Identification of the Chemicals of Emerging Concern Caffeine and Para-Xanthine in the St. Louis River

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Abstract:

The use of caffeine in the United States is very prevalent. People of all ages consume the legal stimulant. Potential residual caffeine and its metabolites in wastewater effluent can contaminate aquatic ecosystems and groundwater supplies. Due to the possible consequences of residual caffeine content in the St. Louis River, caffeine concentration is investigated. Six samples were collected from the St. Louis River and analyzed for caffeine and para-xanthine content using a combined SPE-extraction and GC/MS identification and quantification. Caffeine was identified in six samples, p-xanthine identified in three.

Introduction:

Caffeine use has been steadily increasing over years. Starting in 1985 when Jolt Cola was marketed to the American general public (Jolt Cola, 2014), caffeinated energy drinks have risen in popularity. Energy drinks are a staple in the life of many Americans; college students and young mothers are some of the biggest consumers of these beverages (Heckman, 2010). The debate over the use of caffeine has been long and arduous (Gruber, 2010). What is clear, however, is that caffeine is impacting our world. The consumption of caffeine can come in all forms. While caffeine is often viewed as highly beneficial and pleasurable, it has the potential to cause lasting effects in the environment and human health. Caffeine has been leaking into water sources, such as lakes and rivers in the same way that the other pharmaceuticals substances (Bienkowski 2013). Unlike other pharmaceuticals, the caffeine that has leaked into the water supply is directly from human fecal matter (Potera, 2012). Wastewater treatment plants are designed to remove pathogens and nutrients from sewage, which are not set up to remove chemical compounds such as caffeine or other drugs from the discharge water (Daughton, 1990). The implications of the rise in caffeine and possible caffeine wastewater contamination are not fully known yet. Since caffeine is usually not specifically removed from the wastewater effluent, its concentration in the water may be of concern due to unknown long term effects on biological aquatic ecosystems and possible bioaccumulation over time (Zuccato, 2008). In Hawaii, caffeine was being used to kill the invasive Coqui frog, by spraying it onto the frogs. While using the caffeine as a pest control method, the Hawaii Department of Agriculture was required to monitor the effects of caffeine on other species to insure that it was not killing anything else (Neuroscience, 2001).The caffeine was considered potentially hazardous to applicators because caffeine poisoning is lethal and has no antidote (Zimmerman, 2001). The exemption that allowed them to use caffeine for pest control expired in 2002, and they have not pursued a renewal (Control, 2014).

A study of chemicals of emerging concern in Michigan showed the average caffeine concentration in Lake Michigan within 1 mile of a wastewater treatment plant effluent discharge was 28.15 ng/L (Blair, 2013). As reported in a study done by the USEPA, only 29% of residual caffeine was eliminated due to microbial or photo degradation in a particular estuary over a four week span (Benotti, 2012). The main objective of this research is to identify caffeine and para-xanthine that is mixing into the St. Louis River water. The Western Lake Superior Sanitary District (WLSSD) in Duluth, Minnesota and City of Superior, Wisconsin wastewater treatment plants are possible sources of caffeine and para- xanthine in the water.

Methodology:

Study area and sample collection:

The WLSSD wastewater treatment plant treats about 40 million gallons of wastewater from civilian residences, businesses, and industries every day (Wastewater). To clean the water, WLSSD employs strategies that mimic natural river water flow such as gravity, bacteria, aeration, settling, sand and gravel (Wastewater). The by-product of the WLSSD water purification system is thickened sludge, which they turn into bio solids to be used as fertilizer. The main Superior water treatment plant is much smaller than WLSSD and can only handle up to 5 million gallons of water per day (Process). The superior plant uses many of the same strategies that WLSSD, but they do not produce bio solids as a byproduct. Instead, the Superior water treatment plant removes the solids which are dried and disposed of (Process). The water from both wastewater treatment plants is then pumped into the St. Louis River, which flows into Lake Superior (Fig 1).

