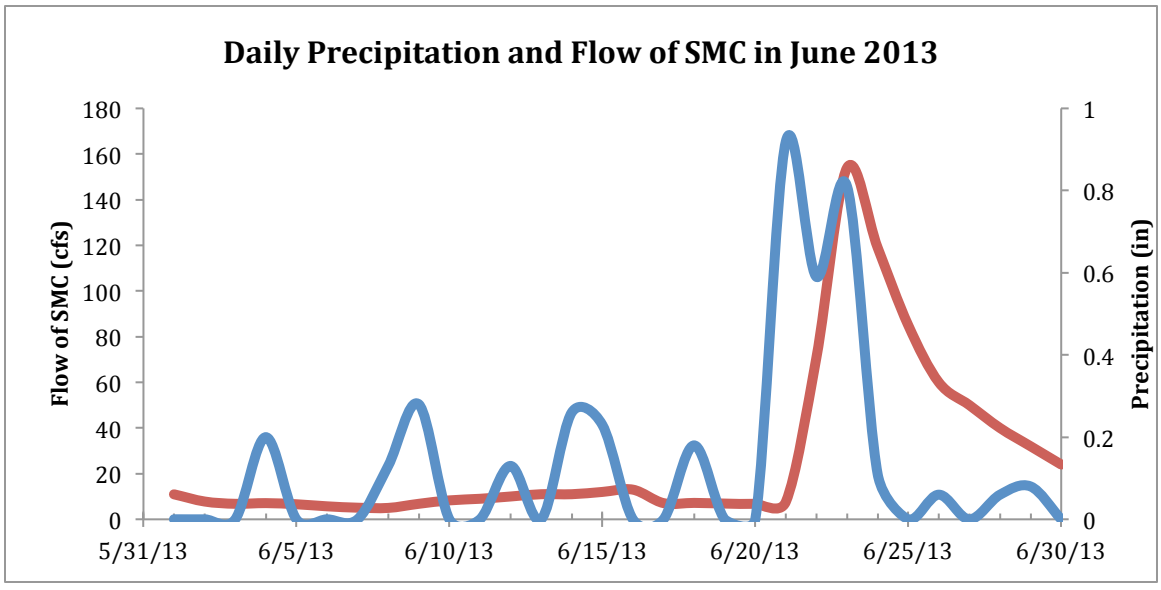

Figure 4b.

