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Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area

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ABSTRACT

Water samples from streams, brooks and storm sewer outfall pipes that collect storm waters across the Island of Montréal were analyzed for caffeine, carbamazepine and fecal coliforms. All samples contained various concentrations of these tracers, indicating a widespread sanitary contamination in urban environments. Fecal coliforms and caffeine levels ranged over several orders of magnitude with a modest correlation between caffeine and fecal coliforms (R^2 value of 0.558). An arbitrary threshold of 400 ng caffeine L^{-1} allows us to identify samples with an elevated fecal contamination, as defined by more than 200 colony-forming units per 100 mL (cfu 100 mL^{-1}) of fecal coliforms. Low caffeine levels were sporadically related to high fecal coliform counts. Lower levels of caffeine and fecal coliforms were observed in the brooks while the larger streams and storm water discharge points contained over ten times more. The carbamazepine data showed little or no apparent correlation to caffeine. These data suggest that this storm water collection system, located in a highly urbanized urban environment, is widely contaminated by domestic sewers as indicated by the ubiquitous presence of fecal contaminants as well as caffeine and carbamazepine. Caffeine concentrations were relatively well correlated to fecal coliforms, and could potentially be used as a chemical indicator of the level of contamination by sanitary sources. The carbamazepine data was not significantly correlated to fecal coliforms and of little use in this dataset.

1. Introduction

Several authors have proposed caffeine (Seiler et al., 1999; Gardinali and Zhao, 2002; Peeler et al., 2006; Wu et al., 2008) or pharmaceuticals products as tracers of human wastes (Seiler et al., 1999; Wu et al., 2008; Kasprzyk-Hordern et al., 2009). Caffeine has the advantage of being ubiquitous and almost entirely human-related, given that there are virtually no agricultural or industrial releases, especially in the Northern hemisphere. Some natural plant sources of caffeine do exist but the background levels thus generated are usually negligible and can thus be disregarded (Peeler et al., 2006). The main sources of caffeine are considered to be coffee, tea, cola, cocoa-containing products and some pharmaceuticals and over the counter medication containing caffeine. The actual contributions from these various sources will vary according to consumption habits. Given that caffeine degrades slowly in the environment with an estimated half-life between 3 d and >3 months – (Benotti and Brownawell, 2009), it has been proposed as a tracer of domestic sanitary contamination.

A large portion of caffeine is metabolized and only about 3% of the ingested molecule is actually excreted through urine (Tang-Liu et al., 1983). Earlier studies have proposed that the contribution from the disposal of unconsumed caffeine-containing beverages and food products may actually be an even greater contributor than actual consumption, given its high metabolism rate, but no actual data were provided to quantify this assertion (Seiler et al., 1999). Concentrations of caffeine have been reported to vary from 20 to 300 μg L^{-1} in raw sewage and 0.1 to 20 μg L^{-1} in treated wastewater effluents (Heberer, 2002; Buerge et al., 2003; Viglino et al., 2007). Reported concentrations in rivers, lakes and seawaters range between 3 and 1500 ng L^{-1} (Buerge et al., 2003) whereas in ground waters values are between 10 and 80 ng L^{-1}. Caffeine has been most studied as a potential tracer of human sewage in surface water including urban/suburban runoff (Standley et al., 2000). Buerge et al. (2003) have shown that caffeine measurements are a direct and sensitive indicator of the presence of wastewater and combined sewer overflow discharges in rivers. Of prime importance when considering using

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caffeine as a wastewater tracer is the persistence of caffeine in the discharge and receiving waters. Caffeine undergoes slow photochemical oxidation, is not likely amenable to significant sorption or volatilization and the main mechanism for its elimination in lake water is biodegradation (Buerge et al., 2003). Caffeine's half-life has been estimated to range from 3.5 d to more than 100 d in estuarine and coastal waters with lower values observed in trophic waters (Benotti and Brownawell, 2009). Persistence in some surface waters can be astonishingly high. Half-lives in lake water estimated from batch incubations and derived from *in situ* changes of concentrations ranged between 120 and 240 d, while estimated half lives from wastewater biological processes are much shorter (0.8–5 h) (Buerge et al., 2003). However, prior exposure to wastewater discharge can significantly increase the biotransformation rate of caffeine in the water column (Bradley et al., 2007).

