# **Stimulated Water**

Development of Protocol for Detecting Caffeine and Nicotine in Surface Water Using the Skidmore Analytical Interdisciplinary Laboratory (SAIL)

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# **Acknowledgements**

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## **Introduction**

*Ecological Impacts of Urbanization* Globally, urban populations

plants.

of equal size (EPA 1996). As rainwater run-off flows across an impermeable surface, it picks up nutrients and pollutants, including nitrogen, phosphorus, ions, pesticides, bacteria, heavy metals, and pharmaceuticals including stimulants such as caffeine and nicotine (Bannerman et al. 1993, Bradley et al. 2002). This water then either flows from impermeable surfaces directly into water bodies or travels through storm drains and pipes into water systems.

Historically, stormwater in the United States was managed through the construction of drains and pipes designed to collect water and remove it from urban areas as rapidly as possible (Roy et al. 2008). In large cities (populations of 100,000 or more), stormwater is treated in the same plants that treat sa

occurring at least every two to five years are likely to leach pollutants and pathogens into groundwater, which may eventually reach streams, rivers, or lakes (Evans et al. 1999 in Petri 2008).

Although septic tanks are not typically found in urban areas, they are commonly used in surrounding suburban areas and rural residential areas that are not supported by municipal wastewater treatment facilities. As of 2007, approximately 26.1 million homes in the United States were served by septic systems (U.S. EPA, Septic). Petri (2008) estimated that there were approximately 10,000 septic tanks in the Saratoga Lake Watershed as of 2007 and that of these 10,000 tanks, only about 5 10% were properly maintained, which means that portions of the watershed could be at high risk for groundwater contamination.

#### *Caffeine*

Caffeine is a naturally occurring stimulant which 80% of the world's population consumes daily for its ability to increase alertness (Heckman et al. 2010). According to the Food and Agriculture Organization, the United States is ranked 10<sup>th</sup> in caffeine consumption, behind countries such as the U.K., Brazil, Canada, Australia, and Japan, countries recognized for heavy coffee and tea consumption (Heckman et al. 2010). Though 71% of caffeine consumed is in the form of coffee, recently developed and heavily advertised energy drinks, sport drinks, and fortified waters have created a new branch of the caffeine market (Heckman et al. 2010). Caffeine also plays an important pharmaceutical role as cough, cold, and headache medicine as well as a cardiac, cerebral, and respiratory stimulant (Buerge et al. 2003).

The abundance of caffeine in our culture increases the likelihood that it will act as an environmental contaminant. Kolpin et al. (2002) previously studied the presence of pharmaceuticals in

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found caffeine to be the 4<sup>th</sup> most frequently found, occurring in 70% of the samples. Since the mean half-life of caffeine is relatively short, approximately 1.5 days, (Moore et al. 2007) it will not likely be environmentally hazardous if its addition to water systems is not consistent. In the human body, caffeine is rapidly filtered from the blood stream by the kidneys and excreted in urine (Heckman et al. 2010). As a consequence of caffeine's high c 0.24 0 0 0 Tm /F2.8.25998-0.5(f) -0.5 (e) 2007). This form of nicotine is a non point source of pollution which has the potential to be processed by microbial activity in aquatic ecosystems (Bradley et al. 2007). Agricultural input from tobacco cultivation is another non-point source of nicotine but this input source is both regionally limited and absent in urban settings. Alternatively, cigarette butt waste is heavily influential in urban settings and densely populated residential areas (Novotny et al. 2009). Since the introduction of filtered cigarettes in the 1950s, consumer preference for this alternative has increased to account for 99% of cigarettes purchased (Novotny et al. 2009). The plastic like cellulose acetate filters prevent a large amount of nicotine and tar inhalation because the carcinogens are retained by the non consumed portion of the cigarette (Novotny et al. 2009), but he dense concet cm BT 50 0heum

detect a wide variety of other novel contaminants in addition to caffeine and nicotine. In order to determine the best methods possible, we experimented with the HPLC, IC, and GC in order to determine which would be the best suited for this variety of research. Our hope is that these methods will contribute to the advancement of understanding of whether novel contaminants are present in the Saratoga Lake Watershed, in what concentrations they are present, and from what sources they are contributed. For the purposes of this project, we focused on streams that are potentially being impacted by leaking septic tanks and Spring Run, which receives high volumes of stormwater run-off.