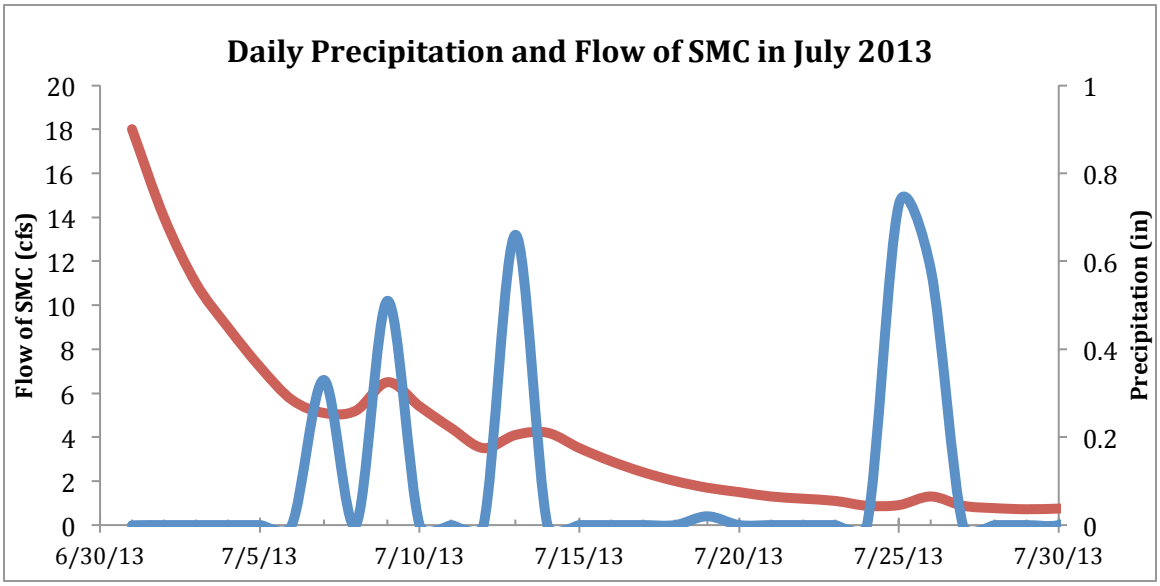

Figure 4c.

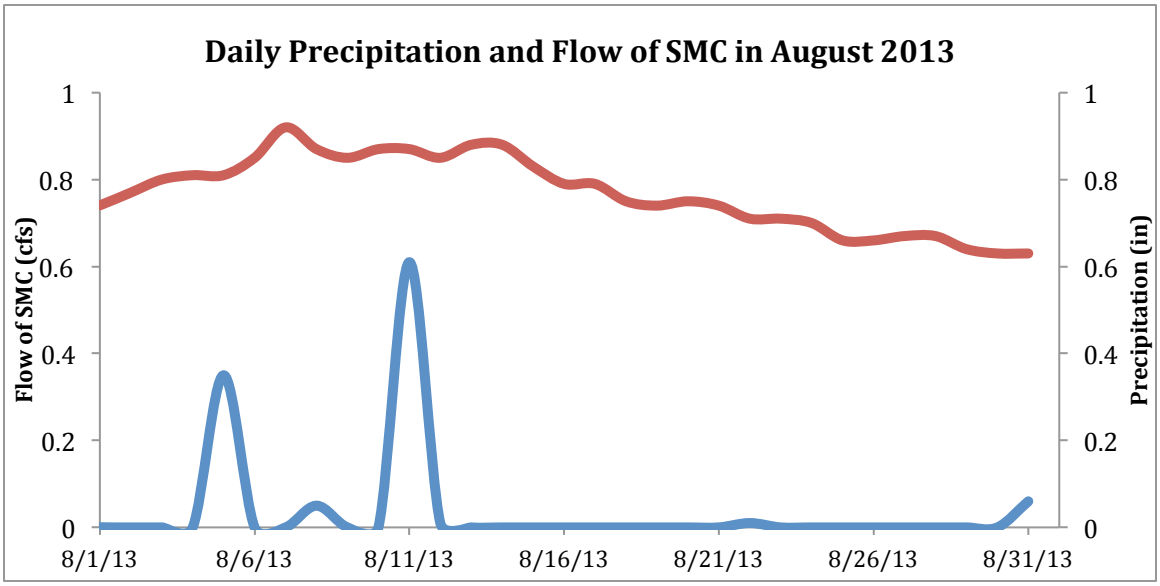

Figure 4d.