Carbamazepine is an anti-seizure drug which is also increasingly used for various psychiatric treatments. Because of its very slow degradation with a half-life estimated to be above 100 d, it has been proposed as an ideal anthropogenic tracer. However, the consumption and usage of this drug will be much lower and less widespread than that of coffee and caffeine-based products, but current analytical procedures can detect ultratrace levels of carbamazepine in surface waters. Reported levels vary between 0.1 and 5 μg carbamazepine L^{-1} in sewage with highly variable removal rates by wastewater treatment plants (WWTP) – with expected concentrations in effluents at similar range (Heberer, 2002; Viglino et al., 2007). Carbamazepine is very slowly biodegraded with half life in estuarine and coastal water exceeding 100 d (Benotti and Brownawell, 2009). Also, carbamazepine is also one of the few compounds which are pretty much unaffected by natural degradation in treatment lagoons (Conkle et al., 2009).

Thermal tolerant coliforms and/or *Escherichia coli* are commonly used to evaluate and regulate the levels of fecal pollution from storm water discharge. Because storm sewers systems collect surface runoff, non-human sources can contribute significantly to fecal indicators such as thermotolerant coliforms and *E. coli*. Storm water discharges can increase the loads of indicators such as *E. coli*. by up to 3–4 log units in receiving waters as compared to background levels measured in dry weather, while median concentrations only increased by about 76% (Aström et al., 2007, 2009) and can contribute significantly to the stream loadings of pathogens such as *Cryptosporidium*, *Giardia* and norovirus (Rechenburg et al., 2006; Aström et al., 2009). More human specific tracers would be extremely useful to establish the sources of contamination and to control them.

Our primary objective was to monitor the levels of a thermotolerant coliforms, caffeine, and carbamazepine in various sections of the storm water collection systems in an urban environment located on the Island of Montréal. The secondary objective was to establish if chemical and microbial indicators could serve as anthropogenic markers in dry weather to assist in identifying potential cross connection in this storm water collection systems.

2. Materials and methods

2.1. Experimental designs

Samples were collected from small streams, brooks, collectors and storm sewer outfall pipes from the storm water system across the Island of Montréal (City of Montreal, QC, Canada) – Fig. 1. The 120 individual samples were collected based on previous surveys so as to: (1) target sites with suspected or confirmed fecal contamination and (2) include a range of fecal coliform densities enabling us to establish whether or not caffeine or carbamazepine could be correlated with thermotolerant coliforms. The samples were collected in June 2008 and October 2008. Samples were taken during wet weather (40) as defined by >15 mm of rain in the 24 h prior to sampling (10/22 – 17 mm, 10/27 – 25&40 mm, 10/28 – 21 mm) and dry weather (80) as defined by <2 mm 24 h prior to sampling (10/05 – 06, 10/20 – 22). All samples were kept at 4 °C and processed for fecal coliforms within 24 h and for caffeine and carbamazepine within 7 d (Aboulfadl et al., 2010) using online solid phase extraction coupled to liquid chromatography and tandem mass spectrometry (SPE-LC–MS/MS).

2.2. Fecal coliforms

Samples were analyzed by the Laboratory of the City of Montreal using Standard Methods 9222 for the detection and enumeration of fecal coliforms on mFC medium by membrane filtration (AWWA, 2005).