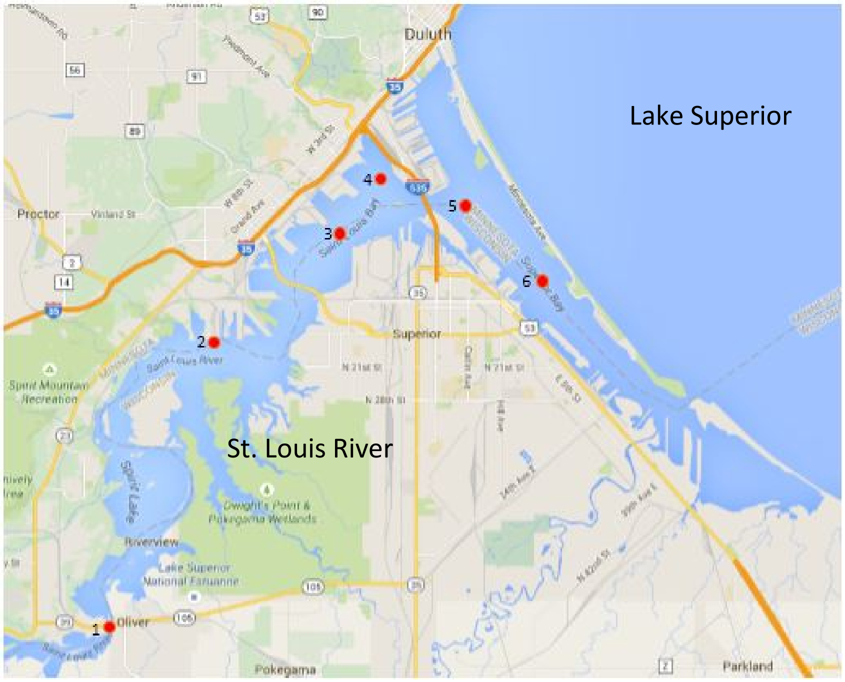

Fig 1. Saint Louis River and Lake Superior

Table No. 1 Sample Details

Sample #	Sample Name	Latitude	Longitude	Time
1	Oliver Bridge	46.65690	92.20167	10:00 AM
2	Dwight's Point	46.71735	92.16655	10:55 AM
3	Erie Pier	46.73794	92.13869	11:05 AM
4	WLSSD	46.75536	92.12079	11:10 AM
5	Blatnik Bridge	46.74911	92.09558	11:40 AM
6	Superior Plant/Barker's	46.72921	92.06885	11:50 AM

Point 1 was taken under the old Oliver Bridge, it was hypothesized that being five km upriver from WLSSD that there should be no caffeine in the sample. Erie Pier and Dwight's point were taken slightly upriver from WLSSD and it was expected that they should have low levels of caffeine. WLSSD and Superior Plant/Barker's were both expected to have the highest levels of caffeine because they were taken directly from the location that the treated wastewater is pumped into the river. Blatnik was taken near Blatnik Bridge and was chosen as a halfway point between the two wastewater treatment plants to test the connection between the two plants.

The sample collectors refrained from consuming caffeine for 72 hours before collecting samples. The samples were collected on July 23, 2014. The samples were taken by placing the 5 liter plastic container under the water and capped. The samples were placed in a refrigerator and shielded from light.

Sample Analysis:

The samples were analyzed according to the method used by Rodriguez del Ray, et al., 2012 with some modifications, in breve: two different SPE columns C18 and ENVI were evaluated using a 1ppm standard solution of caffeine. It was found that the C18 column was less quantitative than the ENVI column to concentrate caffeine from the standard solution. Each sample was passed through five #3 Whatman filters then passed through one glass microfiber filter (GF/F 0.7μm). The caffeine standard stock 1mg/ml and the p-xanthine standard stock 1mg/ml were prepared in methanol. To achieve the dissolving of p-xanthine in the methanol, 3 drops of concentrated hydrochloric acid was added, the mixture was shaken thoroughly and allowed to sit for a week. 1ppm and 0.1ppm solutions were analyzed in GC/MS to test the retention time and detection.

A standard solution sample was prepare and passed through the ENVI SPE column in the following way. The ENVI fused silica column was conditioned by passing 5ml of HPLC grade dichloromethane, 5ml of HPLC grade methanol, and 10ml of milli-q through the column. After conditioning the column, the 1ppm caffeine and xanthine solution was passed through the column followed by 5ml of milli-q. The column was left to dry for 5 minutes before washing with methanol and then eluting with 10ml of dichloromethane. The 10ml of eluate was reduced to 2ml in a warm water bath, under a stream of dry air.1ml of hexane was used to wash any remaining sample into the new test tube. The warm water bath should was changed to a hot water bath. The hexane was evaporated to 0.2ml in the hot water bath under a stream of dry air. This procedure was followed for 0.1ppm standard caffeine and p-xanthine, the blank, and the samples.

The identification was done using an Agilent GC/MS instrument to identify each component. The GC/MS program was run in select ion monitoring (SIM) using one m/z peak to quantification and two peaks for confirmation; m/z 194,109, and 82 to identify caffeine and m/z 180,123,and 68 to identify p-xanthine.

Results:

Table No. 2 GCMS Results

Sample	Identification of para-xanthine	Identification of caffeine
Oliver Bridge	No	Yes
Erie Pier	Yes	Yes
Dwight’s Point	No	Yes
Barker’s/Superior Plant	Yes	Yes
WLSSD	Yes	Yes
Blatnik Bridge	No	Yes

These results are surprising because there should not have been any caffeine under the Oliver Bridge if the only possible sources of caffeine are WLSSD and/or the Superior plant. The goal of this experiment was to identify whether or not caffeine is leaking into the St. Louis River, and it appears that there is indeed caffeine leaking into the river. Given the caffeine found five km upriver from WLSSD, there may be another source of caffeine farther upriver. The identification of para-xanthine is much more interesting because it is far less stable than caffeine. The identification of para-xanthine indicates that the likely sources of contamination in the river are from WLSSD and from the Superior plant. The lack of para-xanthine at Blatnik indicates that the output from the Superior plant is not mobilizing as far up river as the contamination from WLSSD.

Further Research:

More samples from the St. Louis River could be collected closer to the wastewater treatment plants and analyzed using above methods to determine whether or not the caffeine is directly from the wastewater treatment plants. Other research in this area could be conducted by measuring caffeine content in the St. Louis River at different times of day, months of the year, and at other possible sources of waste near the river. Also, the seiche effect between the St. Louis River and Lake Superior could be investigated in relation to the concentration of chemicals of emerging concern. The seiche can cause the river to flow upstream or downstream. The seiche effect could be responsible for pushing contaminates to unexpected places in the watershed.

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