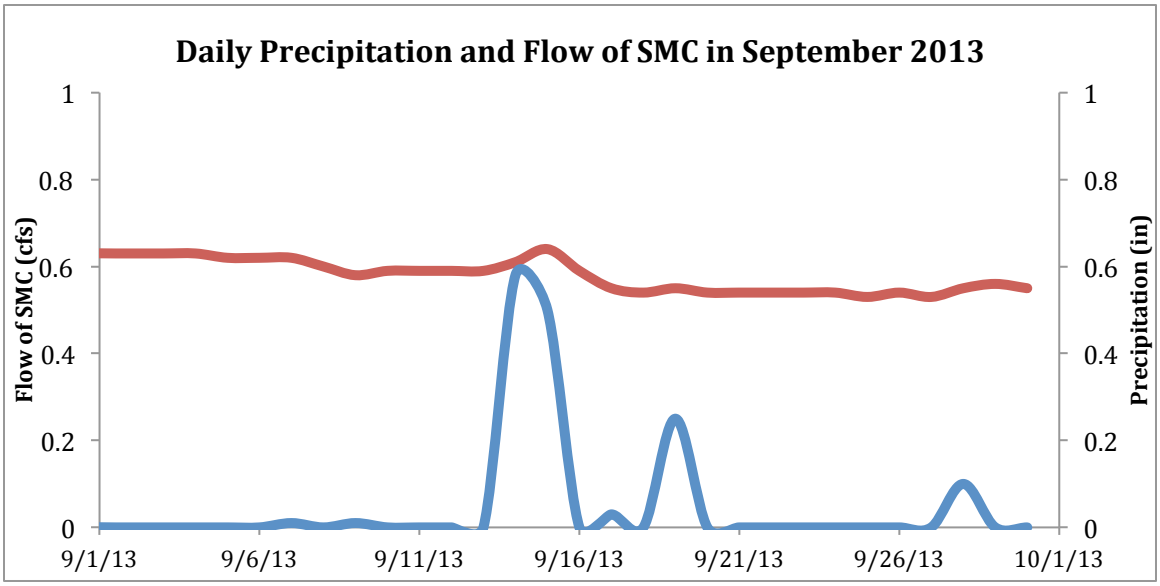

Figure 4e.

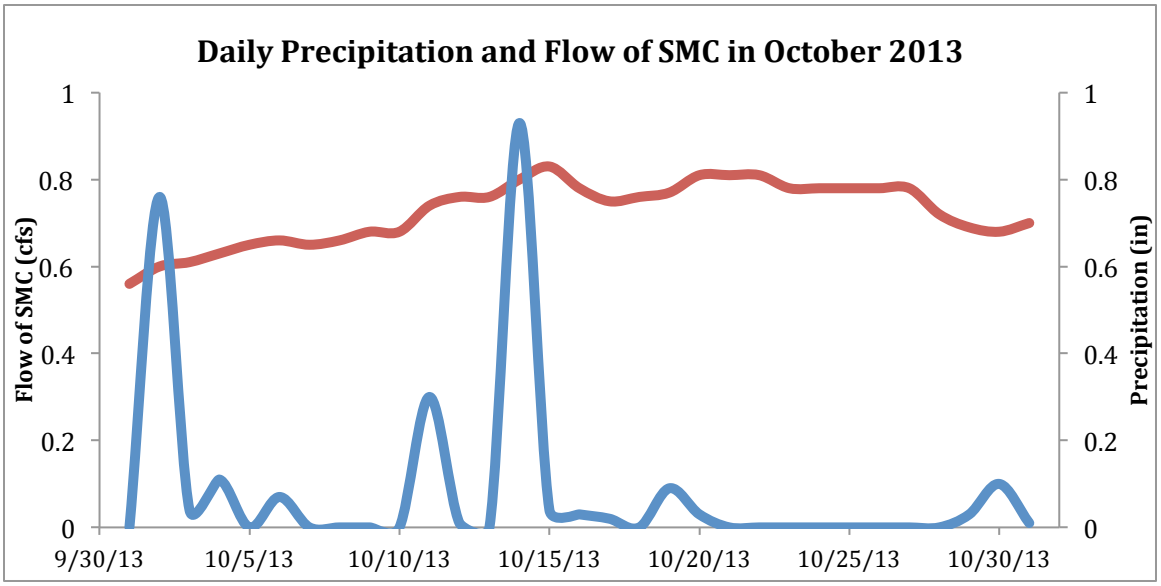

Figure 4f.

Figure 4. Daily precipitation (blue line) as well as the daily flow of SMC (red line) measured from the same location along the main stem for the months of May through October (4a-4f respectively). Note that that Figure 4a, b, and c have different scales for flow (y-axis). (Minnesota Department of Natural Resources 2016).

DISCUSSION

Total Suspended Solids

This study focused on the agricultural aspect of water quality. This is because agricultural practices are the main target of the mitigation tactics being used over the next few years. However, it is not the only factor, especially when it comes to TSS. While the increased flow caused by tile drainage can increase erosion and therefore increase suspended sediments, it is also important to note that ravines play an important role in the sediment loads of SMC. Reducing flow from the fields does not solve the problem completely.