2.3. Chemical analysis

We used an automated solid phase extraction coupled to liquid chromatography and tandem mass spectrometry technique (SPE-LC–MS/MS) based on a method for a larger group of compounds which was slightly modified to accelerate the analysis (Viglino et al., 2007). The compounds were identified by retention-time and by their specific SRM transitions at their respective *m*/*z* ratios. The two most intense transitions were selected for each compound: one for the quantification and another for qualitative confirmation. Standard solutions used for quantification were also preconcentrated using the same procedure as the samples. Before each analysis, an internal standard (IS) was added to correct for variations in sample recovery and instrumental performance. Methanol blanks were also injected after every real sample to clean the columns. The peak areas of analytes were normalized to those of the IS. Five specific concentrations ranging from 0 to 100 ng L^{-1}, with a fixed 70 ng L^{-1} of isotope-labeled internal standard, were injected to build up a calibration curve (R^2 values were at least 0.988). Areas of the analytes and IS were calculated by the LCquanTM 2.5 software (Thermo Fisher Scientific). Limits of detection were estimated as three times the standard deviation of 5 replicates measurements of a real sample and were 9 ng L^{-1} for caffeine and 0.2 ng L^{-1} for carbamazepine. Recoveries in real sample ranged from 87% to 110% and blanks were below or close to our detection limits (see {Viglino, 2008} for details).

All samples were analyzed in duplicates. Water blanks (using HPLC water – Baker (Quebec, Canada)) were included every ten samples. The SPE-LC–MS/MS system uses Thermo Fisher EQUan system, including a six-port switching valve to control an analysis consisting in 1.0 mL injection of sample through a 1-mL sample loop at 1 mL min^{-1} onto a preconcentration column (C18, 12-μm, Hypersil GOLD™ column (octadecyl carbon (C18) bounded silica), 20 mm × 2.1 mm i.d) using a Surveyor LC-Pump (Thermo Fisher Scientific). The pre-concentration column is then washed by flushing at 1 mL min^{-1} with a water/formic acid solution at pH 2.6 for 1.4 min. The valve is then switched to use a Surveyor MS Pump Plus (Thermo Fisher Scientific) to back flush the loading column at 200 μL min^{-1} with 0.1% formic acid–methanol (95:5, v/v) directly into the analytical chromatography column (C18, 3-μm Hypersil GOLD™ column, 50 mm × 2.1 mm i.d. – Thermo Fisher Scientific) which is preceded by similar guard column (2 × 2 mm, 5 μm). The chromatography gradients are detailed in Table 1.

A TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MS, USA) tandem triple quadrupole mass spectrometry fitted with an electrospray ionization source was used for detection. The instrument was operated in positive ionization mode and was directly coupled to the HPLC system at a flow rate 200 μL min^{-1}. Sample analysis was performed in the selective reaction monitoring mode (SRM) – we used for caffeine: SRM 195.0→138.0 with a collision energy of 19 and tube lens of 60

