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SOURCES DE CONTAMINATION FÉCALE DES COURS D'EAU URBAINS

MARIAM HAJJ-MOHAMAD

DÉPARTEMENT DES GÉNIES CIVIL, GÉOLOGIQUE ET DES MINES

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

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M. ZAGURY Gérald, Ph. D., président

Mme DORNER Sarah, Ph. D., membre et directrice de recherche

Mme PRÉVOST Michèle, Ph. D., membre et codirectrice de recherche

M. SAUVÉ Sébastien, Ph. D., membre et codirecteur de recherche

M. COMEAU Yves, Ph. D., membre

M. CABANA Hubert, Ph. D., membre

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RÉSUMÉ

Les contaminations fécales des cours d'eau urbains proviennent surtout de raccordements inversés et de débordements d'égouts unitaires ou sanitaires. Les impacts cumulatifs de ces rejets sur le milieu récepteur sont devenus évidents et méritent de mieux préciser leur origine. Aucune méthode de la boîte à outils du dépistage des sources de contamination fécale dans les secteurs urbains ne représente une solution miracle acceptée comme la meilleure méthode. La combinaison de plusieurs indicateurs dans le but de développer un indice sanitaire est au point pour faire éventuellement l'objet d'une normalisation.

L'objectif principal de ce projet de recherche est de dépister les sources de contamination fécale dans les cours d'eau urbains. Au regard du projet, sept objectifs ont été ciblés. Le premier objectif était de développer et valider une méthode de mesure de dix indicateurs chimiques anthropogènes (acétaminophène (ACE), diclofénac (DIC), carbamazépine (CBZ), aténolol (ATL), caféine (CAF), théophylline (THEO), progestérone (PRO), medroxyprogestérone (MedP), aspartame (APM) et N,N-diéthyl-3-méthylbenzamide (DEET)) dans des matrices complexes telles que les eaux et les sédiments des trop-pleins et des cours d'eau urbains. Avec le deuxième objectif, on cherchait à évaluer l'importance de l'analyse des indicateurs chimiques (ACE, CBZ, CAF et THEO) dans les sédiments par rapport à leur analyse dans l'eau afin de dépister les sources de contamination fécale dans des sites à différents degrés de contamination. Le troisième objectif était d'illustrer le rôle de sédiments des trop-pleins dans le devenir et le transport des contaminants émergents (ACE, CAF, THEO, CBZ et 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH))) lors d'événements pluvieux importants ou lors d'importantes fontes de neige en étudiant leurs phénomènes de sorption dans des microcosmes (eau : sédiments de trop-plein) par la méthode d'équilibres successifs. Le quatrième objectif était de déterminer les indicateurs de contaminations fécales par des raccordements inversés, les plus appropriés parmi des indicateurs chimiques et moléculaires spécifiques aux humains. Le cinquième objectif était d'évaluer les relations possibles entre les indicateurs ciblés pour le dépistage des sources de pollution microbienne (microbiologiques (*E. coli* et coliformes fécaux), chimiques (ACE, CAF, THEO et CBZ) et moléculaires (marqueurs ciblant les *Bacteroidales* (HF183) et l'ADN mitochondrial (Hmt) spécifiques aux humains)) dans des bassins versants à activités différentes par des analyses statistiques comparatives. Le sixième objectif était de

valider le seuil de la caféine (400 ng L^{-1}) proposé dans la littérature pour la priorisation des secteurs problématiques des réseaux d'assainissement séparés, touchés par des raccordements inversés. Il s'agit d'une étude de cas portant sur deux bassins versants de la grande région de Montréal où les égouts sanitaires et les égouts pluviaux sont distincts. On cherchait à déterminer l'origine probable de la contamination fécale humaine observée dans les différents échantillons prélevés. Finalement, le septième objectif était de proposer des valeurs seuils de référence des indicateurs alternatifs ciblés afin d'établir un indice de contamination fécale d'origine humaine par des raccordements inversés et ainsi de prioriser les travaux de réfection du système de drainage le plus problématique.

Le premier objectif a permis de développer une nouvelle méthode d'analyse multi-résidus des contaminants émergents (indicateurs chimiques) dans deux matrices différentes, les sédiments de trop-pleins et de rivières, permettant d'en diminuer les seuils de détection et d'en augmenter la sensibilité. Cette méthode est basée sur l'ultra-chromatographie en phase liquide à haute performance (UHPLC) couplée à la spectrométrie de masse en tandem via la source d'ionisation chimique à pression atmosphérique (APCI) après une étape d'extraction sur phase solide assistée par ultrasons. En se basant sur des critères de consommation, de présence dans l'environnement, d'écotoxicité, dix molécules ayant une large gamme de propriétés physico-chimiques (log K_{OW} allant de -0,02 à 4,9) et appartenant à différentes classes thérapeutiques y compris un anti-épileptique (CBZ), un analgésique (DIC), un anti-inflammatoire (ACE), un stimulant (CAF), un bronchodilatateur (THEO), un ß –bloquant (ATL), deux progestogènes (PRO et MedP), un édulcorant artificiel (APM) et un produit de soin personnels (DEET) ont été étudiées. Des échantillons de sédiments/boues ont été lyophilisés, tamisés en particules de dimension inférieure à 80 µm et dopés avec le mélange des composés sélectionnés. L'extraction a été assistée par ultrasons, suivie d'une étape de purification et de pré-concentration. Les paramètres d'extraction et de purification tels que le nombre de cycles, les solvants d'extraction, le temps et la température de sonication ont été adéquatement optimisés. Concernant l'étape de purification et de concentration, deux types de cartouches (C18 et Oasis HLB) ont été testés tout en appliquant l'extraction sur phase solide (SPE, solid phase extraction) afin d'obtenir une meilleure détection à l'UHPLC/APCI/MS/MS. Les conditions optimales (extraction en cinq cycles par ultrasons combinés avec une étape de nettoyage sur cartouche d'extraction de type Oasis HLB) engendrent des taux de recouvrement qui varient entre 75 et 156 % avec une précision de 10 à 23 %. Les

limites de détection pour les 10 analytes dans les sédiments variaient de 0,01 à 15 ng g^{-1}. Les concentrations les plus élevées de tous les indicateurs excepte DEET ont été observées dans les échantillons des boues de trop-pleins.

Le deuxième objectif à savoir l'intérêt d'analyser les compartiments solides des cours d'eau a permis de mettre en évidence le rôle de sédiments dans les programmes de suivi de la qualité de l'eau, particulièrement pour les cours d'eau où les sources de contamination sont exposés à un niveau élevé de dilution. Dans ces derniers cas, le ratio de la concentration moyenne de l'indicateur chimique sur sa limite de détection (C : LOD) était plus élevé dans les sédiments que dans la colonne d'eau. Par ailleurs, l'analyse des composés testés (ACE, CAF, THEO et CBZ) serait utile dans la colonne d'eau ayant un potentiel de dilution moindre et des sources multiples de rejets pour identifier les sources de contamination fécale humaine.

Le troisième objectif à savoir le rôle des dépôts du trop-plein dans les variations intrinsèques des contaminants émergents (ACE, CAF, THEO, CBZ et CBZ-DiOH) lors d'épisodes de forte pluie ou de fonte des neiges a permis de constater que la dilution par les eaux de ruissellement durant le débordement d'égout unitaire n'est pas assez importante pour que les concentrations rejetées des contaminants restent élevées. Ces contaminants proviennent majoritairement des eaux usées et de la remise en suspension des dépôts d'égout unitaire. De plus, nos résultats démontrent que l'augmentation du contenu organique favorise la sorption de ces composés. Les valeurs de coefficients de distribution solide-eau étaient plus élevées dans Suspended Sediments (SS, matrice très riche en matière organique) que dans Settled Sediments (StS, moins riche en matière organique) ainsi que dans les échantillons dopés par rapport aux échantillons non-dopés. Une vision presque biaisée de la sorption des contaminants a été mise en évidence par le dopage du système eau/solide.

Le quatrième et le cinquième objectifs ont permis de déduire que les indicateurs bactériens classiques comme *Escherichia coli* ne permettent pas une différenciation de l'origine de la pollution fécale dans les eaux du bassin versant urbain doté de réseaux d'égout pluvial et sanitaire séparés. Également, aucun des marqueurs alternatifs ne se révèle suffisant pour déterminer à lui seul l'origine de la pollution fécale. Au moins deux paramètres combinés ont démontré une plus grande efficacité. Le marqueur *Bacteroides* HF183 spécifique à l'humain (100% de précision de classification) et la caféine (75% de précision de classification) ont été les marqueurs les plus

sensibles pour prédire les concentrations de *E. coli* au-dessous et au-dessus du seuil de 235 UFC ou MPN 100 mL^{-1} dans des eaux par temps sec dans les égouts pluviaux, respectivement. Du fait de la présence d'une corrélation significative entre les concentrations des marqueurs alternatifs spécifiques utilisés et les coliformes fécaux, les combinaisons seraient optimales en comportant des marqueurs alternatifs spécifiques (HF183, Hmt, CAF, THEO et ACE) et un indicateur classique de contamination fécale.

L'utilisation de ces marqueurs alternatifs spécifiques serait utile dans les bassins versants ayant un potentiel des sources multiples de contamination fécale dont le secteur résidentiel est un bon exemple. Des différences significatives ont été trouvées entre les différents secteurs étudiés (résidentiel contre industriel /commercial/institutionnel), ce qui suggère l'adoption de différentes stratégies de surveillance des raccordements inversés (le moment idéal, la durée et les méthodes d'échantillonnage, etc.) dans ces secteurs variés.

Le sixième et le septième objectifs ont permis de proposer des valeurs seuil sur les marqueurs alternatifs étudiés (3 Log pour HF183, 2 Log pour Hmt, CAF et THEO, 1 Log pour ACE et -0.3 Log pour CBZ) afin d'identifier les raccordements inversés. Pour la classification des sites, on a proposé un nouvel indice multiparamétrique qui prend en compte l'ensemble des marqueurs alternatifs spécifiques étudiés. Pour un indice supérieur à 0,6, la contamination fécale est exclusivement d'origine humaine via des raccordements inversés. Parmi les sites étudiés ayant des concentrations élevées en *E. coli*, 50% ont montré un indice sanitaire élevé.

Finalement, l'ensemble des résultats obtenus dans le cadre de ce projet de recherche a permis d'établir des recommandations sur la surveillance des cours d'eau urbains ainsi que sur la gestion des rejets du système d'égouts. La mise en place d'un programme d'échantillonnage efficace incluant les compartiments aqueux et solides et l'utilisation des techniques d'analyse sensibles et reproductibles des indicateurs de contamination sont nécessaires pour accomplir le meilleur dépistage des sources de contamination fécale humaine. La remise en suspension des dépôts contaminés dans les systèmes d'égouts devrait être limitée par des travaux de curage et de nettoyage. De plus, le lavage hebdomadaire des rues permettrait de diminuer l'accumulation des dépôts dans les conduites. La combinaison des marqueurs spécifiques de la contamination fécale humaine incluant des marqueurs chimiques et moléculaires devrait être réalisée afin d'optimiser l'approche de dépistage des sources. L'utilisation combinée de différents marqueurs spécifiques

aux humains et aux animaux dans les zones résidentielles, permettrait la discrimination de nombreuses sources potentielles de pollutions fécales (humaine, animale, ou provenant d'hôtes plus spécifiques (par exemple chat, chien, etc.). Les secteurs problématiques du réseau d'assainissement une fois identifiés et hiérarchisés, des investigations poussées impliquant les propriétaires devraient être menées afin d'identifier les raccordements problématiques spécifiques.

ABSTRACT

The fecal contamination of urban streams mainly originates from sewer cross-connections, stormwater and combined sewer overflows. The cumulative impacts of these discharges onto the receiving environment have become strikingly evident and warrant further clarification regarding their origins. No method within the current toolbox for fecal contamination source screening in urban areas presents a miraculous solution accepted as the best method. The combination of multiple indicators in order to develop a sanitary index could eventually become the object of standardization.

The main goal of this research project is the identification of fecal sources of contamination in urban streams. As such, seven specific objectives have been set. The first was the development and validation of an analytical method for ten anthropogenic chemical indicators (acetaminophen (ACE), diclofenac (DIC), carbamazepine (CBZ), atenolol (ATL), caffeine (CAF), theophylline (THEO), progesterone (PRO), medroxyprogesterone (MedP), aspartame (APM), and N,N-diethyl-3-methylbenzamide (DEET)) in complex matrices such as the waters and sediments of combined sewer systems and urban streams. With the second objective, our goal was the evaluation of the importance of chemical indicator analyses (ACE, CBZ, CAF, and THEO) in sediments versus their analysis in water for fecal source tracking at sites having varying degrees of contamination. The third objective was to illustrate the role of combined sewer sediments in the fate and transport of emerging contaminants (ACE, CAF, THEO, CBZ, and 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH)) during precipitation or snowmelt events by studying the sorption phenomenon in microcosms using a batch equilibrium method. The fourth objective was the establishment of the most suitable fecal contamination indicators by sewer cross-connections amongst chemical and molecular human-specific indicators. The fifth objective was the evaluation by comparative statistical analysis the potential correlations between the target fecal indicators (microbiological (*E. coli* and fecal coliforms), chemical (ACE, CAF, THEO, and CBZ) and molecular (markers targeting human-specific *Bacteroides* (HF183) and mitochondrial DNA (Hmt))) in watersheds with different anthropogenic activities. The sixth objective was the validation of the previously suggested caffeine threshold (400 ng L^{-1}) for the prioritization of problematic sectors in the separate sewer systems that are affected by cross-connections. It is a case study involving two watersheds in the Greater Montreal area, where the sanitary and storm

sewers are separate. We sought to identify the probable origin of the human fecal contamination in a variety of collected samples. Finally, the seventh objective was the suggestion of reference threshold values of alternative indicators to establish a sanitary index for the prioritization of remedial work on the most problematic drainage sectors.

The first objective has enabled the development of a new multi-residue analytical method for the determination of emerging contaminants in two different matrices, the combined sewer sludge and stream sediments, which results in the decrease of detection limit and the enhancement of its sensibility. This method is based upon ultra-sonication-assisted extraction and ultra-high performance liquid chromatography (UHPLC)-tandem mass spectrometry operating in positive atmospheric pressure chemical ionisation mode (APCI). Based on their consumption, their presence in the environment, and their ecotoxicity criteria, ten molecules having a wide range of physico-chemical properties (log K_{OW} within a range from -0.02 to 4.9) and belonging to different therapeutic classes including an anti-epileptic (CBZ), an analgesic (DIC), an anti-inflammatory (ACE), a stimulant (CAF), a bronchodilatator (THEO), a ß –blocker (ATL), two progestogens (PRO and MedP), an artificial sweetener (APM) and a personal care product (DEET) were studied. Sediments/sewage sludge samples were lyophilized, screened (particles dimension is below of 80 μm) and subjected to spiking with a mixture of selected compounds. The extraction was assisted by ultra-sonication followed by solid-phase extraction cleanup. We optimized the purification and extraction parameters such as the number of extraction cycles, the extraction solvents, and the time and temperature of sonication. Two solid-phase extraction (SPE) cartridge types (C18 and Oasis HLB) were tested to obtain the best detection by UHPLC/ACPI/MS/MS. The optimal conditions (five ultrasonic extraction cycles combined with Oasis HLB cartridge cleanup step) generate recovery rates ranged from 75 to 156% with relative standard deviations (RSD) of less than 23% for all compounds in stream as well as combined sewer overflow sediment samples. The detection limits for 10 analytes in sediments ranged from 0.01 to 15 ng g^{-1} dry weight (dw) for the compounds of interest. The highest concentrations of all indicators except DEET were observed in sludge samples.

The second objective, based on the interest of solid phase stream analysis, was to highlight the role of sediment in the water quality monitoring programs, especially for rivers where contamination sources are exposed to high dilution levels. In such cases, the ratio of the average concentration of the chemical indicator on its detection limit (C: LOD) was higher in the

sediment than in the water column. Furthermore, analysis of tested compounds (ACE, CAF, THEO, and CBZ) would be useful in the water column of stream with a lower dilution and multiple potential discharge sources, to identify human faecal contamination sources.