To see how much suspended sediment is coming from the ravines and the agricultural fields, TSS should continue to be monitored at the upland sites as well as the main stem. The difference in TSS can be compared in order to give an idea of how much sediment comes from the ravines and how much comes from the agricultural fields. Without knowing this, it would be difficult to determine the effect that the mitigation tactics end up having on the sediment load. Because only the main stem was tested for TSS in the fall and only the upper two sites were tested in the spring, this comparison cannot be made for the TSS data collected in this particular study.

Nitrate

Nitrate, which is a primary contributor to eutrophication, comes in part from the agricultural practices and should be monitored during the agricultural season, but also from groundwater. In future studies, the contribution by both groundwater and agricultural practices to the nitrate load of SMC should be studied using the ion chromatograph method found in Appendix 1C.

E. coli

The ideal number of colonies per plate is between 20 and 60 in order to accurately measure col/100 mL. In order to achieve this result, larger quantities of water must be filtered when concentrations are low. In this study the number of colonies ranged from 0 to 3 per plate. This is not high enough to create accurate results. *E. coli* is not abundant in the spring, which should be taken into account for future studies. In the future *E. coli* should not be measured until later in the spring, or alternatively, much higher quantities of water should be filtered when testing in the early spring. As the concentration of *E. coli* rises, the amount of water filtered can be decreased accordingly.

Precipitation and Flow Response

The discharge of SMC is flashy and not easily predictable. Because discharge determines the total sediment and pollutant load that gets carried from watershed and into the Minnesota River, it is critical to understand the relationship between precipitation, surrounding agricultural practices, and flow. Accurate monitoring of the water quality of the creek is more easily done with an understanding these dynamic relationships.

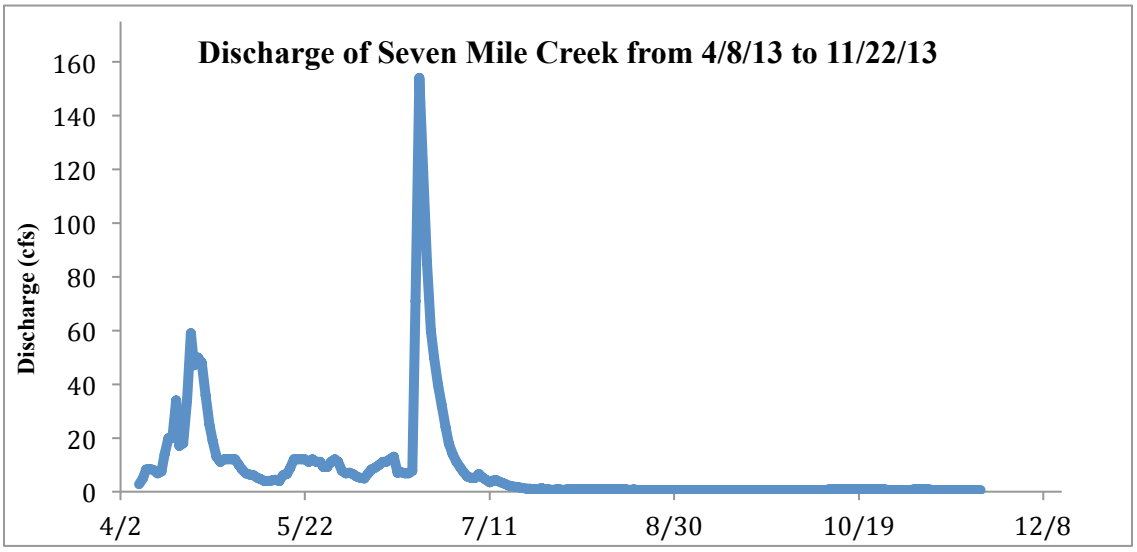

Figure 5. Discharge of Seven Mile Creek from 4/8/13 to 11/22/13 (Minnesota Department of Natural Resources 2016)

Agricultural practices have a big influence on the flow in the creek. In the early to mid-summer, crops are being grown and the drain tiles are open and flowing. This increases the discharge of the creek dramatically and is when the creek experiences its highest levels of discharge (Figure 5).