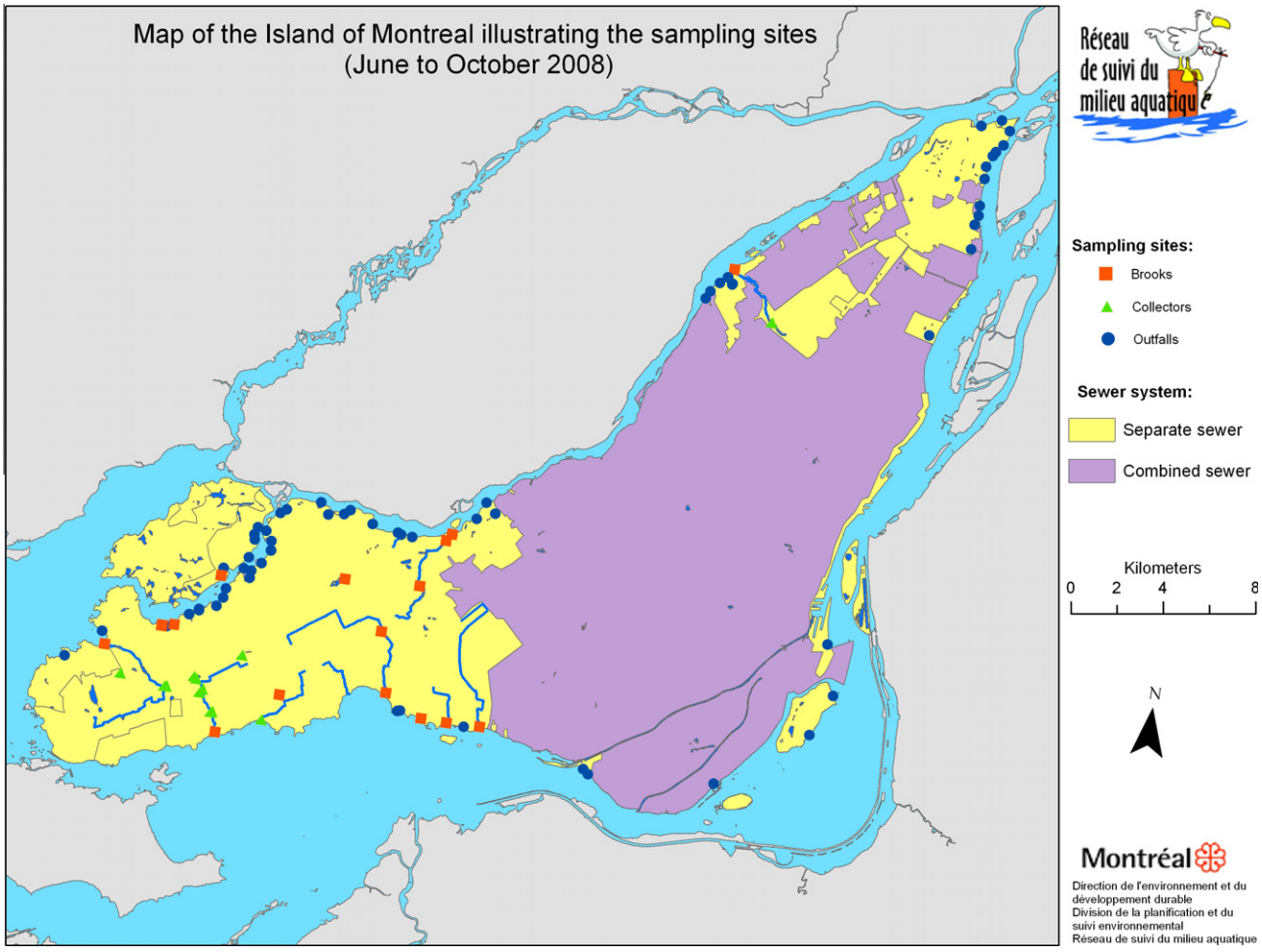

Fig. 1. Map of the Island of Montréal illustrating the sampling sites.

Table 1
Sample pre-concentration program used for the on-line SPE-LC–MS/MS.

Step	Time (min)	Flow rate (mL min^{-1})	Event
LC-pump gradient			
1			Sample preconcentration washing with methanol
2			Conditioning precolumn with Water/F.A pH2.6
3			
Time	Water/F.A (0.1%)	MeOH (%)	Flow rate (μL min^{-1})
MS-pump gradient			
0	95	5	200
2	95	5	200
4	1	99	200
6	1	99	200
7	95	5	200
10	95	5	200

and for carbamazepine: SRM 237.1 → 194.1 with a collision energy of 17 and a tube lens of 70. System control and data acquisition were performed with the Analyst Xcalibur software (rev. 2.0 SP2, Thermo Fisher Scientific).

Three of the samples were below the detection limit for carbamazepine; in those cases we have used half of the detection limit (0.1 ng L^{-1}) as the value for the figures and the statistical analysis.

3. Results and discussion

As the data for the measured parameters span several orders of magnitude, logarithmic transformations were used throughout. Fig. 2 presents the data regrouped per collection system component namely underground collection pipes (collectors), open brooks and discharge points to the river (outfalls). Concentrations of chemical tracers and thermotolerant coliforms confirm a widespread contamination of urban storm waters by wastewater. As expected, caffeine and fecal coliforms concentrations are lowest in urban brooks in which minimal flow is always maintained and provides some dilution, and highest in collectors and outfalls. No clear trend is noted for carbamazepine as concentrations show a systematic but more modest level of contamination across the system with occasional spikes that may be indicative of localized contributions (Fig. 3). Other studies have shown that dry conditions in urban watersheds could be related to higher tracer levels given that high