The third objective, based on the role of the combined sewer deposits in the intrinsic variations of emerging contaminants (ACE, CAF, THEO, CBZ, and CBZ-DiOH) during heavy rain or melting snow events revealed that the dilution by stormwater runoff is not important enough during combined sewer overflow that discharge concentrations of contaminants remain high. These contaminants come mainly from wastewater and the resuspension of combined sewer deposits. Furthermore, our results demonstrate that increasing organic content promotes the sorption of these compounds. The solid-water distribution coefficient values were higher in suspended sediments (SS, high organic matter content) than in settled sediments (StS, low organic matter content) and in spiked samples over native samples. Results showed the importance of working with native sediments as spiking of the water/solid system could lead to important biases.

The fourth and fifth objectives indicated that the traditional bacterial faecal indicators such as *E. coli* do not allow a distinction of the origin of fecal pollution in watersheds with separated sewer systems. Also, none of the alternative markers has proved sufficient to determine alone the origin of fecal pollution. At least two combined parameters have shown a greater efficiency. The human-specific HF183 *Bacteroides* 16S rRNA genetic marker (100% classification accuracy) and caffeine (75% classification accuracy) were the most sensitive markers to predict the *E. coli* concentrations below and above the threshold of 235 UFC or MPN 100 mL^{-1} in dry weather water runoff in storm sewers, respectively. Due to the presence of a significant correlation between the concentrations of human specific alternative markers and fecal coliforms, the combinations comprising specific alternative markers (HF183, Hmt, CAF, THEO and ACE) and a classical contamination fecal indicator, are optimal.

Using these alternative specific markers would be useful for watersheds with multiple potential faecal contamination sources which the residential sector is a good example. Significant differences were found between the various sectors (residential versus industrial/commercial/institutional), suggesting the adoption of different monitory strategies for the identification of sewer cross-connections (the ideal timing, duration and sampling methods, etc.) in these various sectors.

The sixth and seventh objectives allowed to propose threshold values on the human specific alternative markers (3 log for HF183, 2 Log for Hmt, CAF, and THEO, 1 Log for ACE, and -0.3 log for CBZ) to identify cross-connections. For the classification of sites, we proposed a new multi-index which takes into consideration all the specific alternative markers. For an index greater than 0.6, fecal contamination is exclusively from human via cross-connections. Among the sites with high concentrations of *E. coli*, 50% showed a high sanitary index.

Finally, all the results obtained in the framework of this research project generated recommendations for urban streams supervision and sewer discharge management tools. The establishment of an efficient sampling program including aqueous and solid compartments and the use of sensitive and reproducible analysis techniques for fecal indicators are needed to do the best fecal contamination source tracking. Resuspension of contaminated deposits in sewer systems should be limited by flushing and cleaning work. In addition, the weekly washing of streets would reduce the accumulation of deposits in pipes. The combination of specific markers of human fecal pollution including chemical and molecular markers should be performed to optimize the source tracking approach. The use combining different human and animal specific markers in residential areas, allow the identification of many potential sources of fecal pollution (human, animal, or from specific hosts (*e.g.*, cat, dog, etc.). Once sectors are identified and prioritized, additional investigations involving property owners could be undertaken to identify the specific problematic connections.

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ACE	Acetaminophen/Acétaminophène
ADBI	Célestolide
ADN	Acide désoxyribonucléique
ads	Adsorption
AGI	Maladie gastro-intestinale aiguë
AHTN	Tonalide
ANC	Actives non cultivables
APCI	Ionisation chimique à pression atmosphérique
APHA	American Public Health Association
APM	Aspartam
ARN	Acide ribonucléique
app	Apparent
ATL	Atenolol
AWI	Anthropogenic waste indicator
CAF	Caffeine/Caféine
CBZ	Carbamazepin/Carbamazépine
CBZ-DiOH	10,11-dihydro-10,11-dihydroxycarbamazepine
Cd	Cadmium
CEC	Capacité d'échange cationique
CFU	Colony forming units
CID	Collisioninduced dissociation
C_{sed}	Sediment concentration in Settled Sediment
CSO	Combined sewer overflow
CSS	Combined sewer system
C_{SS}	Sediment concentration in Suspended Solid
CTL	Citalopram
Cu	Cuivre
$C_{W, Sed}$	Aqueous Concentration in Settled Sediment
$C_{W, SS}$	Aqueous Concentration in Suspended Solid
DAD	Diode-array detector
DBO_5	Demande biochimique en oxygène pendant 5j

DCF	Diclofenac
DCO	Demande chimique en oxygène
DEET	N,N-diethyl-3-methylbenzamide
des	Desorption
DL	Detection limit
DOCA	Acide deoxycolique
DOM	Dissolved organic matter
D_{OW}	Distribution coefficient, octanol-water
DSPM	Dépistage des sources de pollution microbienne
DW	Dry weather
dw	Dry weight
DZP	Diazépame
E1	Estrone
E2	17β-Estradiol
E. coli	*Escherichia coli*
EE2	17α-Ethinylestradiol
ERY	Erythromycine
ESI	Electrospray ionization
FC	Coliformes fécaux
FIB	Fecal indicator bacteria
FLX	Fluoxétine
f_{OC}	Teneur en matière organique
FS	Streptocoques fécaux
FT	Coliformes totaux
FWAs	Agents fluorescents d'avivage
GenBac	General *Bacteroidales*
HAP	Hydrocarbures aromatiques polycycliques
HCT	Hydrocarbure totaux
HF183	ARN ribosomal 16s de *Bacteroides* spécifique à l'homme
HHCB	Galaxolide
Hmt	ADN mitochondrial spécifique à l'homme
IBP	Ibuprofène
ICI	Industrial/commercial/institutional

IPM	Iopromide
K_d	Coefficient de partition (adsorption-desorption)
$K_{d,app}$	Apparent solid-water distribution coefficient
K_F	Coefficient de sorption de Freundlich
K_{OC}	Organic carbon–water partition coefficient
K_{OW}	Octanol–water partition coefficient
LC	Chromatographie en phase liquide
LCA	Acide lithocolique
LOD	Limit of detedtion
LP	Medium sand with fine-grained sand and little fines
MAE	Microwave extraction
MAMROT du territoire	Ministère des Affaires municipales, des Régions et de l'Occupation
ME	Matrix effect
MedP	Medroxyprogesterone
MES	Matières solides en suspension
MF	Filtration sur membrane
MOD	Matière organique dissoute
MRM	Multiple reaction monitoring
MS-MS	Spectrométrie de masse en tandem
NPP	Le nombre le plus probable
NPX	Naproxène
NTK	Azote total kjeldahl
OMS	Organisation Mondiale de la Santé
On-line SPE-LC–MS/MS	Online solid phase extraction-liquid chromatography-tandem mass spectrometry
Pb	Plomb
PCB	Polychlorobiphényles
PCR	Polymerase chain reaction
pH	Potentiel de l'hydrogène
pKa	Potentiel d’ionisation
PLE	Pressurized liquid extraction
PPCP	Pharmaceuticals and personal care products

PPSPs	Produits pharmaceutiques et de soins personnels
PRO	Progesterone
PX	Paraxanthine
qPCR	Quantitative real-time PCR
R	Residential
ROX	Roxithromycine
RSD	Relative standard deviation
RSM	Mode de balayage sélectif d'ions fragments
SMX	Sulfamethoxazole
SPE	Solid phase extraction
SRM	Selected reaction monitoring
SS	Suspended sediment (solid)
ST	Fine sand with medium sand and little fines
STEP	Station d'épuration des eaux usées
STP	Sewage treatment plant
StS	Settled Sediment
THEO	Theophyllin/Théophylline
TMP	Triméthoprime
TOF	Time of flight
TSA	Tryptone soybean agar
UFC	Unité formant colonie
UHPLC	Ultra-chromatographie en phase liquide à haute performance
UPW	HPLC Grade water
USE	Ultrasonic-assisted extraction
UV	Ultra-violet
VBNC	Bactérie viable mais non cultivable
WRRF	Water resource recovery facility
WW	Wet weather
WWMP	Watewater micropollutant
WWTP	Wastewater treatment plant
Zn	Zinc

CHAPITRE 1 INTRODUCTION

1.1 Mise en contexte

L'eau c'est la vie, comme l'a dit Le Saint Coran dans Sourate 21; Verset 30 : "…et nous avons fait de l'eau toute chose vivante…". Malheureusement, elle l'est aussi pour les micro-organismes pathogènes où ils peuvent se reproduire ou se déplacer causant alors diverses maladies et épidémies d'origine hydrique.

L'homme devient plus sensible aux problèmes de l'eau. Il veut une vie saine et moderne où toute utilisation accrue des capacités des milieux aquatiques devrait être offerte. Ce qui pousse les chercheurs à mettre en place une méthodologie permettant un suivi permanent de la qualité des eaux, surtout celles affectées par les activités anthropogènes.

Durant plusieurs décennies, des efforts considérables ont été entrepris dans le domaine de l'assainissement urbain par la construction de réseaux unitaires et séparés et de stations d'épuration des eaux usées. Malheureusement, si le problème a été en très grande partie résolu, il subsiste autant en temps de pluie qu'en temps sec. D'une part, lorsque la capacité d'interception et de traitement des eaux unitaires est limitée lors d'évènements pluvieux importants ou lors d'importantes fontes de neige, il s'en suit des déversements qui ont des impacts importants sur le milieu naturel. D'autre part, lorsque les égouts sanitaires déversent les eaux usées dans le réseau pluvial par des mauvais raccordements. L'eau usée déversée ainsi dans le réseau pluvial séparé s'achemine sans traitement aux milieux aquatiques contribuant grandement à la dégradation de la qualité de l'eau. Les contaminations d'origine urbaine sont donc principalement représentées par les surverses des systèmes d'égouts unitaires et les eaux usées des habitats dont l'assainissement n'est pas conforme ou raccordé aux collecteurs pluviaux séparés. La maîtrise du risque sanitaire des eaux lié à ces apports représente donc un enjeu majeur pour les municipalités tout en prenant compte des aspects relatifs au développement économique et écologique.

Un aspect important de qualité du milieu aquatique est la qualité microbiologique des eaux de surface. Celle-ci est soumise à de grandes variations quel que soit le lieu donné. Les événements transitoires qui sont responsables de ces variations sont souvent difficiles à expliquer par la surveillance régulière. C'est le cas des bassins versants urbains complexes où il peut y avoir des

sources ponctuelles et diffuses très variables, certaines continues, d'autres irrégulières, chacune avec un temps de parcours différent jusqu'au point d'échantillonnage.

L'origine humaine ou animale de la contamination fécale, une fois déterminée, constituerait une partie importante de la gestion de la qualité de l'eau réceptrice et permettrait de mettre en place des actions correctives ciblées et économiques à but écologique (Edge & Schaefer, 2006). Les coliformes totaux, les coliformes fécaux et les entérocoques ont été historiquement adoptés en tant qu'indicateurs de contamination fécale pour évaluer la qualité microbiologique des eaux de recréation, surveiller sa dégradation et prévenir le risque d'exposition aux pathogènes (Santé Canada, 2009). Toutefois, ces microorganismes indicateurs sont présents à la fois dans les matières fécales humaines et animales et même dans l'environnement, avec des taux de survie différents de ceux des autres pathogènes. Leur détection dans les eaux peut conduire à une surestimation de la contamination fécale. Par contre, ces indicateurs peuvent devenir non cultivables, ce qui conduit à une sous-estimation de la contamination fécale par les méthodes analytiques classiques basées sur la culture. Dans les deux cas, les répercussions économiques et sanitaires sont négatives (Alhamlan et al., 2015; Sercu et al., 2009). Notons entre autres, des toxi-infections alimentaires collectives lors de la consommation des fruits de mer (Le Guyader et al., 2010) ou des aliments produits avec de l'eau contaminée (Munro et al., 2012), des maladies intestinales, cutanées, ou pulmonaires par contact ou ingestion d'eau (Hlavsa et al., 2011) et des fermetures ou déclassements de zones de baignade (Réseau de suivi du milieu aquatique. Ville de Montréal, 2014). Des recherches avancées étudient l'opportunité de se fier principalement ou exclusivement sur les indicateurs microbiologiques spécifiques, génétiques et/ou chimiques de la contamination fécale de l'eau afin d'évaluer la qualité microbiologique de l'eau, d'identifier les sources de contamination et de sélectionner les procédés de traitement convenables (Glassmeyer et al., 2005; Haack et al., 2009; Young et al., 2008a).

Le travail présenté ici s'inscrit dans ce contexte très général puisqu'il porte sur les méthodologies d'analyse des parties dissoutes et particulaires des indicateurs chimiques dans les cours d'eau urbains et les trop-pleins (Chapitre 4), la dynamique de ces indicateurs dans les trop-pleins (Chapitre 5) et le dépistage de raccordements inversés dans des bassins versants urbains par la combinaison des indicateurs microbiologiques, génétiques et chimiques (Chapitre 6). Il répond alors à quatre questions majeures :

Est-ce que l'analyse des indicateurs chimiques à usage humain dans le compartiment solide (sédiment) des cours d'eau contaminés apporte des informations plus performantes sur l'ampleur de la contamination fécale plutôt que l'analyse du compartiment liquide (eau) ?

Quel est le rôle potentiel que les sédiments peuvent jouer dans le devenir des indicateurs chimiques dans les réseaux d'égouts unitaires ?

Est-il possible de différencier l'origine des contaminations en zone urbaine et tout particulièrement dans les réseaux d'égouts unitaires et séparés ?

Existe-t-il une relation entre les indicateurs classiques de contamination et les indicateurs alternatifs spécifiques (les indicateurs chimiques, les marqueurs *Bacteroidales* et les marqueurs des ADN des mitochondries) ?

L'objectif à long terme de ce travail était de développer un indice de contamination sanitaire dans un milieu naturel récepteur tel que les cours d'eau urbains et les réseaux d'égouts, afin d'aider les municipalités à prioriser les secteurs les plus contaminés par des surverses ou des raccordements inversés. De plus, ce nouvel indice impose aux communes de définir des profils de vulnérabilité pour chaque bassin versant étudié. Ces profils consistent en un recensement et une hiérarchisation des sources potentielles de contaminations fécales des eaux.

Pour les travaux expérimentaux nécessaires au développement de l'indice, nous avons choisi comme sites d'étude les ruisseaux US_1 et US_2 et la rivière US_3, des trop-pleins dans le bassin versant de la rivière US_3, un canal d'eau potable ainsi que des collecteurs pluviaux dans deux bassins versants (MEA et DEN) des ruisseaux urbains US_2 et US_4 respectivement. Nous avons, par ailleurs, choisi de porter notre attention sur des indicateurs particuliers des sources de contamination fécale, les indicateurs microbiologiques traditionnels (les coliformes fécaux et les *Escherichia coli*) et les indicateurs alternatifs chimiques (carbamazépine, caféine, théophylline, acétaminophène, etc.) et génétiques (marqueurs ciblant les *Bacteroidales* et les ADN des mitochondries des cellules eucaryotes de PCR en temps réel) car ils sont aujourd'hui considérés comme les meilleurs indicateurs alternatifs de contamination fécale des eaux qui ne sont jamais préalablement combinés ensemble dans une seule étude. Ils sont sensibles et spécifiques et persistent dans l'environnement suffisamment longtemps pour permettre leur détection et l'identification de l'origine de la pollution au niveau des eaux urbaines.