Precipitation also affects flow in the creek, but the extent to which it influences flow is dependent on the time of year due to agricultural activities in the watershed. As seen in Figure 4a and Figure 4b, precipitation increases the flow of the creek. However, heading into the rest of the summer and the fall, precipitation does not appear to show the same level of influence. This is due to the fact that crops are growing during this time and they use up most of the precipitation, meaning it does not make it into the stream itself to increase discharge. However, this is a simplification of the relationship, as other factors such as soil saturation also have an effect.

Monitoring Recommendations

One of the main objectives of this study was to figure out the best way to accurately gauge water quality. Moving forward, there should be roughly four stages throughout the monitoring season. The first stage is in the spring when the snow begins to melt and the flow of the creek begins to pick up. This could begin anytime in late February, March or April. Any preparations that need to be done before monitoring begins should be done in February, in case of early snowmelt. From this time up until the snow has all mostly melted, TSS and flow should be monitored at least once a week, and additionally in the case of precipitation events, which could increase the rate of snow melt as well as the mobilization of sediment and flow.

The second stage in the monitoring season is after snowmelt is complete and goes up through approximately mid to late June, when agricultural practices begin to increase flow dramatically. This stage is when precipitation is influential and should be monitored carefully. Regular monitoring should occur at least once a week, as well as during and after precipitation events. For light precipitation monitoring should be done immediately following it as well as the day afterwards. For heavier or more extended events, more frequent monitoring should be done both during and after the precipitation.

Once the crops have taken root and start to suck up most of the precipitation, it is no longer as important to keep track of precipitation events and monitoring can become

more scheduled. This is the third stage, during which flow is at its highest for the year. This takes places from roughly mid June to late July or early August (Figure 1). This is most likely an important phase for *E. coli* and nitrate due to the high agricultural activity. Nitrate and *E. coli* were not analyzed enough to demonstrate that fact, so future studies should be sure to analyze nitrate and *E. coli* to a further extent.

In the final stage of the monitoring season, which goes from roughly August through October, flow has decreased and somewhat stabilized, and precipitation continues to have some influence. At this point monitoring can be done approximately once a week and in the case of heavy or prolonged precipitation events.

CONCLUSION

Seven Mile Creek varies throughout the seasons and successfully monitoring it requires altering monitoring frequency depending on the time of year, the flow, agricultural practices, and precipitation patterns. The timeline of the four monitoring stages is not exact. Every year will be different due to variations in snowmelt and precipitation as well as how those affect the agricultural season. Additionally, the recommendations are based on simplified versions of the complex relationship between pollutants, sediment load, flow, agricultural practices, and precipitation. The recommendations are meant to serve as general guidelines and should be adjusted according to variations.

Monitoring the water quality of SMC before, during, and after mitigation efforts in the watershed is crucial to understanding the ways that water quality can be improved on a larger scale. Improving the quality of smaller watersheds will contribute to an overall improvement of water quality in the larger rivers, lakes, and oceans to which they flow. Understanding the dynamics of Seven Mile Creek is the first step.

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APPENDICES

Appendix 1. Methods

A. Fecal Coliform Procedure
From 18th Ed. Standard Methods, 9222D

Safety equipment
A lab coat, eye protection, and laboratory gloves should be worn at all times during this procedure. Keep bottles of ethanol away from open flame. Use caution when immersing filtration devices in boiling water.

Reagents and supplies needed
Filtration manifold and sterile filtration receptacles
500 or 1000 mL filtration flasks
Sterile filtration membranes
Sterile forceps
Ethanol flame lamp
Squeeze bottle of ethanol
M-FC medium
Petri dishes
Disposable pipettes with bulb
Ample supply of distilled water
Large beaker of boiling distilled water for sterilization of funnels
Graduated cylinders for measuring samples and water
Volumetric pipetters for measuring samples
Pipette tips
Sharpie marker for labeling
Incubator
Low-power microscope

Preparation of medium
Prepared M-FC medium is used to ensure reproducibility.