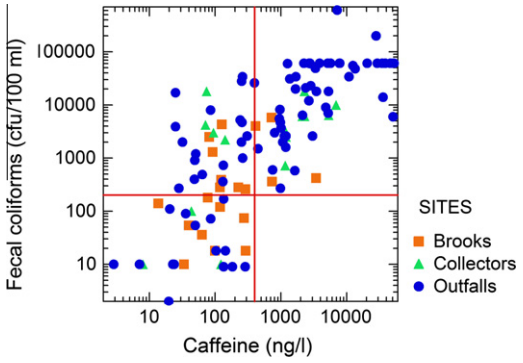

Fig. 2. Fecal coliform counts as a function of caffeine concentrations, $R^2 = 0.558$. The red lines represent an arbitrary threshold limits for fecal coliforms at 200 cfu 100 mL^{-1} and the tracer at 400 ng caffeine L^{-1}.

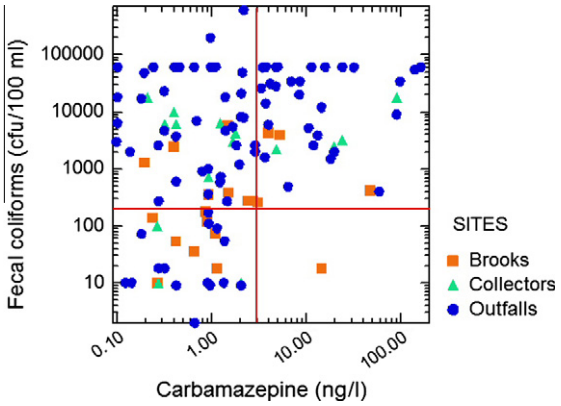

Fig. 3. Fecal coliforms counts as a function of carbamazepine concentrations, $R^2 = 0.072$. The red lines represent an arbitrary threshold limits for fecal coliforms at 200 cfu 100 mL^{-1} and the tracer at 3 ng carbamazepine L^{-1}.

Table 2
Concentrations of fecal coliform bacteria, caffeine and carbamazepine per component of the stormwater collection system.

Brooks $n = 20$	Fecal coliforms (100 mL)	Caffeine (ng L^{-1})	Carbamazepine (ng L^{-1})
Brooks n = 20			
Min	10	13.7	0.2
Max	5800	3400	47
Median	270	121	1.1
Mean	1027	367	4.4
Collectors n = 15			
Min	10	8.0	0.2
Max	18000	6800	90
Median	3200	1164	1.2
Mean	5369	1529	9.8
Discharge outfalls n = 85			
Min	2	2.9	0.1
Max	610000	53000	161
Median	6000	1066	1.4
Mean	29297	6264	9.9

rains lead to more dilution from added water than increase from combined sewer overflows (Benotti and Brownawell, 2007). Table 2 summarizes the values observed. The comparison between the three components of the storm water collection system shows about a 1 log increase in the mean concentration of thermotolerant coliforms from the lowest at 275 cfu/100 mL measured in brooks to 1600 and 3600 cfu/100 mL in collectors and outfalls.

Fig. 2 shows the modest correlation ($R^2 = 0.56$) found between the concentrations of caffeine and thermotolerant coliform bacteria counts. Considerable spread is notable at low concentrations of caffeine and coliform bacteria. However, caffeine concentrations above 400 mg L^{-1} are systematically found in samples containing coliform counts above 200 cfu 100 mL^{-1}. However, some samples containing less than 400 ng L^{-1} of caffeine may contain low levels of fecal coliforms while other exhibit higher levels of fecal coliforms. The absence of a clear relationship between fecal indicators and persistent pharmaceuticals may either be the result of coliform die-off or be attributed to contributions from non-human fecal sources. Several sites sampled are along recreational parks where domestic animals such as dogs or wild fauna (birds and raccoons) could contribute fecal coliforms without any caffeine inputs. These observations reinforce the notion that caffeine in surface waters is a more specific indicator of contamination by probable sanitary sewage discharge than fecal coliforms, as proposed by others for a much smaller dataset ($n = 3$) (Wu et al., 2008). The overall observed correlation over the same range of caffeine and coliforms than in this study is better than the reported correlation for three Baltimore watersheds (Young et al., 2008) where the R^2 values varied between 0.16 and 0.37.