### **Methods and Protocol Development**

#### *Site Identification and Sample Collection*

To identify and select the stream sites for sample collection within the Saratoga Lake

Watershed, we used a hotspot map of improperly installed septic systems (LA Group in Petri

2008). This map identified septic tanks that are located on improper soils or within 200 feet of a

stream and created hotspots based on the relative density of septic tanks (Figure 1).



**Figure 1.** Ten sampling sites in the Saratoga Lake Watershed selected along septic tank density continuum (based on LA Group in Petri 2008) indicated by yellow circles.

We selected streams along a continuum ranging from low density to high density of septic systems. To serve as a control, we choose a non-urban stream site that was not near improperly installed septic systems. Additionally, we selected two locations along Spring Run, one in Congress Park, upstream of downtown Saratoga Springs, and one adjacent to EBI,

downstream of downtown Saratoga Springs.

In total, we collected samples from each of the 12 locations on four occasions during February and March 2011, twice after high input events

#### *High Performance Liquid Chromatograph (HPLC)*

The first instrument we used was the HPLC because stream water samples require very

little preparation to be processed by this instrument. Also, we believed we would be able to

detect caffeine, cotinine, and nicotine using the same wavelength setting.



**Figure 2.** HPLC results of caffeine and cotinine standard mix; he injection peak appears just before 2 minutes, the caffeine peak appears around 3.5 minutes, and the cotinine peak appears around 6 minutes

To prepare our stream water samples for the HPLC, we filtered 5 mL of each sample through a 20 micron filter to remove all suspended particles. We ran a sample from Spring Run at the downstream location by EBI following the same methods used for the standard solutions. Because of its State 303 (d) List designation, we expected Spring Run to be highly likely to contain at least one of our novel contaminants of interest. However, we did not detect caffeine or cotinine in the Spring Run at EBI sample.

In addition to the Spring Run sample, we ran samples from Wheeler Creek and Mud Creek, two locations included within the intermediate range of the septic influence continuum. We used a different solvent ratio (70% water and 30% methanol) and a different HPLC retention column to alter retention time in hopes of achieving a higher resolution between contaminants. The standards must be run using the same methods that are used for the stream water samples to ensure accurate identification of the peaks as their hypothesized contaminants, so we ran each standard and the mix of standards again following the new methods. The HPLC functions best when the solvent ratio is close to the sample it is processing, so we diluted the standards with

methanol in a 70:30 ratio. Unfortunately, we did not detect caffeine or cotinine in the Wheeler or Mud Creek samples (Figure 3).



**Figure 3.** Based on the methods used for caffeine and cotinine standard solutions at 1 mg/L concentrations, neither caffeine nor cotinine were in the Wheeler Creek (top) or Mud Creek (bottom).

We determined that the HPLC was not an ideal instrument for our research purposes. We were not able to separate the nicotine peak from the injection peak. Previous research into novel contaminants has tested for cotinine instead of nicotine (Barnes et al. 2002, Kolpin et al. 2002, Kolpin et al. 2004) justifying our decision to proceed without identifying nicotine in a standard

solution. However, we would ideally be able to detect both cotinine and nicotine using the same methods and same instrument.

There are several possibilities for why we were unable to detect our novel contaminants using the HPLC. The column that is currently installed on the instrument may not be designed to process stream water samples that potentially contain numerous contaminants and can stress the instrument. By filtering our water samples, we anticipated being able to avoid this problem. Another possibility is that the instrument's pump is not appropriate to process stream water. The pump pressure builds to too high of a level, causing the instrument to shut down. We could not alter the pump pressure from its default setting, which may have been why we could not interpret the results of our sample. The HPLC that is currently installed in SAIL functions best with a higher methanol to water solvent ratio. To procure results with a high enough resolution to confirm identity of caffeine, cotinine, and nicotine peaks, we needed to alter the solvent ratio. Since we were having many technical difficulties and since we were not confident in the limited results we were getting, we moved on to another instrument.