1.2 Structure de la thèse

Cette thèse est divisée en plusieurs chapitres qui correspondent à l'évolution des questionnements et des approches choisies pour dépister les sources de contamination fécale dans les cours d'eau urbains et bâtir des listes de marqueurs alternatifs. Il s'agit d'une thèse par articles :

Le Chapitre 1 introductif est dédié à la présentation du contexte, de la problématique de la thèse et présente son organisation. Le Chapitre 2 est consacré à la présentation d'une synthèse des données bibliographiques. Le Chapitre 3 présente les objectifs de recherche ainsi que la démarche expérimentale. Les Chapitres 4, 5 et 6 présentent l'essentiel des résultats de la recherche sous forme de trois publications scientifiques couvrant le développement et l'optimisation d'une méthode analysant des indicateurs chimiques dans les sédiments, la répartition de certains de ces indicateurs entre les deux compartiments solide et liquide et finalement, leur utilisation avec d'autres indicateurs traditionnels et alternatifs (moléculaires) afin de dépister les sources de contamination fécale humaine dans des bassins versants aux réseaux d'égouts séparés. Enfin, le Chapitre 7 offre une synthèse des travaux ainsi qu'une discussion générale pour finalement, mener à la conclusion et aux recommandations au Chapitre 8.

CHAPITRE 2 REVUE DE LA LITTÉRATURE

Dans cette synthèse bibliographique des données, plusieurs grands axes sont abordés : le portrait général de la problématique de déversements des systèmes d'égout unitaires et des mauvais raccordements aux systèmes d'égouts pluviaux, les indicateurs de la contamination fécale, leurs caractéristiques, les facteurs influençant leur persistance, les méthodes analytiques ; traditionnelles versus modernes et microbiologiques versus chimiques et génétiques, utilisées pour le dépistage des sources de pollution microbienne (DSPM) ainsi que les avantages et les inconvénients de ces méthodes.

2.1 Types et problématiques des réseaux

2.1.1 Réseaux unitaires, pseudo-séparatifs et séparatifs

Les réseaux unitaires étaient systématiquement les premiers réseaux construits dans les grandes villes du monde. Ils permettent d'évacuer conjointement les eaux de ruissellement et les eaux usées. Pour éviter l'acheminement des volumes d'eau trop élevés à la station d'épuration lors d'épisodes de pluie abondante ou de fonte rapide de neige, le surplus d'eau appelé surverse est rejeté directement dans les cours d'eau sans aucun traitement préalable. Toutes les eaux des égouts collecteurs unitaires sont dérivées vers l'intercepteur en période de temps sec, excepté lors d'incidents (pannes, urgences, etc.).

Dans le réseau pseudo-séparatif, les apports d'eaux pluviales sont divisés en deux parties : les eaux de ruissellement de la voirie, trottoirs et surfaces perméables sont acheminés dans les collecteurs pluviaux tandis que les égouts sanitaires collectent les eaux domestiques, commerciales, industrielles et les eaux de ruissellement provenant des drains de fondation, des drains de toits plats et des entrées de garage situées sous le niveau du sol. Comme dans le cas du réseau unitaire, le réseau pseudo-séparatif doit être dimensionné de manière à recueillir les rejets d'eaux usées, de même que les eaux pluviales dont le débit est beaucoup plus grand que celui des eaux usées.

En 1969, Waller avait estimé que 6.7 millions de canadiens étaient desservis par des égouts unitaires (Waller, 1969). Actuellement, la construction de réseaux d'égouts unitaires et pseudo-séparatifs est interdite car il contribue à la complication de l'épuration des eaux d'égout du point

de vue technique et économique et mène à des débordements d'eaux usées non traitées au milieu récepteur (Brière, 2012).

Afin de réduire la charge de polluants déversés dans les milieux aquatiques, le réseau d'assainissement doit être séparatif (au Québec, depuis 1965 et en Ontario, depuis 1985) (Environment Canada, 2001). L'évacuation de l'eau de ruissellement se fait à travers des égouts collecteurs pluviaux qui sont séparés des égouts collecteurs sanitaires. L'eau pluviale est alors rejetée directement dans les milieux récepteurs sans aucun traitement. Considérant les charges polluantes générées, notamment les matières en suspension, que ces eaux peuvent transporter en milieu urbain et qui dépassent dans certains cas celles produites par les eaux usées après traitement (Ministère du Développement durable, 2013), il paraît aujourd'hui contre-indiqué de retourner directement à l'environnement les eaux de ruissellement urbain. Un grand nombre de technologies ont été développés pour le traitement de ces eaux (Imran et al., 2013; Nnadi et al., 2015).

Ajoutons que l'état dégradé de réseaux unitaires et sanitaires cause des infiltrations d'eaux parasites en passant dans des zones sous le niveau de la nappe phréatique (afflux direct). Dans ce cas-là, les eaux parasites de drainage de la nappe souterraine se mélangent avec l'eau usée, surchargeant alors le réseau d'assainissement et son ouvrage d'épuration. Autres charges qui représentent des entrées régulières à l'égout (afflux constant) et qui ne peuvent pas être analysées séparément, sont les purges des tours de refroidissement et les eaux provenant du drainage des fondations et des sources (Metcalf and Eddy, 2003).

2.2 Portrait général de la problématique des déversements des réseaux unitaires et séparatifs dans l'agglomération montréalaise et la grande région de Montréal

Le problème de déversements d'eaux usées brutes est très courant au Canada. Environnement et Changement Climatique Canada a rapporté qu'à chaque année, plus de 150 milliards de litres d'eaux usées non traitées et insuffisamment traitées (eaux d'égouts) sont rejetés dans nos cours d'eau y compris le fleuve, la mer et les Grands Lacs. Parmi les villes canadiennes qui ont vécu cette situation, citons entre autres, des villes du Québec (MAMROT, 2012), Halifax, Victoria ,Winnipeg , Toronto, etc. (MacQueen K., 2006; Public Works and Government Services Canada,

2003). Attirant l'attention sur l'agglomération montréalaise qui a déversé récemment (mi-octobre 2015) dans le fleuve Saint-Laurent (déversements programmés par la Ville) plusieurs milliards de litres d'eaux usées non traitées, elle possède un réseau d'égout de 6 400 km. Les arrondissements et les villes de l'agglomération montréalaise sont majoritairement dotés de systèmes d'égouts unitaires. La proportion des systèmes d'égouts unitaires et séparatifs sur l'île de Montréal est de 63 % (154 ouvrages de débordement) contre 37 % qui sont principalement développés dans les parties extrêmes Ouest et Est de l'île (Ciment Québec, 2005). Les mauvais raccordements et les débordements des réseaux déversent une quantité importante d'eau usée non traitée. Ils sont la cause de rejets de polluants nuisibles pour les écosystèmes et limitent les possibilités d'usages en rive. L'ampleur du problème est souvent inconnue puisque les volumes des débordements des ouvrages de surverse ne sont pas mesurés et les raccordements inversés ne sont pas tous répertoriés (Union Saint-Laurent Grands Lacs et al., 2009).

2.2.1 Origine, transport et évolution spatiale des caractéristiques des polluants dans les réseaux d'assainissement urbains

Pendant les périodes de temps sec, de très nombreux polluants d'origines multiples (les retombées atmosphériques sèches, la circulation automobile, les industries, les déchets solides, l'érosion des sols, la végétation et les animaux) s'accumulent sur les surfaces des bassins versants urbains. Ces polluants se présentent sous forme dissoute, particulaire ou adsorbée sur des particules dont la taille varie de 1µm à plusieurs millimètres.

Au cours des événements pluvieux, les polluants sont transportés, par le ruissellement, sur les surfaces urbaines jusqu'aux réseaux d'assainissement séparatifs ou unitaires où ils s'ajoutent aux polluants des eaux usées, des éventuels dépôts des réseaux qui sont susceptibles d'être remis en suspension par l'augmentation des débits (Figure 2.1). La Figure 2.2 schématise les différents mécanismes qui interviennent lors du transport des solides en suspension (Guichard & Mouchel, 1993).

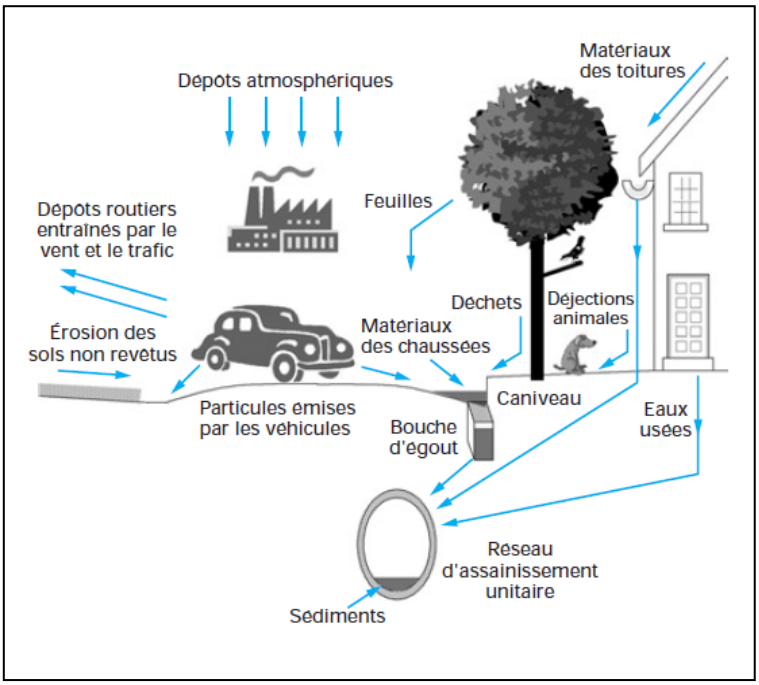

Figure 2.1: Principales entrées dans le réseau d'assainissement unitaire, tirée de (Chocat et al., 2007).

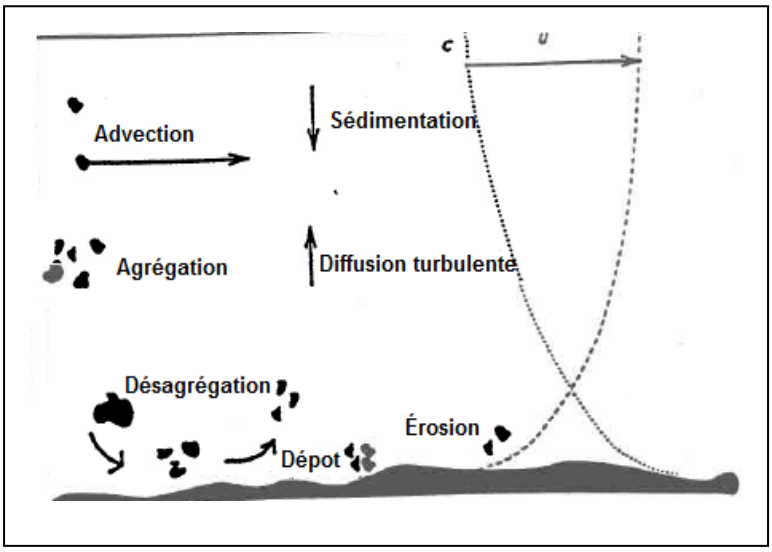

Figure 2.2. Mécanismes intervenant durant le transport des matières solides en suspension, tirée de (Guichard & Mouchel, 1993).

La majorité des polluants est également présente dans la phase particulaire (Droppo et al., 2002). Ils sont associés à des particules cohésives de diamètre supérieur à 0,45 µm et inférieur à 63 µm (Bédard, 1997). Des études ont montré que 70 à 80 % de la masse des particules en suspension

ont une taille inférieure à 100 µm avec un diamètre médian d_{50} de l'ordre de 30 à 40 µm (Chocat et al., 2007).

Les flux polluants issus des systèmes unitaires sont chargés en matières solides en suspension (MES), en micropolluants minéraux (métaux lourds) et organiques (hydrocarbures aliphatiques et aromatiques polycycliques, pesticides chlorés ou azotés, solvants, etc.), en microorganismes éventuellement pathogènes et en nutriments. Leurs concentrations sont généralement supérieures par rapport à celles trouvées dans les systèmes séparatifs. Le Tableau 2.1 présente les ordres de grandeur de la fraction associée aux particules (taille > 0,45 µm), en masse, de certains polluants présents dans les rejets urbains par temps de pluie (Chocat et al., 2007; Marsalek & Rochfort, 2004).

Tableau 2.1. Ordres de grandeur de la fraction particulaire en masse de quelques polluants contenus dans les rejets urbains par temps de pluie, tiré de (Chocat et al., 2007).

Polluants	**Fraction particulaire**
Demande chimique en oxygène, DCO	0,80 – 0,90
Demande biochimique en oxygène pendant 5j, DBO_5	0,75 – 0,95
Azote total kjeldahl, NTK	0,48 – 0,80
Plomb, Pb	0,80 – 0,98
Zinc, Zn	0,15 – 0,40
Cuivre, Cu	0,35 – 0,60
Cadmium, Cd	0,20 – 0,60
Hydrocarbure totaux, HCT	0,80 – 0,90
Hydrocarbures aromatiques polycycliques, HAP	0,75 – 0,97
Polychlorobiphényles PCB	0,90 – 0,95

La mise en place d'un dispositif expérimental sur une série de six bassins versants à Paris a permis d'étudier la variabilité des flux et de la nature des polluants transférés par temps sec et par temps de pluie dans les réseaux d'assainissement unitaires, à différentes échelles spatiales et également d'évaluer la contribution des trois sources (eaux usées, eaux de ruissellement et stocks de dépôt dans le réseau) à la pollution de temps de pluie. Les résultats obtenus indiquent une importante contribution des sédiments érodés, constitués par temps sec et véhiculés par temps de pluie dans les réseaux d'assainissement unitaires, aux flux des matières en suspension, des matières organiques et du cuivre, suivie par la contribution des eaux usées puis celle des eaux de ruissellement. Ces dernières sont considérées comme étant la source principale du plomb, du zinc et du cadmium (Kafi-Benyahia et al., 2008). Quant aux microorganismes d'origine fécale, leurs

concentrations augmentent dans le milieu récepteur après un événement pluvieux (Jeng et al., 2005).

De nombreux processus physiques (érosion, sédimentation, agglomération, adsorption, etc.), chimiques (oxydoréduction, etc.) et biochimiques (biodégradation des matières organiques, etc.) modifient, temporellement et spatialement, la nature (particulaire versus dissoute), l'espèce et les concentrations des différents polluants transportés par l'écoulement au sein des réseaux d'assainissement urbains (Chocat et al., 2007).

La variabilité spatiale de l'état de sédiments des surfaces urbaines a été étudiée par Droppo et ses collègues. Ils ont observé le passage de sédiments de l'état non floculé à l'état floculé durant leur transport de la rue vers les eaux de ruissellement. Ceci dépend des propriétés cohésives des solides en suspension (vecteurs de pollution). Les flocs augmentent de taille au fur et à mesure qu'ils s'avancent dans le système d'égout (Droppo et al., 2002).

Le mécanisme de floculation peut modifier considérablement les caractéristiques hydrodynamiques des particules. La masse volumique diminue, alors que la porosité, la vitesse de chute et la teneur en matière organique et en eau croissent lorsque la taille de floc augmente (Droppo et al., 2002).

Un examen microscopique montre que les flocs sont composés de polymères extracellulaires, des microorganismes et des substances organiques et inorganiques adsorbés. Les amas de matières fibreuses de polymères permettent de renforcer la structure de flocs en augmentant la surface disponible pour la sorption des nutriments et des contaminants (Droppo, 2004; Liss et al., 1996).

2.2.2 Dépôts dans les réseaux d'assainissement unitaires

Le diagramme de Hjulström basant sur la vitesse du courant et la taille des particules, résume les conditions de transport, de sédimentation et formation de dépôt et de remise en suspension des particules déposées au fond du cours d'eau (Figure 2.3). La sédimentation d'une particule est directement proportionnelle à sa taille et inversement proportionnelle à la vitesse du courant. Cette dernière doit descendre sous un seuil critique pour favoriser la sédimentation de particules.

C'est la loi de Stokes qui régit la vitesse de chute d'une particule en suspension ou la vitesse de sédimentation. Elle est proportionnelle à la différence entre sa densité et celle de l'eau, inversement proportionnelle à la viscosité de l'eau (plus la température de l'eau diminue, plus sa

viscosité augmente) et proportionnelle au carré du diamètre moyen de la particule (Frontier et al., 2008). La relation empirique est la suivante :

$$v = 2/9\ g.r^2\ (\rho-\rho')\ \mu^{-1},$$

où v est la vitesse de sédimentation de la particule ($m.sec^{-1}$), r est le rayon de la particule (m), ρ est la densité de la particule ($Kg.m^{-3}$), ρ' est la densité du fluide ($Kg.m^{-3}$), μ est la viscosité dynamique du milieu ($Kg.m^{-1}.sec^{-1}$) et g est l'accélération de la pesanteur ($m.sec^{-1}$).