Sterilization of materials (9030B)
Metal and glass instruments to b used in this procedure should be sterilized by autoclaving. Instruments should be autoclaved at 137°C for 60 minutes. Cover the ends of graduated cylinders with aluminum foil prior to sterilization. Autoclave the following instruments prior to beginning the procedure:

Forceps (>3 sets)
Disposable pipettes (50)
Glass rods (20)
Pipette tips, 0-200 uL and 200-1000 uL
Graduated cylinders (10 50 mL and 5 100 mL)

All sampling containers, incubation media, Petri dishes, membrane filters, and absorbent pads must be sterile prior to use. If these supplies are not received in sterile form, they must be sterilized by the appropriate method.

Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid contamination. A filtration series is considered to be interrupted when an interval of 30 min or longer elapses between sample filtrations. After interruptions, treat any samples as a new series and sterilize all membrane filter holders in use. Sterilize by flowing or boiling water for 5 min.

Selection of sample size (9222D.2.a)
Select a volume of water sample to be examined using Table 9222:III or previous data from site. These volumes are intended to yield counts between 20-60 fecal coliform colonies per member. If the bacterial density is unknown, filter several decimal volumes to establish fecal coliform density.

For sample sizes smaller than 10 mL, dilute sample with sterile water (in a sterile bottle or graduated cylinder) to a volume of approximately 50 mL prior to filtration.

Table 9222:III

Water	Volume (X) to be filtered in mL						
Source	100	50	10	1	0.1	0.01	0.001
Lakes, reservoirs	X	X					
Wells, springs	X	X					
Water supply intake		X	X	X			
Natural bathing waters		X	X	X			
Sewage treatment							
Secondary effluent			X	X	X		
Farm ponds, rivers				X	X	X	
Storm water runoff				X	X	X	
Raw municipal sewage					X	X	X
Feedlot runoff					X	X	X

For Seven Mile Creek, the volume used ranged from 50 to 0.1 mL depending on the time of year

Preparation of culture dish
Using a Sharpie marker, label the bottom of the Petri dish with the sample number and volume of sample used, if necessary.

Remove an ampule of M-FC medium from it Styrofoam holder. Swab each ampule with ethanol prior to use. Break open the glass ampule along the score mark and pipette the contents (2 mL) to saturate each pad. Carefully remove any excess liquid from the culture dish (experience has shown that this step is generally unnecessary).

Filtration (9222D.2.b)
Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of filter receptacle. Place the matched funnel unit over receptacle and lock it in place. Filter sample under a partial vacuum. With filter still in place, rinse funnel by filtering three 20-30 mL portions of sterile dilution water. After the final rinse and filtration, disengage the vacuum and unlock and remove the funnel.

Re-sterilize the forceps by saturating with ethanol and passing through a flame after each use.

Insert a sterile rinse water sample (100 mL) after filtration of a series of 10 samples to check for possible contamination and cross-contamination. Incubate the control membrane culture under the same conditions as the sample.

Remove the membrane filter from the filtration apparatus with sterile forceps and place it grid-side up on the sterile pad impregnated with medium. Use a rolling motion to avoid entrapment of air beneath the membrane.

Incubation
After placing membrane filter on absorbent pad, replace Petri dish lid, invert dish, and place in incubator at 44.5 +/- 2°C for 24 +/- 2 hours. Place a label on the incubator indicating the time that incubation began, and a warning against opening of the incubator.

Colonies
Remove dish from incubator and examine colonies under a low-power dissecting microscope and reflected light source.

Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Pale yellow may be atypical cultures of *E. coli. E. coli* presence can be verified by gas production in mannitol at 44.5°C.

Non-fecal coliform colonies are gray to cream colored. Normally few non-fecal will be observed on M-Fc medium because of selected action of the elevated temperature and addition of rosolic acid salt reagent. Therefore, gray colony counts are not an accurate indication of total coliform abundance.