#### *Ion Chromatograph (IC) on Liquid Chromatography Mode*

Using the IC, we processed our standard solutions and a stream water sample from Spring Run. To prepare the samples for the instrument, we poured approximately 5 mL of each of the standards and of the mix into plastic vials and capped them. These vials can be loaded into an auto-sampler which rotates through each of the samples, draws up a fraction of each sample, and injects the sample into the instrument in a mobile phase. Based on the polarity of each molecule, the instrument separates each component of the solution and yields the time and wavelength at which each molecule can be detected.

We ran trials of the dilutions of caffeine and cotinine standards at 1 mg/L. We also ran a mix of equal parts of the caffeine and cotinine standard solutions to see if we could detect and

identify both contaminants in one trial run. Although we were able to detect caffeine and cotinine on the IC in our standard solutions and

**Figure 4.** GC output display of caffeine, just before 13 minutes, and cotinine, around 11 minutes, both at 1 mg/L concentrations.

Our initial standard solutions were at concentrations of 1 mg/L. Once we verified that the

GC would be able to detect this concentration of our contaminants, we further diluted our

caffeine standard to 100

developed, we did not expect to detect cotinine in the concentration of the 1 L Bell Brook sample. When we ran the stream water sample from Bell Brook, we did not detect caffeine or cotinine (Figure 6).



Figure 6. Following a 1 L concentration and extraction from a stream sample from Bell Brook, caffeine and cotinine were not detected.

The detection level of 10  $q/L$  is close to the high end of the range that caffeine has been detected in the environment. The maximum concentrations we found in our literature review were 7.99 g/L at low flow (Glassmeyer et al. 2005) and 6.00 g/L at high flow (Kolpin et al. 2002). While we cannot definitively say that caffeine is not present in Bell Brook, we can say that the concentration must be relatively low. Since Bell Brook is in the most densely concentrated area of septic tanks in the watershed, it is unlikely that caffeine would be found in higher concentrations in other streams. While we had hoped to conduct a survey of streams in the watershed, the time consuming nature of our methods limited us to running only one sample.

#### **Suggestions for Future Research:**

In order to continue research on novel contaminants in the Saratoga Lake Watershed, we believe that SAIL should invest in a carboy apparatus, which would filter samples through the cartridge via gravity. This would make it possible to lower the standard detection levels to 1.0 g/L, which is closer to what has been previously detected. Our project was limited to collecting samples in the winter and early spring, but we believe that collecting samples at baseline during the summer might yield higher concentrations of novel contaminants.

We also recommend that future researchers pursue investigation of how caffeine and cotinine are transported into surface water through a comparison of stormwater and run-off, septic system influence, and wastewater effluent. Samples from Spring Run at Congress Park and EBI could be analyzed because this stream received high input from stormwater and urban run-off. Water running into storm drains during rain events can be collected and analyzed. Samples from the wastewater treatment plants can also be analyzed. While no wastewater is discharged in the Saratoga Lake Watershed, wastewater is discharged into the Hudson River and samples could be collected downstream of these locations. Furthermore, the 10 sampling sites Figure 1) identified in this project can be used to examine septic tank influence.

While we limited our study to caffeine and cotinine, future research could also look into the possible presence of other novel contaminants in the watershed. Additionally, the ecological impacts of novel contaminants has been largely uninvestigated. We believe that an analysis of the ecological impacts of caffeine, cotinine and other novel contaminants is important, considering their prevalence in water bodies across the United States. There are many potential ways to quantify these impacts. We outlined methods for assessing changes in foraging behavior of fish exposed to caffeine and nicotine (Appendix A

Based on our methods development process, we created a list of information that should be obtained prior to starting a project involving the use of the instruments in the SAIL facilities. This list of recommendations for future research endeavors is included (Appendix B).

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## **Appendix A**

**Methods for Assessing Changes in Fish Foraging Habits after Exposure to Caffeine and**