In our dataset, of the 120 samples that we analyzed, 93 exceeded 200 cfu 100 mL^{-1} and 32% of those had less than 400 ng L^{-1} of caffeine. It must be emphasized that we deliberately included a large proportion of sites suspected of sanitary contamination so as to evaluate if the chemical tracers could actually identify those sites. Those proportions do not in any way represent actual occurrence of contaminated sites on the Island of Montréal. Nevertheless, this dataset suggests that a water sample shown to contain more than 400 ng L^{-1} of caffeine, has a 100% chance of being contaminated with more than 200 cfu 100 mL^{-1} of fecal coliforms, the standard reference value for bathing and recreational use applicable in the Montreal area (but the U.S. EPA equivalent is 235 cfu 100 mL^{-1} – (USEPA, 1986)). At the other end of the spectrum, 30 out of 57 samples (53%) below the 400 ng caffeine L^{-1} threshold had more than 200 cfu 100 mL^{-1}. This would suggest that the presence of a significant concentration of caffeine is an excellent indicator of the presence of fecal coliforms but there might be other sources of fecal coliforms for which caffeine would not be a good tracer and which would not be picked up by caffeine. Agricultural activities could certainly be a major caffeine-free contributor but there are very few sites where agriculture could be a significant contributor on the Island of Montréal.

Fig. 3 shows that the data for carbamazepine are more dispersed with no clear correlation with fecal coliform bacteria ($R^2 = 0.072$) or caffeine ($R^2 = 0.004$). In this situation, carbamazepine is not a useful tracer of fecal coliforms. The same arguments for non-sanitary sources of fecal coliforms apply to carbamazepine, but in this case it is also possible that in some cases, anthropogenic inputs of fecal coliforms were not systematically accompanied by carbamazepine. Carbamazepine is prevalent in wastewater-impacted environments and very persistent (Conkle et al., 2009). As it is mainly used to treat patients with seizure disorders such as epilepsy, it is clearly not as widely used in a population as caffeine, resulting in localized discharges in the collection system from the limited households including such patients. On the other hand, households in which coffee, tea, colas or chocolate are not consumed are few. In the case of small sub basins such as the ones studied here, relying on the concentrations of a persistent but not widely used compound may not be applicable. The higher K_{ow} of carbamazepine might also contribute to a better retention to sediments and particulates, thus in some cases, this might reduce its concentrations below the threshold. Inversely, its exceedingly low degradation rate might also in some cases lead to the disappearance of the coliforms before the carbamazepine was actually eliminated. These results are in

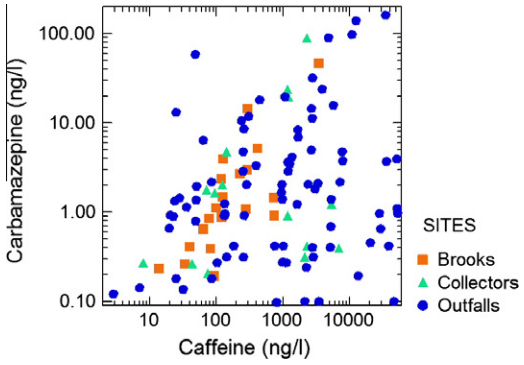

Fig. 4. Carbamazepine as a function of caffeine concentrations.

contrast with those of (Young et al., 2008) who compared caffeine with triclosan and triclocarban as tracers of microbial indicators. They observed that the compounds which more readily sorbed to particulate organic carbon (i.e. triclosan and triclocarbon) were better correlated to microbial indicators with reported R^2 of 0.45 to 0.55 whereas for caffeine they reported R^2 values in the range 0.16 to 0.37. Thus, in selecting a suitable chemical tracer of fecal contamination, it is necessary to consider its sources, fate and transport characteristics and it might be useful to test tracers with more polar characteristics, less prone to adsorb onto sediments and particles. In our dataset, the correlation between caffeine and carbamazepine is very weak (Fig. 4).