Elevating temperature to 45.0 +/- 2°C may be useful in eliminating environmental *Klebsiella* from the coliform population, if necessary.

Calculations (9222B.6)
Compute the count, using membrane filters with 20 =-60 fecal coliform colonies and not more than 200 colonies of all types per membrane, by the following equation

$$\text{Fecal coliform colonies/100mL} = \frac{\text{fecal coliform colonies counted x100}}{\text{mL sample filtered}}$$

With water of good quality, fecal coliforms should be minimal. Therefore, count all colonies present, disregarding the lower limit of 20 colonies above, and calculate as above. If replicate samples have been analyzed, use the total number of colonies on all places and divide by the total volume used.

If confluent gr4owth occurs, report results as "confluent growth with (or without) fecal colonies, coliforms plus non-coliforms, exceeds 200 per membrane, or if the colonies are not discrete enough for accurate counting, report results as "too numerous to count" and request another sample from the same location.

B. Total Suspended Solids Procedure

Standard Operation Procedure For Measuring TSS and TVS
Gustavus, June 2013

This protocol was designed for measuring total suspended and total volatile solids in surface waters.

Sample collection

- Collect samples in clean plastic bottles
- Cap tightly, no preservatives
- Refrigerate until analysis

Ideally, samples are filtered and placed in drying over within 24 hours of collection. Maximum holding time is 1 week.

Filter and weighing cup preparation

- Place aluminum weighing cups and Pyrex dishes in furnace, burn at 550°C for 1 hour
- Lightly stack ~10 glass fiber filters on clean (burned) Pyrex petri dish
- Burn at 550°C for 10 minutes
- When cool remove from furnace, cover with clean petri dish cover and store until use

Filtering

- Place one prepared filter into aluminum weighing cup. Label cup by scratching number or letter onto tab. (Note: if you use sharpie to write a label it may burn off in the furnace; therefore, in that case you should also record the position of the cups in the furnace before you burn them.)
- Tare balance. Weigh filter in cup, record total mass to the nearest 0.01g.
- Assemble filter apparatus with that prepared filter. Be sure to keep track of which number filter is in each filter apparatus (e.g., place labeled aluminum cup next to filter)
- Vigorously shake sample bottle to suspend sediment. Immediately pour water into graduated cylinder to desired volume. Record volume. (*The volume you use depends on how much sediment is suspended in the water. For samples that are*

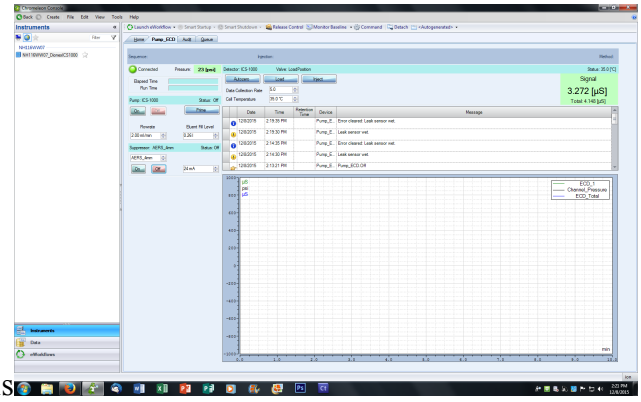
not turbid, you may want to use up to 200 mL of water. For samples that are very turbid, 25 mL may be adequate.)

- Turn on vacuum pump and immediately pour measured sample volume into filter apparatus. (Do not allow suspended sediment to settle in the graduated cylinder.) If entire water volume does not fit at once, carefully swirl in graduated cylinder until you can pour it into the filter apparatus. You may also use DI to rinse remaining sediment out of cylinder.
- When all water has passed through filter, turn off pump. Use tweezers to carefully move filter into its appropriate aluminum cup, without losing any sediment on tweezers or elsewhere.
- Place aluminum cups with wet filters in drying oven at 105°C at least 6 hours or overnight.
- Weigh dried cups with filters. Record mass.
- Place cups with filters in furnace, burn at 550°C for 2 hours.
- When cool, weigh cups with filters. Record mass.
- For each batch of 20 filters, prepare one "blank" by "filtering" 100 mL of DI water through a pre-weighed filter, then treating the filter as a sample.