Les argiles de type montmorillonite marquées à l'holmium ont été utilisées récemment comme des traceurs géochimiques afin de suivre les dynamiques de sédiments (flocs de petite taille < 63 μm) dans le bassin de rétention des eaux pluviales urbaines. Elles ont été conçues pour imiter les sédiments naturels. En outre, elles peuvent former des flocs présentant une taille et une vitesse de sédimentation similaires à celles de sédiments. Elles ont alors les mêmes caractéristiques dynamiques que ces derniers (Spencer et al., 2011).

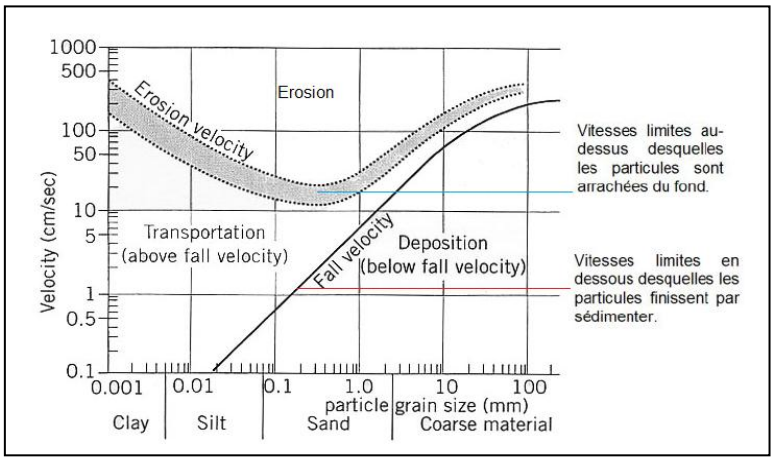

Figure 2.3. Diagramme de Hjulström, tirée et modifiée de (Hjulström, 1935).

Les dépôts présents dans les réseaux d'assainissement unitaires contribuent annuellement de 25% à 45% des matières en suspension des flux polluants globaux (Chocat et al., 2007). La Figure 2.4 présente les différents types de dépôts classifiés selon la granulométrie des particules: 1- «dépôt de type A» est le dépôt grossier, minéral, plus ou moins consolidé et qui se trouve au fond de la canalisation, 2- «dépôt de type C» qui a été identifié sous différentes formes selon les références, citons entre autres *near bed solids*, *dense undercurrent* et *fluid sediment*, est une couche organique fortement hétérogène (papiers, matières fécales, etc.), abrite une activité biologique, facilement érodable, et située à l'interface eau/sédiment et 3- «dépôt de type D» ou biofilm se développant par temps sec sur les parois rugueuses de réseaux dans la zone de battement des eaux

usées de temps sec, présente une teneur élevée en matière organique et devient avec le temps très chargé en métaux lourds et en micropolluants (Crabtree, 1989).

La principale source de pollution des eaux transportées par temps de pluie à l'exutoire du bassin versant est la couche organique ou le dépôt de type C (Ahyerre et al., 2000). Plus la teneur en matière organique est élevée dans le dépôt, plus ce dernier est résistant à l'érosion. Elle permet la stabilisation de la structure du sédiment par la formation de ponts entre les particules et les agrégats. Également, elle joue un rôle dans l'apport de substrats aux microorganismes pour la production de métabolites tels que les polysaccharides qui permettent à leur tour la floculation des argiles (Huang, 2004).

La présence de fines particules dans le dépôt qui viennent occuper les espaces vides entre les grosses particules donnent un matériau plus compact. De plus, les microorganismes qui sont principalement liés aux limons et aux argiles, renforcent l'effet de cohésion interne des particules. Entre ces dernières, il existe alors des pores dont leurs tailles affectent la survie des microorganismes. L'eau circule difficilement dans les petites pores (comme par exemple dans l'argile) et par la suite l'accessibilité des substrats aux microorganismes est plus difficile (Huang, 2004). La Figure 2.5 présente les tailles de différentes particules associées dans les sédiments.

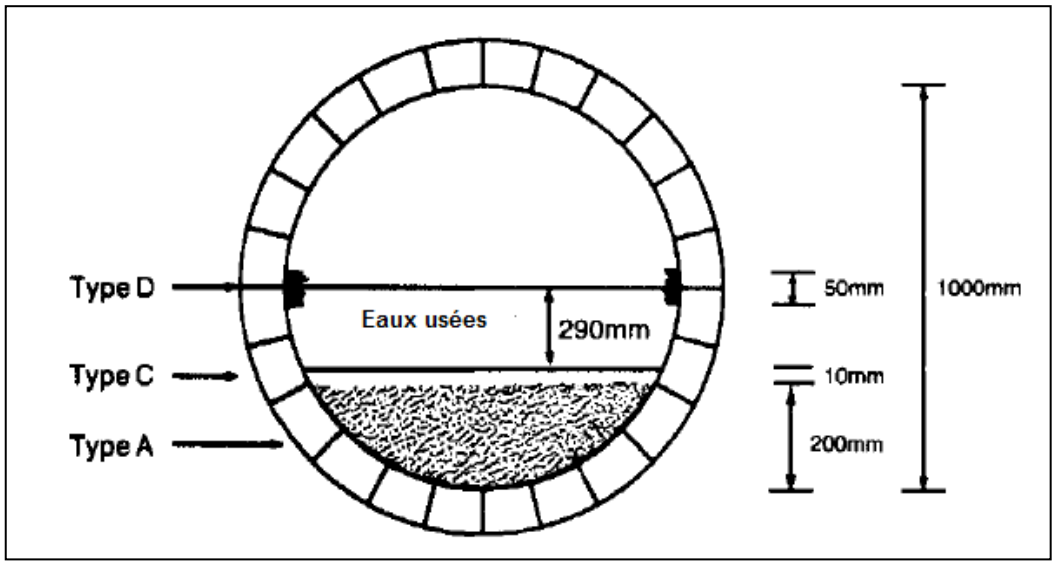

Figure 2.4. Types de dépôts dans les réseaux d'assainissement unitaires, tirée de (Crabtree, 1989).

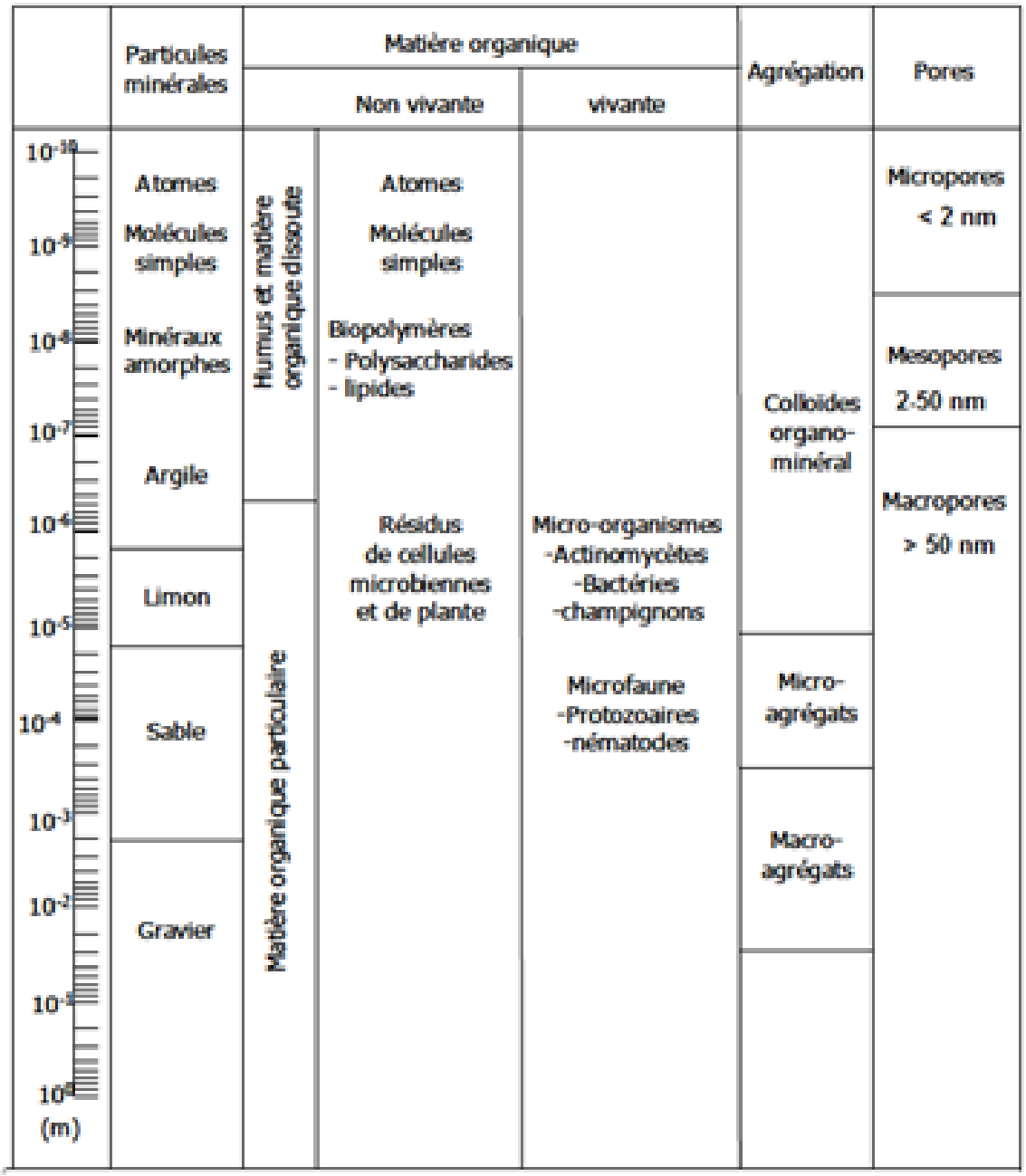

Figure 2.5. Gammes de taille des particules associées de sédiments, tirée de (Huang, 2004).

2.2.3 Défaillance environnementale des réseaux d'assainissement

La défaillance environnementale la plus remarquable des réseaux d'assainissement survient par suite de déversement des eaux sanitaires par les collecteurs pluviaux en temps sec, ou des eaux unitaires par les trop-pleins en temps humide (Gibson III et al., 1998; Mulliss et al., 1997; Petersen et al., 2005; Sercu et al., 2009). La fréquence annuelle des déversements (sans traitement) ne doit pas dépasser la valeur prescrite par le Ministère des Affaires municipales, des Régions et de l'Occupation du territoire MAMROT (0 rejet et 4 débordements par an par temps sec et humide, respectivement).

Les surverses sont l'une des principales sources ponctuelles de la pollution du milieu récepteur (Ritter et al., 2002). Les impacts du débordement d'orage sur le cours d'eau récepteur dépendent de la dynamique du rejet, donc de l'événement pluvieux. Leur sévérité est également fonction de la réaction du milieu, ce qui est également le cas pour les déversements en temps sec.

On peut distinguer deux types d'impacts attribuables aux rejets d'eaux de débordement : Les impacts immédiats et les impacts différés ou chroniques. Les impacts immédiats sont ceux subis par le milieu pendant une période de temps inférieure à l'intervalle entre deux événements pluvieux. Le terme chronique fait référence à un impact dont la durée est supérieure au temps de déversement et à l'intervalle entre deux pluies (CEGEO Technologies inc., 1993). Ils peuvent être classés selon trois catégories : les impacts esthétiques, les impacts physico-chimiques et les impacts microbiens (Chocat et al., 2007).

2.2.3.1 Impacts esthétiques

Les impacts esthétiques sont des signes d'avertissement qui nous préviennent de la qualité de rejets. Les pollutions visuelles et olfactives sont attribuées à la forte turbidité des eaux, à la présence de débris sanitaires et flottants qui entraînent le plus souvent des odeurs désagréables et incommodantes pour les riverains ou les usagers du milieu. Ces odeurs sont causées, entre autres, par la dégradation de la matière organique contenue dans les sédiments (Chocat et al., 2007).

2.2.3.2 Impacts physico-chimiques

Le régime hydrologique du milieu récepteur est affecté par les débits importants des eaux de débordement qui sont fortement chargées en matières en suspension. Ces particules solides

véhiculées ont des granulométries et des vitesses de sédimentation élevées. D'après (Benoist & Lijklema, 1990), de 30% à 50% des solides contenus dans les débordements de réseaux unitaires sont de nature à sédimenter à proximité des émissaires. Deux conséquences peuvent se produire, dues principalement au caractère irrégulier des précipitations: d'une part, l'érosion de la berge et du fond et d'autre part, l'augmentation localisée des dépôts solides (Chocat et al., 2007).

Les eaux de débordement sont chargées en matière organique biodégradable qui consomme l'oxygène dissous dans le milieu aquatique. Par conséquent, ce milieu devient sous-oxygéné ou anoxie entraînant alors la mort des espèces aquatiques sensibles (Chocat et al., 2007). De même, la présence de nutriments ($11{,}8.10^3$ tonnes/année azote et $2{,}3.10^3$ tonnes/année phosphore) dans les eaux de surverses accélère l'eutrophisation du milieu récepteur (Ritter et al., 2002).

Les micropolluants toxiques minéraux (particulièrement les métaux lourds) et organiques (composés pharmaceutiques, hormones, pesticides, etc.) ont des effets toxiques chroniques (tératogènes et cancérigènes). Ceci se traduit par une perte de biodiversité dans la faune et la flore (Ritter et al., 2002) par l'effondrement d'espèces, citons comme exemple les populations de poissons sauvages étudiées par Kidd et al. dans des lacs expérimentaux en Ontario (Kidd et al., 2007).

2.2.3.3 Impacts microbiens

Les microorganismes pathogènes sont classés au troisième rang après les nutriments et les sédiments parmi les principaux polluants affectant la qualité des eaux de surface aux États-Unis (Baker, 1992). La contamination d'environ 32% des rivières polluées est attribuée à ce type de polluants (Ritter et al., 2002). Les matières fécales d'origine humaine et animale sont les principales sources de pollution microbienne (Arnone & Walling, 2007). Les agents pathogènes provoquant des épidémies d'origine hydrique sont les bactéries (organisme unicellulaire sans noyau ou procaryote et mesure rarement plus de quelques micromètres), les virus (organisme acellulaire formé d'un assemblage d'acides nucléiques et de protéines qui protègent ces acides nucléiques et lui donnent son pouvoir infectieux. Il est souvent de l'ordre d'une dizaine ou d'une centaine de nanomètres) et les parasites qui peuvent être unicellulaires (les protozoaires) ou multicellulaires (Chocat et al., 2007). Le Tableau 2.2 résume les maladies transmises par des principaux agents bactériens pathogènes, des virus et des protozoaires excrétés dans les fèces.

Au cours des cinquante dernières années, plus de la moitié des épidémies d'origine hydrique aux États-Unis ont été précédées par des périodes de pluies abondantes (Curriero et al., 2001). Également, deux épidémies au Canada ; une à Walkerton en 2000 et une autre à North Battleford en 2001. L'épidémie à North Battleford avait été provoquée par une inondation qui avait fait déborder des égouts et souillé le réservoir d'eau potable de la ville, diffusant alors des bactéries pathogènes responsables de maladies hydriques. À Walkerton, l'épidémie causait la mort de sept personnes et 2300 cas de gastroentérites (Hrudey & Hrudey, 2007), tandis qu'à North Battleford, 35,8% de la population a été infecté (Wagner et al., 2005).