Various approaches and indicators have been proposed for microbial source tracking. Isolates for antibiotic resistance have been proposed as a means of differentiating fecal coliforms of human vs. animal origin, presuming that resistance to pharmaceuticals ought to be related to human coliforms (Field and Samadpour, 2007). Unfortunately, tests for wild animals in urban environments have shown no significant differences in their antibiotic resistance (Souza et al., 1999). Another potential means of differentiating the origin of the fecal coliforms would be to evaluate the fecal sterol profiles along with that of caffeine (Suprihatin et al., 2003). This might help to determine whether the false positives (i.e., low caffeine, high fecal coliforms) are related to seemingly animal and non-human fecal sources. Various PCR-based methods might be able to differentiate the fecal coliforms coming from human versus those of animal origins (Field and Samadpour, 2007), but a promising option could be to use a PCR-based method for the detection of animal epithelial cells (Kortbaoui et al., 2009). Such an approach would in theory allow the exact determination of the species of origin for the contamination found in the high coliform/low caffeine samples (in reality this forensic approach is not easily put into place). Even an improved and simplified PCR-based approach would only make sense to validate the caffeine tracer; it would be unpractical as a means of detection of cross-connections, given the high labor, expertise and costs involved.

4. Conclusions

The water samples we collected show a widespread contamination by human-based tracers and fecal coliforms across this urban environment. Caffeine and carbamazepine concentrations in surface brooks, streams, stormwater collectors and discharge points were correlated to fecal coliform counts with an R^2 value of 0.55 for caffeine and nearly no correlation with carbamazepine (R^2 of 0.07). Setting an arbitrary threshold value of 400 ng caffeine L^{-1}, it is possible to identify samples systematically contaminated with fecal coliforms above an arbitrary level of 200 cfu/100 mL for fecal coliforms (corresponding to the threshold used by the applicable Environmental Ministry regulations). A lower concentration of caffeine is not necessarily indicative of the absence of coliforms, as is expected due to background contamination from other sources or coliform die-off. Carbamazepine does not seem to be a useful coliform tracer and this might be due in part to its higher K_{ow}, lower degradability, affinity for solids or differences in prevalence of use. It would be useful to integrate more polar tracers to see if they would outperform caffeine. Further research efforts could certainly be combined with a PCR-based approach to confirm the sources of the fecal coliforms.

It is nevertheless clear from this data that any water sample containing more than 400 ng caffeine L^{-1} can be considered contaminated with fecal coliforms above the 200 cfu 100 mL^{-1} threshold. This sort of analysis could be a useful forensic tracer for the detection of cross-connected sewers, or for other tracing purposes of the sources of sanitary contamination. But given the high levels of variability and uncertainty, this should not be considered a definitive proof and would remain indicative until confirmed through other means. Nevertheless, a caffeine sampling program would be relatively easy to implement and might provide a useful tool to identify sanitary contamination sources and help reduce surface water contamination within a urban watershed.

In a confined urban watershed with relatively short residence time (<4 h), the definition of a threshold of caffeine or another chemical tracer appears preferable to the interpretation of levels of *E.* coli or fecal coliforms. Background levels likely to be found in these urban systems without any wastewater discharges should be very low, equivalent to those found in drinking water or less (which are around 20 ng caffeine/L and ~0.2 ng carbamazepine/L on the Island of Montréal (Garcia-Ac et al., 2009).

Equivalent contamination was observed in both dry (<2 mm in previous 24 h) and wet periods (>17 mm in previous day) in all components of the system (data not shown). The presence of elevated concentrations of caffeine and carbamazepine in dry periods is certainly indicative of cross connections and the observed concentrations in the presence of low dilutions could be used to estimate the discharged volumes (Conkle et al., 2009). The situation in wet weather may be more complex as it includes ongoing contributions from cross connections, scouring of accumulated sediments and potential sewage overflows.

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