C. Nitrate

Ion Chromatograph Operation Manual

Start-Up

1. Open Chromeleon Console software and click on the instruments tab
    a. It should look like this 

Prepping the IC

1. Prime
    a. Open the waste pump one turn (bottom left)
    b. Click prime button
    c. Prime for 10 minutes (should see air bubbles moving through the pump waste tube)
    d. Turn pump off (on computer) and close waste pump by turning to the right until it is tight
2. Set the flow rate of the pump to 2.0 mL/min
    a. Turn on pump

3. Set suppressor to AERS 300_4mm and the voltage to 24 mA
    a. Turn on suppressor (shortly after turning on the pump)
4. Do not run samples until the pressure levels off between 2000 and 3000 psi (this takes 5 to 10 minutes)
5. Open data tab, click create sequence

Preparing Samples (can be done while IC is priming and/or while waiting for pressure to level off)

1. Fill tube within 1 centimeter of the top
2. Push cap down flush to the edges of the tube
3. Place samples into a rack in the order that matches the created sequence
4. Place rack in automated sampler and be sure that the black dot is just underneath the sampling apparatus
5. See picture below to make sure all the settings on the automated sampler are correct (disregard the red light next to empty, this will change when you load your samples)

Running the Samples

1. Check to make sure the suppressor and pump are both still running and that the pressure is still between 2000 and 3000 psi with only minor fluctuations
2. Hit the start button in the data tab to start sequence
    a. Hit hold/run button on the automated sampler right after you hit start
        i. If the settings on the sampler are correct, the sequence should progress and sample automatically
    b. Each sample takes 12 minutes to run, but will begin with a negative run time and count up to 12 (during the negative time is when the automated sampler will move on to the next sample)
3. Allow sequence to be completed
    a. The sequence data will save automatically
4. Turn off suppressor and pump

*When finished with run, put the IC into either short term or long term storage based on time between use

Short Term Storage (3-7 days)

1. Disconnect cell out to regent in and disconnect eluent in
2. Use plastic syringe and push 5 mL of deionized water through the regent in port on the suppressor - until air bubble are removed
3. Run 3 mL of deionized water through the eluent in port on the suppressor - until all air bubble are removed
4. Unplug the other two ports - regent out and eluent out
5. Cap all four ports

Short term to long term shut down steps

1. Reconnect the cell in tube to eluent out, bottom of the suppressor
2. Reconnect the cell out tube to regent in
3. Reconnect regent out to waste

Long-term Shut down (7+ days)

1. Disconnect the tube connecting the column to the suppressor (says eluent in on it)
2. Rinse and refill the milli Q container (milli Q can be found in 207- biochemistry lab)
3. Connect milli Q water to the intake tube (just replace the cap)
4. Disconnect the injection valve from the column (leave the guard attached with the column) and connect the injection valve to the suppressor, tighten lightly with the wrench
    a. Column is left disconnected
5. On Chromeleon console click prime, open waste valve, click ok
6. Set the pump to a flow rate of 1mL/min
7. Prime until there are no air bubbles coming out if the waste tube
8. Stop prime, close the waste valve
9. Turn on the pump at 1.0 mL/min - do not turn on the suppressor
10. Run the pump for 10 minutes
11. Turn off the pump
12. Disconnect all four tubes from suppressor
13. Cap the four ports - tighten lightly with wrench
    a. Make sure the caps are caps (no passage) not connectors - they look similar

Prepping the IC from shut down

1. Connect injection valve to guard in
2. Connect column out to eluent in
3. Connect cell in to eluent out
4. Connect cell out to regent in
5. Connect regent out to waste

Start hydration process (prepping the IC)