En milieu urbain, lors des événements pluvieux intenses, une forte charge en microorganismes est entraînée par le ruissellement sur des surfaces imperméables. Ces eaux peuvent contenir jusqu'à $10^{6,1}$ UFC d'*E. coli* 100 mL^{-1} (Kim et al., 2005). Cependant, la contamination des eaux de surface par les coliformes fécaux provient majoritairement des réseaux d'égout unitaires (Ritter et al., 2002). Une comparaison entre les microorganismes en temps humide et en temps sec, montre que les concentrations de ces derniers augmentent de 2 Log en aval du point de rejet de surverse en temps humide (Rechenburg et al., 2006). Quelle que soit la taille des réseaux d'égout unitaires, la charge annuelle de bactéries et de parasites provenant de ces derniers a été trouvé significativement supérieure à celle de l'effluent traité de l'épuration des eaux usées (Figure 2.6).

La durée de l'événement pluvial joue également un rôle dans la détermination de la qualité de l'eau réceptrice. Une étude menée par (Wu, Rees, et al., 2011) a démontré que la densité d'*E. coli* était plus élevée pendant les événements intenses de courte durée en comparaison avec des événements modérés de longue durée. Dans le même contexte, Wu et al. ont trouvé que les sources contribuant à la contamination fécale de l'eau en temps sec et humide sont différentes. En temps humide, la contamination fécale d'origine humaine prédomine (24,43% en temps humide versus 9,09% en temps sec).

Tableau 2.2. Principaux agents pathogènes bactériens, virus et protozoaires présents dans les fèces et les maladies transmises, tiré de (Ritter et al., 2002).

	Organisme	Maladie	Sources primaires
Bactéries	***Campylobacter***	Gastro-entérite	Matières fécales humaines
	***Salmonella* (1700 espèces)**	Fièvre typhoïde/salmonellose	Matières fécales humaines et animales
	***Shigella* (4 espèces)**	Dysenterie bacillaire	Matières fécales humaines
	Vibrio cholerae	Choléra	Matières fécales humaines
	***Escherichia coli* (souches entéro-pathogènes)**	Gastro-entérite	Matières fécales humaines
	Yersinia enterocolitica	Gastro-entérite	Matières fécales humaines et animales
Virus	***Adénovirus***	Maladies respiratoires et gastro-intestinales	Matières fécales humaines
	***Enterovirus* (71 types)**	Poliomyélite méningite aseptique	Matières fécales humaines
	Hepatitis A	Hépatite infectieuse	Matières fécales humaines
	Virus de Norwalk	Gastro-entérite	Matières fécales humaines
	Reovirus	Maladies respiratoires et gastro-intestinales	Matières fécales humaines et animales
	Rotavirus	Gastro-entérite	Matières fécales humaines
	Virus de Coxsackie	Méningite aseptique	Matières fécales humaines
Protozoaires	***Balantidium coli***	Dysenterie	Matières fécales humaines
	Cryptosporidium entamoeba histolytica	Dysenterie	Matières fécales humaines
	Giardia lamblia	Gastro-entérite	Matières fécales humaines

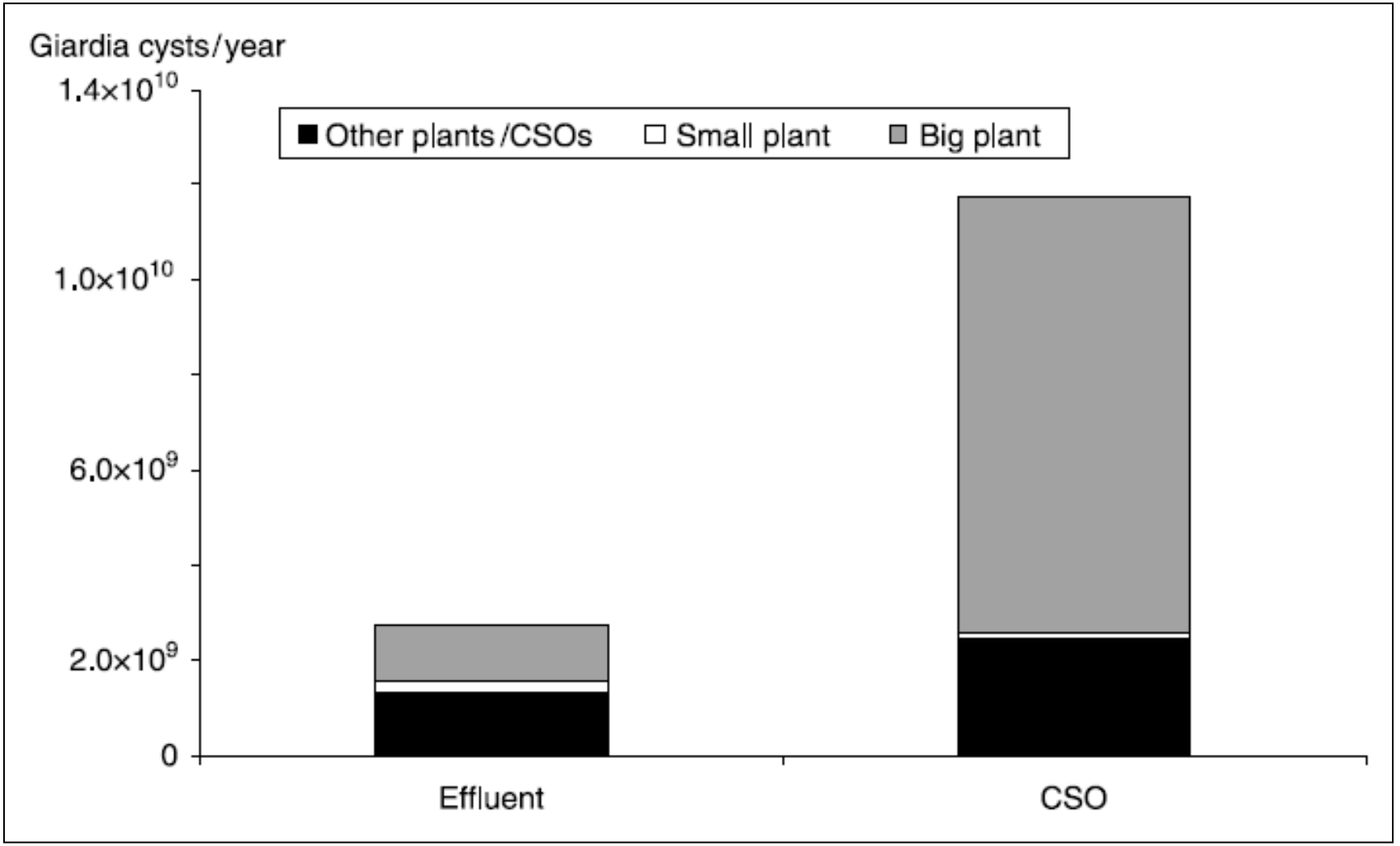

Figure 2.6. Comparaison de la charge annuelle des kystes de *Giardia* dans la rivière provenant des stations d'épuration et des débordements des égouts unitaires, tirée de (Rechenburg et al., 2006).

2.3 Qualité microbiologique des eaux et discrimination de l'origine des contaminations fécales

A l'échelle de la planète, la préoccupation majeure d'eau potable reste la contamination microbienne ou fécale plutôt que les autres types de pollution (Parajuli et al., 2009; Stevens et al., 2004). Les risques de décès et de maladies hydriques causés par des agents pathogènes dans l'eau non traitée sont respectivement de 100 à 1000 fois et de 10 000 à 1 000 000 fois plus grands que le risque de cancer des sous-produits de chloration de l'eau potable (Regli et al., 1993). Selon l'OMS, l'eau contaminée est la première cause de maladie et de mortalité au monde (World Health Organization, 2001). Au Canada, 90 décès et 90 000 cas d'infections aigües d'origine hydrique sont enregistrés annuellement (Environnement Canada, 2001, 2009). Une étude récente a estimé 334 966 cas ayant une maladie gastro-intestinale aiguë (AGI) associée à des systèmes d'eau potable municipaux canadiens (Murphy et al., 2015). Une autre étude a démontré une association significative entre les précipitations extrêmes (dans la période de 8 jours suivants) et le taux de visites à l'urgence pour une maladie gastro-intestinale pour tous les âges seulement dans des régions où les sources d'eau potable sont touchées par des surverses (Jagai et al., 2015).

2.3.1 Évaluation de la qualité microbiologique des eaux

L'identification de tous les microorganismes pathogènes responsables de maladies d'origine hydrique est impossible pour les raisons suivantes : (i) la grande variété et diversité de microorganismes pathogènes qui peuvent être présents dans l'eau contaminée ; (ii) la faible abondance de chaque espèce dans l'échantillon analysé, ce qui conduit à utiliser des échantillons d'eau de grand volume pour concentrer les pathogènes et (iii) l'absence de méthodes standardisées et rapides pour la détection de ces microorganismes pathogènes, malgré le développement de la biotechnologie (Ortega et al., 2009; Stevens et al., 2004).

Les laboratoires de recherches et d'analyses ont substitué la détection des pathogènes par des indicateurs de contamination fécale car la détection de tous les pathogènes potentiels est très difficile et incertaine. La microflore intestinale compte jusqu'à 100 000 milliards de bactéries, ce qui permet de l'utiliser comme indicateur de la contamination fécale d'origine humaine ou animale (Gilpin et al., 2002).

Un indicateur microbiologique idéal devrait répondre à des critères stricts tels que : (i) faire partie de la microflore intestinale ; (ii) être non pathogène ; (iii) sa présence est fortement associée à celle de microorganismes fécaux pathogènes et plus nombreux qu'eux ; (iv) être au moins aussi résistant aux stress environnementaux et à la désinfection que les pathogènes ; (v) ne sont pas susceptibles de se multiplier dans l'environnement ; (vi) rapidement détectable et facilement dénombrable à faible coût ; (vii) identifiable sans ambiguïté dans tous les échantillons ; (viii) être distribué de manière aléatoire dans l'échantillon à analyser et enfin (ix) posséder des caractéristiques de cinétique similaires aux microorganismes pathogènes (Resnick & Levin, 1981).

Les chercheurs ont utilisé des méthodes traditionnelles pour le dénombrement de coliformes totaux, de coliformes fécaux ou *Escherichia coli* et d'entérocoques, afin de déterminer la qualité microbiologique de l'eau et d'évaluer les risques sanitaires liés à la présence de microorganismes pathogènes d'origine fécale dans les eaux (Gilpin et al., 2003; Plummer & Long, 2009). Il existe deux grands types de méthodes basées sur la mise en culture : (i) la détermination du nombre le plus probable (NPP) et (ii) la méthode de filtration sur membrane (MF). L'eau ne devrait pas contenir d'*Escherichia coli* ni de coliformes totaux dans les systèmes d'approvisionnement en eau potable (Comité fédéral-provincial-territorial sur l'eau potable, 1968). Dans les eaux d'irrigation, les recommandations canadiennes pour les coliformes fécaux et les coliformes totaux sont respectivement de 100 UFC 100 mL^{-1} et de 1000 UFC100 mL^{-1} (Le Conseil Canadien des Ministres de l'Environnement, 2002). En eaux douces de baignade, le nombre de coliformes fécaux ne doit pas dépasser 200 UFC100 mL^{-1} avec un maximum de 10% des échantillons supérieurs à 400 UFC100 mL^{-1} (Ministère de l'Environnement et de la Faune, 1998).

Les méthodes classiques de dénombrement sont limitées par la turbidité élevée de l'eau, la présence d'autres microorganismes qui entrent en compétition avec les indicateurs dénombrés et la présence de facteurs inhibiteurs comme les métaux lourds et les phénols (Prescott et al., 2003).

2.3.1.1 Indicateurs microbiologiques classiques

Les indicateurs microbiologiques classiques de la contamination fécale ne permettent pas d'identifier l'origine des contaminants dans l'environnement car ils sont présents à la fois dans les selles humaines et les excrétions des animaux à sang chaud et à sang froid (Field &

Samadpour, 2007). Ils sont sélectionnés pour détecter la présence d'une pollution fécale, citons entre autres les coliformes totaux, les coliformes fécaux et les entérocoques.

Les coliformes totaux

L'adoption de coliformes totaux (FT) par le Public Health Service aux États-Unis remonte à 1914 (Bitton, 2005). Ils sont utilisés comme indicateurs de la contamination fécale de l'eau de surface et permettent d'évaluer l'efficacité d'une filière de traitement. Ils représentent 1% de la flore totale des matières fécales. Ce sont des bactéries en forme de bâtonnet, Gram-négatives, anaérobies facultatives ; non sporulantes, capables de fermenter le lactose avec production d'acide et de gaz à 35 °C en 48 heures (Prescott et al., 2003). Le Tableau 2.3 présente les différents genres de coliformes qui ont été classifiés selon leurs origines. Des coliformes peuvent exister et proliférer dans les sols et les eaux naturelles (en grand nombre dans les eaux de surface) comme dans le système intestinal (Kampfer et al., 2008; Stevens et al., 2004).

Tableau 2.3. Classifications de coliformes totaux selon leurs origines.

Coliformes totaux	***Klebsiella***	***Enterobacter***	***Citrobacter***	***Yersinia***	***Serratia***	***Hafnia***	***Pantoea***
Humaine/ Animale	X	X	X	X	X	X	X
Environnementale	X	X	X	X	X	X	X

Coliformes totaux	***Escherichia***	***Kluyvera***	***Cedecea***	***Ewingella***	***Moellerella***	***Leclercia***	***Rahnella***	***Yokenella***
Humaine/ Animale	X	X						
Environnementale		X	X	X	X	X	X	X

Les coliformes fécaux ou coliformes thermotolérants

Les coliformes fécaux (FC) sont des coliformes intestinaux provenant d'animaux homéothermes et capables de se multiplier à la température plus restrictive de 44,5 °C (Prescott et al., 2003). Parmi les coliformes fécaux, *Escherichia coli* (*E. coli*) est uniquement capable de produire l'indole à partir de tryptophane (Garcia-Armisen et al., 2007; Pugsley et al., 1973). Il représente l'espèce prédominante et est considéré comme l'indicateur classique le plus spécifique d'une contamination fécale d'origine humaine ou animale puisqu'il n'existe que rarement dans l'environnement, citons par exemple l'eau chaude tropicale (Stevens et al., 2004). En moyenne, *E. coli* constituait 77% des coliformes fécaux dans des échantillons d'eau douce contaminés de façon différentielle (Garcia-Armisen et al., 2007).

Des milieux spécifiques classiques sont utilisés pour la détermination du NPP ou des UFC, possédant du lactose et un indicateur pour identifier la production d'acide, comme par exemple le

milieu mTEC. D'autres milieux spécifiques permettant d'augmenter la spécificité et diminuer le temps de réponse des méthodes basées sur la mise en culture, possèdent des substrats chromogéniques et fluorogéniques pour la détection d'une activité enzymatique spécifique (β-D-glucuronidase, enzyme spécifique des *E. coli*). L'hydrolyse de ces substrats par l'enzyme spécifique donne lieu à des produits colorés ou fluorescents détectés sous illumination UV (Manafi, 2000).

E. coli demeurent moins longtemps dans le milieu naturel en comparaison avec les virus et les protozoaires (Stevens et al., 2004). Cette bactérie est plus sensible à la lumière solaire, la prédation, et la salinité plutôt que le froid (Anderson et al., 2005; Harwood et al., 2005; Muela et al., 2000).

Des expériences *in situ* et *in vitro* menées par (Jenkins et al., 2011) ont montré que les souches pathogènes *E. coli* O157:H7 sont plus persistantes dans les eaux de surface que les indicateurs classiques, *E. coli* et les entérocoques fécaux. Ces derniers sont affectés par la prédation et la lumière solaire, alors que les souches pathogènes ont montré une forte résistance.

La turbidité et l'attachement aux sédiments peuvent améliorer la survie d'*E. coli* dans l'eau (Craig et al., 2004). Leur décadence est deux fois plus élevée à l'état libre que lorsqu'elles sont associées aux particules en suspension (Garcia-Armisen & Servais, 2009).

Les streptocoques ou les entérocoques fécaux

Les entérocoques sont parfois considérés comme plus résistants aux conditions environnementales et aux désinfectants que les autres indicateurs (comme les coliformes) (Anderson et al., 2005; Metcalf, 1978). Le ratio de coliformes fécaux à streptocoques fécaux (FC/FS) a été utilisé pour différencier entre les sources de contamination fécale humaine et animale. Pour un ratio supérieur ou égal à 4, la contamination est considérée d'origine humaine. La contamination est d'origine animale avec un ratio inférieur à 0,7 (Edwards et al., 1997).

Les concentrations de FC et de FS peuvent être influencées par des variables telles que les saisons et le débit lors de l'échantillonnage (Edwards et al., 1997). Tyagi et al. (2009) ont présenté de nombreuses limites, raisons pour lesquelles APHA (American Public Health Association) n'a plus recommandé l'utilisation de ce ratio depuis l'année 1998 sauf que pour une pollution fécale très récente : (i) le ratio se modifie graduellement avec le temps. Il peut alors

évoluer avec la survie des coliformes et des streptocoques dans l'environnement ; (ii) il est affecté par la désinfection des eaux usées et par les méthodes de dénombrement de FS ; (iii) le taux de survie est variable entre les différentes espèces de streptocoques fécaux ; (iv) les variations de concentrations des streptocoques en fonction des individus, sont liées aux différents régimes alimentaires et ; (v) les valeurs intermédiaires sont difficilement interprétables.

Atherholt et al. ont montré que la pollution fécale des eaux souterraines a été détectée le plus souvent par le test de FT, suivie par les test de FC, *E. coli* et d'entérocoques. L'absence de FC, d'*E. coli* et/ou des entérocoques et la présence de FT et/ou des coliphages n'indiquent pas absolument la présence d'un risque sanitaire (Atherholt et al., 2003).

Enterococcus faecalis (*E. faecalis*) et *E. faecium* sont les deux espèces les plus souvent identifiées chez l'humain. *Streptococcus bovis* (*S. bovis*), *S. equinus*, *S. gallolyticus* et *S. alactolyticus* ont été identifiées chez les bétails, les chevaux et les volailles. Parfois, elles peuvent être présentes chez l'humain, en particulier *S. bovis* (Groupe scientifique sur l'eau, 2002).

La diversité de sources de ces indicateurs fait détourner les études vers des indicateurs de plus en plus spécifiques. D'où l'utilisation des indicateurs microbiologiques spécifiques, moléculaires et/ou chimiques afin de dépister les sources de contamination fécale dans les eaux (Tyagi et al., 2009).

2.3.2 Dépistage des sources de pollution microbienne

Le dépistage des sources de pollution microbienne (DSPM) consiste à déterminer et contrôler les sources ponctuelles et diffuses de la contamination fécale dans l'eau. « Il permet d'établir un degré de similitude entre les microorganismes recueillis dans les écosystèmes aquatiques et les microorganismes provenant de sources voisines connues de pollution fécale pour en arriver à déduire la source probable de la contamination fécale » (Edge & Schaefer, 2006). À ces fins, les deux principales étapes consistent à déterminer et identifier l'indicateur à rechercher, puis choisir la méthode compatible pour la mettre en évidence dans les échantillons environnementaux étudiés. Les indicateurs peuvent être des microorganismes, des composés chimiques (pharmaceutiques) ou moléculaires (les ARN 16S ribosomaux des microorganismes, les ADN des mitochondries des cellules eucaryotes de l'organisme hôte, etc.).

2.3.2.1 Indicateurs microbiologiques spécifiques

Le Tableau 2.4 présente les caractéristiques des indicateurs microbiologiques spécifiques d'espèces les plus communes. Ces indicateurs peuvent être utilisés individuellement ou en combinaison afin de déterminer les sources de contamination fécale. Ils comprennent des bactéries et des virus.

L'indicateur possédant des caractéristiques biochimiques et de survie similaires à celles d'un microorganisme pathogène peut être utilisé pour détecter la présence de ce dernier. Citons par exemple, les bactériophages qui peuvent être utilisés comme indicateurs de la présence de virus entériques dans l'eau. La présence de pathogènes causant des maladies spécifiques pour une espèce donnée peut être détectée par le suivi d'un indicateur spécifique à cette espèce.

Certains indicateurs peuvent se trouver chez les espèces sauvages. Ils peuvent être beaucoup plus résistants aux stress environnementaux et peuvent se multiplier dans l'environnement (Young et al., 2008b). Leur détection dans les eaux conduit à une surestimation de la contamination fécale. D'autres indicateurs sont plus sensibles et ne peuvent persister trop longtemps; meurent ou entrent dans un état de dormance et deviennent non cultivables. Leur détection par les méthodes analytiques classiques qui sont basées sur la culture, conduit alors à une sous-estimation de la contamination fécale. Dans les deux cas, les répercussions économiques sont négatives (Sercu et al., 2009).

Comportement des indicateurs microbiologiques dans l'environnement

La Figure 2.7 schématise les différents processus contrôlant le devenir des indicateurs microbiologiques, une fois rejetés dans l'environnement aquatique. Ces processus peuvent être classés en trois catégories : processus hydrodynamiques, biotiques et physiologiques (Troussellier et al., 1998).

Processus hydrodynamiques

Parmi les processus hydrodynamiques, citons entre autres la dilution, la dispersion, la sédimentation et la remise en suspension. Les deux premiers processus dépendent de l'hydrodynamique du système, alors que les deux derniers sont accommodés par l'attachement de microorganismes à des particules en suspension.

La liaison microorganisme/surface est un processus complexe dépendant du microorganisme, de la surface solide et de la phase liquide (Ling et al., 2002). Elle peut être divisée en plusieurs phases : (i) l'attachement primaire et réversible par l'intermédiaire des forces électrostatiques et des adhésines situées à la surface cellulaire comme les pili bactériens, les fimbriae et les couches capsulaires ; (ii) l'adhésion secondaire et irréversible, résultant de la liaison permanente des exopolysaccharides (diamètre individuel varie entre 2 à 20 nm) développée après le stade de la colonisation de surface et enfin (iii) la formation de biofilm (Droppo et al., 2009; Dunne, 2002).

Les facteurs affectant l'adsorption de microorganismes sont nombreux, citons entre autres (i) l'hydrophobicité de la surface cellulaire, (ii) la charge électrostatique, (iii) l'état physiologique, la forme et la taille de la cellule, (iv) la présence des protéines spécifiques à la surface de la cellule et des polymères extracellulaires, (v) l'aire de la surface disponible et la charge à la surface des particules du sol et (vi) les ions dans les sédiments (Ling et al., 2002).

Les forces attractives et répulsives des surfaces sont principalement fonctions de la composition minérale et organique du sol (particule) et de l'équilibre ionique de la phase liquide (Guzmán et al., 2009). La majorité des études ont montré que l'adsorption de microorganismes augmente avec l'augmentation de la teneur en argile dans le sol, du chlorure de sodium dans la colonne d'eau et la force ionique d'une part et la diminution de la taille de particules du sol et de microorganismes d'autre part. En plus, la teneur en argile est plus importante que la matière organique dans la détermination de l'adsorption de microorganismes (Ling et al., 2002).

Une comparaison de la sorption de l'indicateur classique (*E. coli*) sur deux sols différents (14% d'argile avec 0,84% de matière organique et 35% d'argile avec 0,54% de matière organique) a permis d'établir une relation linéaire entre le coefficient de partition K_d du modèle linéaire de Freundlich et le logarithme de la teneur en argile. Dans cette étude, les expériences ont été menées à l'aide de bactéries cultivées sous forme planctonique. La teneur en matière organique dans les sols était faible, ce qui conduisait à une sous-estimation de la sorption d'*E. coli*. Pour une faible adsorption, K_d était égal à 0,33 et 127 mL g^{-1} dans les sols à 14% et 35% argile respectivement. Par ailleurs, pour une forte adsorption, K_d était égal à 0,62 et 25,0 mL g^{-1} respectivement (Ling et al., 2002). Par comparaison avec d'autres bactéries (*Bacillus* sp., *Pseudomonas* sp., *Serratia* sp. et *Chromobacteria* sp.), la sorption d'*E. coli* aux différents sols était la plus faible (Marshall, 1971).

Pour mieux comprendre le devenir et le transport d'*E. coli* dans l'environnement après l'épandage des excréments animaux, une étude récente menée par (Guzmán et al., 2009) a permis d'établir une équation générale estimant les coefficients de l'isotherme de Freundlich en fonction de la teneur en argile. À ces fins, ils ont utilisé deux sols naturels (sable loameux et loam sableux) et sept sols artificiels (diffèrent par leur teneur en argile : le Kaolinite, et en matière organique : la mousse des tourbières) et les mélangés à la température de la pièce (23 °C) avec un effluent d'épandage (dilué dans de l'eau distillée avec des ratios de 1:5, 2:4, 3:3, 5:1 et 6:0). Deux séries de microcosmes ont été préparées avec les sols artificiels à 0%, 5%, 10% et 20% argile (avec et sans mousse) et deux autres avec les sols naturels (avec et sans matière organique). La relation entre la sorption d'*E. coli* et la teneur en argile était non linéaire. Pour les sols artificiels à 5% et 10% argile, la sorption a augmenté après l'addition de la matière organique mais avec des concentrations initiales spécifiques d'*E. coli* ($\geq$ 5000 NPP mL^{-1} pour 5% argile et $\geq$ 3500 NPP mL^{-1} pour 10% argile). Tandis que la sorption restait la même pour les sols à 20% argile (avec et sans mousse). Ceci a été expliqué par une compétition de sorption aux particules colloïdales de l'effluent, existant entre la matière organique ajoutée (CEC = 108 à 162 $cmol_c$/Kg, surface de contact = 200 m^2/g) et la kaolinite (CEC = 1 à 15 $cmol_c$/Kg, surface de contact = 8,8 m^2/g). Cette compétition est fonction du pH et de la force ionique. La mousse des tourbières possédant une capacité d'échange cationique plus élevée, contrôlait le pH de la solution. Lorsque la concentration de l'effluent a augmenté (augmentation de la concentration d'*E. coli* et du pH), les charges des surfaces de kaolinite devenaient plus négatives et la kaolinite entrait en compétition avec la mousse des tourbières pour s'attacher aux ions, aux colloïdes et aux composés organiques dans la solution. En outre, avec une teneur élevée en argile (20%), le nombre de moles de charges de l'argile était approximativement équivalent à celui de la mousse. D'où la minimisation de l'effet de la matière organique ajoutée (mousse des tourbières) sur la sorption d'*E. coli*.

Tableau 2.4. Caractéristiques des indicateurs spécifiques d'espèces les plus communs.

Indicateurs	Source identifiée	Taux de survie	Avantages /Limites	Références
Streptococcus bovis	Chez les animaux et rarement chez les humains	Bas, représente une contamination fécale récente	Coût d'analyse de faible à moyen	(Sargeant, 1999)
***Bifidobacterium* sp.**	Chez les humains (sources ponctuelles et non ponctuelles) *B. breve et B. adolescentis* (humains) et *B. thermophilum* (animaux) *Bifidobacterium* fermentant le sorbitol (chez les humains, rares chez les cochons)	Bas (non détectable) surtout à haute température Représentent une contamination fécale récente Ne se prolifèrent pas dans l'environnement	Prédominante de la microflore intestinale Coût élevé, longue manipulation avec les méthodes de culture traditionnelles, condition anaérobie stricte, aucun milieu de culture défini, faible présence dans l'eau Différenciation difficile avec les méthodes microbiologiques et biochimiques traditionnelles, plus facile avec les méthodes d'hybridation sur membrane	(Field & Samadpour, 2007; Gavini et al., 1991; Gilpin et al., 2002; Long et al., 2003; Lynch et al., 2002; Mara & Oragui, 1983; Resnick & Levin, 1981; Rhodes & Kator, 1999)
Bacteroides* sp. et Bacteriophages de *B. fragilis	Chez les humains (*Bacteroides-Prevotella*, *B. fragilis* HSP40) Absent ou rare chez les animaux *B. thetaiotaomicron* (chez les humains et les chiens)	Bas, représente une contamination fécale récente	Bonne association avec la source et ne se prolifèrent pas dans l'environnement Nombre restrictif dans les eaux usées nécessite un grand volume d'échantillon Utilisation des méthodes indépendantes de culture (PCR basée sur la spécificité aux hôtes) Manque des amorces spécifiques surtout aux espèces sauvages	(Field & Samadpour, 2007; Gilpin et al., 2003; Gilpin et al., 2002; Kreader, 1995; Tyagi et al., 2009)
Rhodococcus coprophilus	herbivores (croissance sur les excréments des herbivores) et animaux domestiques dans les régions tropicales	Modéré dans l'environnement	Viable *in vitro* pendant 17 semaines dans l'eau fraiche à 5° C, 20° C et 30° C Coût élevé et longue manipulation avec les méthodes de culture traditionnelles (21 j) Différenciation facile avec les méthodes de PCR, mais avec ADN de haute qualité (application limitée pour les rivières)	(Gilpin et al., 2002; Long et al., 2003; Mara & Oragui, 1981)

Tableau 2.4. Caractéristiques des indicateurs spécifiques d'espèces les plus communs (suite).

Indicateurs	Source identifiée	Taux de survie	Avantages /Limites	Références
Clostridium perfringens	Chez les humains et les animaux Pollutions ponctuelles	Elevé (forme sporulée : jusqu'à des années), ne représente pas une contamination fécale récente Concentration diminue avec la distance du point de déversement	Coût moyen, condition anaérobie	(Atherholt et al., 2003; Edwards et al., 1997)
Coliphages males spécifiques (F^+) à ARN (I-IV) **Coliphages males spécifiques F^+ à ADN (FDNA)**	chez les animaux (I, II et IV) et les humains (II, III et IV). Chez les porcs (I et II). 90% de FRAN sont du groupe I dans les eaux de surface. Absents chez les oiseaux FDNA de type M13 (humaine)	Peuvent se multiplier dans les systèmes d'égout (eaux usées fraîches) et dans l'environnement	Coût élevé, rare (échantillon individuel). Pas de corrélation entre leur nombre et le nombre de virus entériques pathogènes	(Atherholt et al., 2003; Grabow et al., 2001; Long et al., 2005; Long et al., 2003; Tyagi et al., 2009)
Virus entériques humains	Chez les humains (lien étroit)	Stable (Adenovirus : Virus à ADN)	Mesure directe Faible nombre dans les environnements aquatiques Coût élevé et manipulation longue et difficile	(Gilpin et al., 2002; Tyagi et al., 2009)
***Pseudomonas aeruginosa* (PA)**	Chez 11 -16% des adultes et rare chez les animaux Ratio PA: CF	Production des enzymes qui détruisent *E. coli* Très sensible au rayonnement UV	Mise en culture sur agar sélectif, Nombre élevé dans les eaux non traitées et diminue beaucoup dans les eaux usées traitées (évaluation du procédé de traitement)	(de Victorica & Galvan, 2001; Deller et al., 2006; Howard et al., 2004; Long et al., 2002)

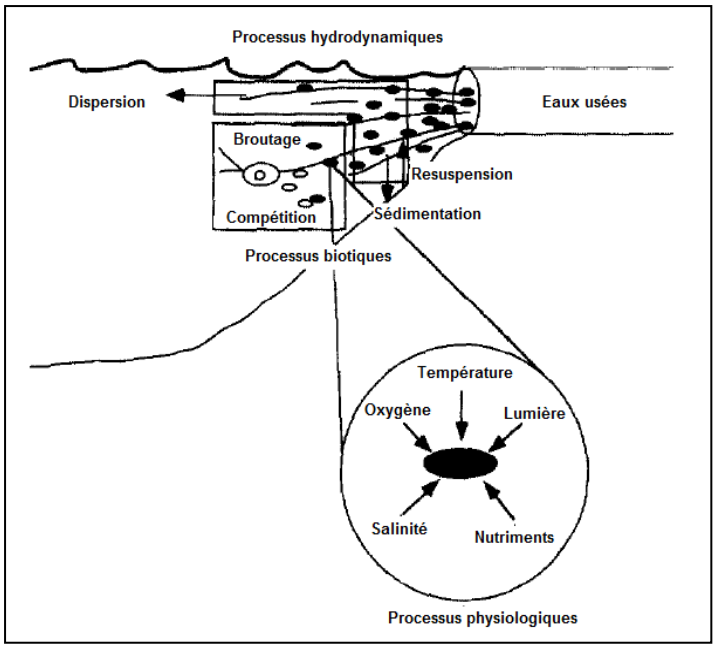

Figure 2.7. Principaux facteurs environnementaux impliqués dans le devenir des indicateurs microbiologiques fécaux rejetés dans les milieux aquatiques, tirée de (Troussellier et al., 1998).

Dans les sols naturels, l'enlèvement de la matière organique (par addition de peroxyde d'hydrogène) a augmenté la sorption de bactéries aux particules en suspension. Par hypothèse, l'augmentation du pH (après l'élimination de la matière organique) et la libération de la surface des particules d'argile étaient à l'origine de cette variation dans la sorption d'*E. coli*. Ajoutons que le pH du mélange (sol et effluent) était plus bas que le pH du sol, ce qui conduisait à la diminution de charges variables à la surface minérale du sol et favorisait alors l'attachement de bactéries au substrat organique. Les chercheurs ont conclu que la relation entre les coefficients de sorption de Freundlich «K_F» et la teneur en argile dépend de la présence et de l'absence de la matière organique. K_F était plus élevé en présence de la matière organique à condition que la teneur en argile demeure ≤ que 10%. En d'autres termes, plus la teneur en argile est élevée, plus la différence entre les valeurs de K_F avec et sans matière organique diminue. Les équations empiriques suivantes permettent de décrire la sorption d'*E. coli* avec des teneurs variables en matière organique (de 0% à 0,2% en carbone total) et en argile (de 0% à 20%) :

$$\left.\begin{aligned} \ln(KF) &= 0{,}8 + 2{,}1\ln(\%\ \text{argile})^{0{,}7} \\ {}^{1}/_{nF} &= 1{,}0 - 0{,}4\ln(\%\ \text{argile})^{0{,}3} \end{aligned}\right\} \quad 0\% < \text{Carbone total} < 0{,}2\%$$

Boutilier et al. (2009) ont calculé les coefficients de partition d'*E. coli* en microcosmes contenant des eaux usées différentes. Ils ont trouvé K_d = 19 000 mL g^{-1}, 324 000 mL g^{-1} et 293 mL g^{-1} respectivement dans l'effluent d'une fosse septique, l'effluent traité de lagune et les eaux usées d'une laiterie.

Généralement, la majorité des indicateurs microbiologiques (65% à 85%) restent en suspension dans la colonne d'eau (Jeng et al., 2005). Le pourcentage des indicateurs associés à des particules ayant tendance à sédimenter, varie légèrement d'une étude à l'autre. Il était estimé entre 15% et 30% d'après (Cizek et al., 2008), entre 25% et 35% d'après (Krometis et al., 2010) ou bien entre 16% et 47% d'après (Schillinger & Gannon, 1985). Des bactéries attachées à des particules en suspension (à concentration ≥ 50 mg L^{-1}), comme *E. coli*, avaient une vitesse de sédimentation constante (0,066 m/h) (Garcia-Armisen & Servais, 2009).

Des études ont montré que les indicateurs microbiologiques (coliformes fécaux, *Escherichia coli* et entérocoques fécaux) ont été adsorbés à des particules en suspension (de 0,45 µm à 30 µm de diamètre) dans les eaux de surverse afin d'atteindre le fond de l'estuaire par sédimentation. Ils ont traversé une distance de 1,61 km à 2,41 km du point de rejets de surverse. Les entérocoques sont préférentiellement fixés aux particules d'un diamètre qui varie entre 10 µm et 30 µm, tandis que les coliformes fécaux et *E. coli* ont tendance à s'adsorber à une vaste gamme de particules en suspension de diamètres différents. 20% et 90% d'*E. coli* ont été libres dans différentes eaux usées ou associées à des particules < 5 µm. Par contre, 10% à 50% d'*E. coli* ont été associées à des particules > 5 µm ayant des vitesses de sédimentation très basses (Boutilier et al., 2009; Jeng et al., 2005).

Le nombre d'*E. coli* attachés (21,8% à 30,4%) était plus élevé par rapport aux coliformes fécaux (9,8% à 27,5%) et aux entérocoques (8,3% à 11,5%) (Jeng et al., 2005). Les spores de *Clostridium perfringens* ont montré une forte affinité d'adsorption aux particules (50%) par rapport aux autres indicateurs microbiologiques (Cizek et al., 2008).

L'attachement aux sédiments permet de protéger les microorganismes contre la photolyse et de prolonger leur survie d'au moins sept jours (Jeng et al., 2005; Schillinger & Gannon, 1985). Le nombre de coliformes fécaux dans les sédiments côtiers peut devenir 10 à 10 000 fois plus élevé que leur nombre dans la colonne d'eau sus-jacente. Cependant, cette augmentation est dépendante de la composition granulométrique de sédiments et de la teneur en carbone organique

et en azote (Craig et al., 2002). L'augmentation de la concentration des entérocoques (> 100 fois plus) dans la rivière en amont du point de décharge de la station d'épuration (distance allant jusqu'à 0,5 km) est primordialement attribuée à la croissance *in situ* des entérocoques dans les sédiments du lit de la rivière (Litton et al., 2010).

Processus biotiques

La prédation par des protozoaires, la compétition avec des flores autochtones et l'infection par des bactériophages conduisant à la lyse bactérienne, sont les principaux processus biotiques qui contrôlent la disparition des indicateurs microbiologiques dans l'environnement aquatique. Les sédiments constituent un abri pour les microorganismes attachés contre la prédation (Schillinger & Gannon, 1985).

Processus physiologiques

Les conditions stressantes rencontrées dans l'environnement aquatique sont gérées par plusieurs paramètres, tels que la température de l'eau, la lumière solaire, la carence en nutriments, le pH, la salinité (eaux marines ou saumâtres) et l'oxygène dissous, etc. Les microorganismes ont démontré une capacité à évaluer l'environnement autour d'eux, de filtrer ce qui est pertinent et ce qui ne l'est pas et de prendre des décisions qui assurent la survie de la colonie dans son ensemble. Une réponse à ces conditions stressantes est l'entrée de microorganismes dans un stade de VBNC «Bactérie Viable mais Non Cultivable» ou ANC «Actives Non Cultivables» (Balleste & Blanch, 2010).

Les études, menées *in vitro*, sur les effets des paramètres environnementaux sont contradictoires. Il est très difficile d'imiter les conditions environnementales dans le laboratoire. En plus, les protocoles expérimentaux établis par les chercheurs sont très différents et difficilement comparables. Ils diffèrent par les conditions expérimentales, les souches bactériennes provenant des milieux différents et les méthodes de dénombrement utilisées.

Une étude en microcosmes a montré que les indicateurs microbiologiques de contamination fécale tels que les coliformes totaux, *Escherichia coli*, les entérocoques et les coliphages mâles spécifiques F^+ peuvent être inactivés sous l'influence de la température et la lumière solaire, indépendamment de leurs origines (affluent et effluent d'une station d'épuration, eaux de ruissellement dans un milieu urbain). Les entérocoques sont les plus persistants parmi ces

indicateurs en absence de la lumière. Une fois incubés en présence de la lumière solaire, ils se dégradent rapidement en comparaison avec *E. coli* et les coliphages mâles spécifiques F^+. Leur dégradation est lente à 14 °C (hiver) plutôt qu'à 20 °C (été) (Noble et al., 2004). D'autres études ont montré qu'une température élevée peut favoriser la survie de bactéries fécales dans les eaux naturelles (Oliver et al., 1995).

Par lagunage, l'inactivation naturelle de coliformes fécaux (*E. coli*) était la principale voie de leur élimination (de 52% jusqu'à >99%) en comparaison avec l'adsorption et la sédimentation. Elle est influencée par la température et la composition des eaux usées traitées (Boutilier et al., 2009).

Ballesté et Blanch ont effectué deux types d'expériences afin d'étudier la survie de *Bacteroides fragilis* (*B. fragilis*), *B. thetaiotaomicron*, et des espèces environnementales de *Bacteroides* : (i) sur le terrain (rivière), dans lequel les bactéries ont été exposées à des changements de plusieurs paramètres environnementaux et (ii) en microcosmes à température contrôlée. Les études *in situ* ont montré différents modèles de survie pour les souches cultivables de *Bacteroides*. *B. fragilis* ont été fortement affectés par l'effet combiné de la température élevée et de prédateurs (plus actifs dans des conditions plus chaudes). Ils possèdent la capacité de produire des enzymes qui leur permettent de croître en présence de faibles concentrations d'oxygène (Rocha et al., 2003). Par contre, *B. thetaiotaomicron* cultivables et les espèces environnementales de *Bacteroides* ont été plus touchés par la concentration de l'oxygène dissous dans l'eau. La durée de survie de ces dernières était plus élevée, grâce à une meilleure adaptation aux conditions environnementales. En comparaison avec les coliformes fécaux et les entérocoques, les *Bacteroidales* sont considérées comme indicateurs de contaminations fécales récentes (Balleste & Blanch, 2010).

Indicateurs microbiologiques *versus* microorganismes pathogènes

Des études ont montré que la présence et le comportement de coliformes fécaux, *Escherichia coli* et entérocoques fécaux dans l'environnement sont étroitement corrélés avec les salmonelles (Krometis et al., 2010). De même, *Giardia* et *Cryptosporidium* présentent certains comportements de transport similaires que les coliformes fécaux, l'*Escherichia coli*, les entérocoques fécaux et les spores de *Clostridium perfringens* (Cizek et al., 2008). Cependant, une étude statistique de 40 ans de données sur les corrélations entre les indicateurs microbiologiques et les microorganismes pathogènes, a révélé que les corrélations entre les coliformes et les parasites étaient en général faibles ou inexistantes (Wu, Rees, et al., 2011).

2.3.2.2 Indicateurs chimiques

Un indicateur chimique idéal présente une concentration constamment détectable dans l'eau usée, tout en étant épuré en même temps que les microorganismes pathogènes. De plus, cet indicateur ne doit pas être affecté par les changements environnementaux (pH, température, etc.) et sa détection dans l'environnement aquatique doit être corrélée à l'existence de pathogènes. Finalement, son taux de persistance dans l'eau doit être comparable à ceux de microorganismes pathogènes (Young et al., 2008b).

Parmi les indicateurs chimiques, citons entre autres, les antalgiques, les anti-inflammatoires, les antibiotiques, les bactériostatiques, les antiépileptiques, les béta-bloquants, les lipidorégulateurs, les produits de contraste, les cytostatiques, les contraceptifs oraux, les produits de soins personnels, etc. (Garric & Ferrari, 2005). Généralement, la classification de ces indicateurs est basée sur leur utilisation.

Origine des indicateurs chimiques

Les indicateurs chimiques, consommés ou utilisés en quantités très importantes, sont rejetés ou excrétés à l'état de traces dans le milieu aquatique soit sous forme active, soit sous forme conjuguée hydrosoluble et inactive (Defert & Huart, 2009; Khetan & Collins, 2007). Par exemple, plus de 90% de la dose administrée d'antibiotiques est excrétée dans les selles et les urines (Drillia et al., 2005).

Des études ont été réalisées pour la mise en présence de plus de 80 composés pharmaceutiques et métabolites dans les milieux aquatiques (Defert & Huart, 2009). Ces composés sont divisés en deux groupes : fécaux ou sous-produits de métabolisme et non fécaux provenant des activités humaines (Gilpin et al., 2002; Tyagi et al., 2009).

La Figure 2.8 montre les voies possibles d'introduction de ces polluants dans les milieux aquatiques (Agence Francaise de Sécurité Sanitaire des Aliments (afssa), 2010). Les stations d'épuration des eaux usées sont les principales sources de dispersion de ces composés dans les milieux aquatiques. Les excréments humains et animaux, les eaux de ruissellement urbaines et rurales, les effluents industriels, l'infiltration des eaux usées de surface dans les eaux souterraines et les lixiviats de décharges municipales d'ordures ménagères contribuent également à la pollution des eaux (y compris l'eau potable) (Alighardashi et al., 2008). Une analyse individuelle

de cours d'eau a indiqué que les concentrations des composés pharmaceutiques en aval de rejets augmentent durant les périodes où le débit est lent, ce qui montre que la majeure source de composés pharmaceutiques trouvés dans les eaux de surface provient de rejets urbains dans les rivières et les cours d'eau (Kolpin et al., 2004).

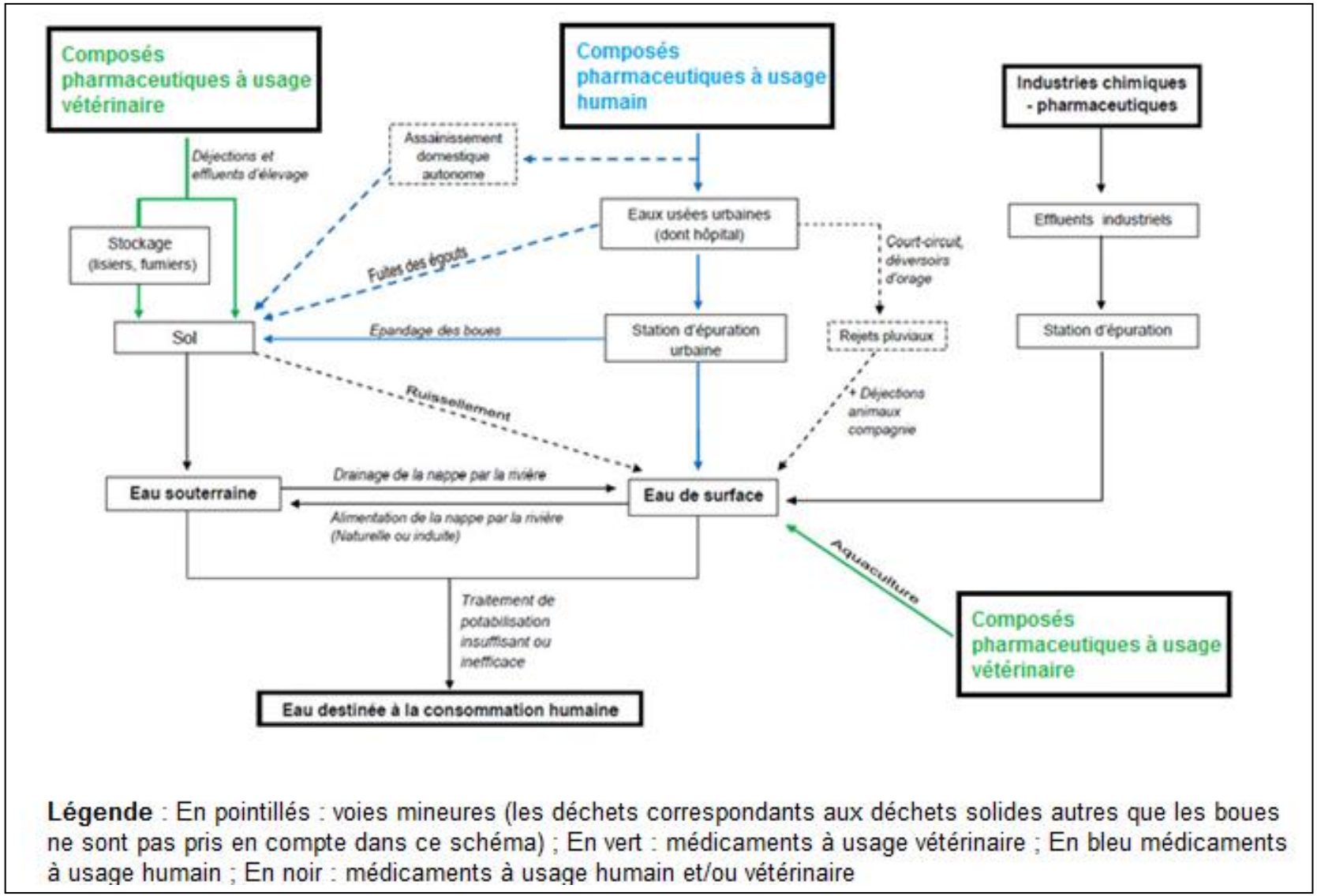

Figure 2.8. Origines des composés pharmaceutiques émergeant dans l'environnement aquatique, tirée de (Agence Francaise de Sécurité Sanitaire des Aliments (afssa), 2010).

Revue des propriétés des indicateurs chimiques

Les propriétés physico-chimiques et le comportement des indicateurs dans l'environnement sont des éléments clés pour leur caractérisation. Ils permettent d'évaluer le devenir de ces indicateurs dans les eaux (Figure 2.9). En d'autres termes, le degré et le type de l'atténuation naturelle (dilution, hydrolyse, photolyse, biodégradation, sorption irréversible et parfois décomposition radioactive) permettent de déterminer la présence subséquente et la distribution des indicateurs chimiques dans l'environnement (Lin et al., 2010).

Les caractéristiques physico-chimiques et environnementales d'un composé sont résumées dans les points suivants :

- la structure moléculaire ;

- la volatilité décrite par la tension de vapeur à 20°C et la constante de Henry ;

- la mobilité de la molécule décrite par la solubilité dans l'eau, le potentiel d'ionisation pKa, le log D ou D_{OW} (forme hydrosoluble à pH 7) et le coefficient de partage octanol/eau K_{OW} (caractérisant le caractère hydrophile/hydrophobe) ;

- Les interactions avec les constituants du milieu (Scheytt et al., 2005):

  - adsorption à la matière organique notamment dans le sol, l'eau ou les boues activées des stations d'épuration des eaux usées, traduite par la valeur du K_{OC},
  - adsorption sur le sol traduite par le K_d : liée aux caractéristiques du sol (texture), à la présence ou non d'argile et à la granulométrie des constituants,
  - formation de complexes avec les cations divalents (Ca^{2+}, Mg^{2+}) ou les éléments de transition présents dans l'environnement (Fe, Mn, etc.),

- les processus de dégradation abiotique (hydrolyse, photolyse),

- les processus de biodégradation (aérobie et anaérobie) ;

- la bioaccumulation (Agence Francaise de Sécurité Sanitaire des Aliments (afssa), 2010).

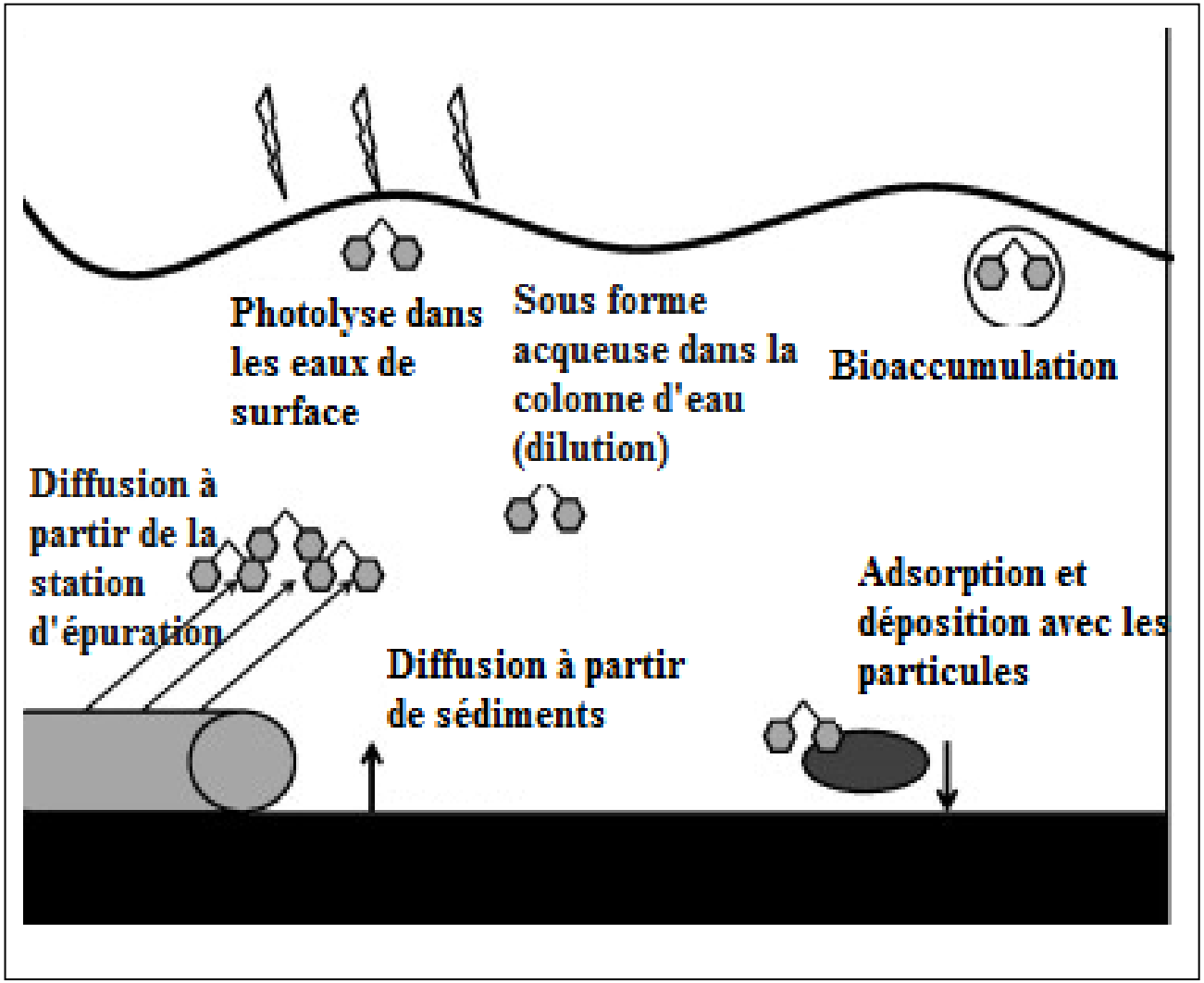

Figure 2.9. Modèle de système pour le devenir des indicateurs pharmaceutiques dans la colonne d'eau, tirée de (Wilson et al., 2009).

Le Tableau 2.5 résume les caractéristiques des indicateurs chimiques de la pollution fécale d'origine humaine qui sont fréquemment cités dans la littérature (Monteiro & Boxall, 2010; Suárez et al., 2008; Suarez et al., 2010). Une analyse de données sur des indicateurs chimiques utilisés pour le dépistage de contamination fécale des eaux de surface est présentée dans le Tableau 2.6.

Mécanismes d'adsorption sur une matrice solide

L'adsorption de substances polluantes dissoutes en milieu aquatique est un phénomène complexe faisant intervenir de nombreux mécanismes physico-chimiques au niveau des liaisons entre les molécules dissoutes et la phase solide du milieu récepteur (sédiment ou sol). Les principales liaisons physico-chimiques sont : les forces de Van der Waals et de Coulomb; les liaisons hydrogène; l'échange de ligands; les liaisons covalentes; les interactions dipôle-dipôle; et les liaisons hydrophobes (Tableau 2.7).

Le coefficient de partage (K_d) permet de caractériser le rapport entre la concentration en substance polluante adsorbée au niveau de la matrice du milieu récepteur, et la concentration en substance polluante dissoute dans l'eau (Ramaswami et al., 2005). Le Tableau 2.8 compile les valeurs de coefficients de partition des composés chimiques les plus étudiés.

La majorité des composés chimiques utilisés sont organiques (Monteiro & Boxall, 2010). Les phénomènes de leur adsorption sur des particules solides du milieu récepteur sont de type liaisons hydrophobes (Scheytt et al., 2005). L'adsorption de ces composés électriquement neutres et possédant des solubilités dans l'eau très faibles, est fortement dépendante de la teneur en matière organique (f_{OC}) du milieu (Zhang et al., 2010).

Les paramètres qui affectent plus ou moins l'adsorption de polluants sur la matrice solide sont nombreux. Leur contribution dépend des caractéristiques de polluants. On peut citer parmi ces paramètres : le pH, la force ionique, la composition du sol, l'épandage de boues sur le sol et la mobilité des contaminants (Monteiro & Boxall, 2010).

Tableau 2.5. Caractéristiques physico-chimiques des indicateurs chimiques, tiré de (Karnjanapiboonwong et al., 2010; Suárez et al., 2008; Williams, Ong, et al., 2009).

Composé pharmaceutique	Solubilité dans l'eau (mg/L)	Constante de Henry H (µg m^{-3} air/ µg m^{-3} eaux usées	Constante de dissociation pKa	Log K_{OW}
Antibiotiques				
Triméthoprime (TMP)	400	$9{,}8.10^{-13}$	6,6-7,2	0,9-1,4
Roxithromycine (ROX)	0,02	$1{,}0.10^{-24}$	9,2	2,1-2,8
Sulfamethoxazole (SMX)	610	$2{,}6.10^{-11}$	5,6-6,0	0,5-0,9
Erythromycine (ERY)	1,4	$2{,}2.10^{-27}$	8,9	2,5-3,0
Antidépresseurs				
Fluoxétine (FLX)	60	$3{,}6.10^{-6}$	10,1	4,05
Citalopram (CTL)	31	$1{,}1.10^{-9}$	9,6	2,9-3,7
Contraceptifs oraux				
Estrone (E1)	30	$1{,}6.10^{-8}$	10,4	3,1-3,4
17β-Estradiol (E2)	3,6	$1{,}5.10^{-9}$	10,4	3,9-4,0
17α-Ethinylestradiol (EE2)	11,3	$3{,}3.10^{-10}$	10,5-10,7	2,8-4,2
Anti-inflammatoires				
Ibuprofène (IBP)	21	$6{,}1.10^{-6}$	4,9-5,7	3,5-4,5
Naproxène (NPX)	16	$1{,}4.10^{-8}$	4,2 (a)	3,2
Diclofenac (DCF)	2,4	$1{,}9.10^{-10}$	4,0-4,5	4,5-4,8
Antiépileptiques				
Carbamazépine (CBZ)	17,7	$4{,}4.10^{-9}$	13,9	2,3-2,5
Muscs				
Galaxolide (HHCB)	1,8	$4{,}5.10^{-3}$	-	5,9-6,3
Tonalide (AHTN)	1,2	$5{,}1.10^{-3}$	-	4,6-6,4
Célestolide (ADBI)	0,22	$7{,}3.10^{-1}$	-	5,4-6,6
Tranquillisants				
Diazépame (DZP)	50	$1{,}5.10^{-7}$	3,3-3,4	2,5-3,0
Produits de contraste				
Iopromide (IPM)	23,8	$4{,}1.10^{-27}$	-	-
Stimulants psychomoteurs				
Caféine (CAF)	$2{,}16.10^{4}$		10,4	-0,07

Tableau 2.6. Analyse de données sur des indicateurs chimiques de la contamination fécale des eaux de surface.

	Indicateurs	Sources	Taux de persistance	Avantages/Limites	Références
Indicateurs fécaux	**Stérols et Stanols**	Coprostanol/cholestanol > 1 (humaine) Coprostanol/cholestanol ≤ 1 (essentiellement non humaine) Chez les humains (60% coprostanol : produit de réduction de cholestérol) Chez les animaux supérieurs (l'épicoprostanol est le produit de réduction du cholestérol) Chez les herbivores (24-ethylcoprostanol et 24-ethylepicoprostanol : produits de réduction de 24-ethylcholestérol dérivés des plantes) Chez les animaux carnivores (absence de 5β-stanols)	Leur persistance est fonction des conditions environnementales (aérobies versus anaérobies) Dégradation dans 2 semaines dans les colonnes d'eau Stables en conditions anaérobies (sédiments) exemple coprostanol, sa demie-vie est de 450 j à 15 °C et en aérobiose (cours d'eau): < 10 j à 20 °C	Les ratios coprostanol/24-ethylcoprostanol et Coprostanol/épicoprostanol sont fiables Les ratios stérols/stanols dépendent du régime alimentaire, de la capacité des animaux à les métaboliser et de la composition de la microflore intestinale biohydrogénant les stérols en stanols à configurations variables Réduction de cholestérol et de 24-ethylcholestérol en cholestanol et 24-ethylcholestanol respectivement dans l'environnement Présence naturelle dans les sédiments Concentration diminue avec la dilution	(Atherholt et al., 2003; Düreth et al., 1986; Gilpin et al., 2002; Sargeant, 1999; Tyagi et al., 2009)

Tableau 2.6. Analyse de données sur des indicateurs chimiques de la contamination fécale des eaux de surface (suite).

	Indicateurs	Sources	Taux de persistance	Avantages/Limites	Références
Indicateurs fécaux	**Acides biliaires secondaires : acide lithocolique (LCA) et acide deoxycolique (DOCA)**	Chez les humains et les animaux supérieurs Les ruminants (DOCA) et les non ruminants (LCA) Chez les porcs (absence de DOCA et présence des acides hyocholiques)	Plus stables aux dégradations que les 5β-stanols et trouvés à des concentrations plus élevées Persistent dans les sédiments des milliers d'années	Tests plus sensibles en comparaison avec 5β-stanols	(Bull et al., 2002; Tyagi et al., 2009)
	Caféine	Se présente naturellement dans plus de 60 espèces de plantes, y compris les graines du café, cacao, les arbres du cola et dans les feuilles de thé Ajoutée aux boissons et aux ingrédients pharmaceutiques (consommation de 80-400 mg/personne/jour) Déchet agro-industriel majeur généré par les stations de traitement du café et du thé (décaféination) 20-300 mg/L dans les eaux usées, ≤0,15 mg/personne par jour dans les eaux usées traitées Les surverses de réseaux unitaires sont les principales sources de la caféine	Biodégradable par des bactéries de genres *Pseudomonas* et Serratia, par *Klebsiella* sp. et *Rhodococcus* sp. (isolées des eaux usées), par *Alcaligenes* sp. CF8 (isolée de l'eau de surface) et par des champignons appartenant aux genres *Aspergillus, Penicillium, Rhizopus* et *Stemphyllium* (par des enzymes dé-méthylase ou oxidase) Dégradable en présence de la lumière solaire (photolyse indirecte, $t½ = 1,5 \pm 0,4$ j) Adsorption aux sols: *Pahokee* Peat Soil et *Elliott Silt Loam Soil* (K_F =16,85 (mg/g)(L/mg)$^{1/n}$; 1/n = 1,05 et K_F = 15,32 ; 1/n = 1,28 respectivement). Se présente dans les eaux de surface et souterraines (à concentrations plus basses)	Soluble dans l'eau Traité efficacement dans les STEP (> 99%) Par ozonation (dose 100 μM) : 100% d'élimination après 5 min Exige sa présence en concentration élevée et près de la source de contamination Pas de sorption au carbone organique particulaire, donc ne peut pas suivre les microorganismes décantés (dans les sédiments) surtout pendant la tempête Production des denrées alimentaires contenant de la caféine à proximité d'une source en cours d'évaluation limite son utilisation comme indicateur Utilisé comme indicateur de contamination fécale des eaux de surface par les eaux usées non traitées (surverses de réseaux unitaires, rejets directs, etc.) mais sa sensibilité dépend des conditions régionales et diminue avec la diminution de l'efficacité de son abattement dans les STEP Corrélation avec la présence de pathogènes dans les eaux de surface	(Buerge et al., 2006; Buerge et al., 2003; Field & Samadpour, 2007; Gokulakrishnan et al., 2005; Lam et al., 2004; Madyastha et al., 1999; Mazzafera, 2002; Mohapatra et al., 2006; Piocos & de la Cruz, 2000; Scott et al., 2002; Soh et al., 2011; Young et al., 2008b)

Tableau 2.6. Analyse de données sur des indicateurs chimiques de la contamination fécale des eaux de surface (suite).

	Indicateurs	Sources	Taux de persistance	Avantages/Limites	Références
Indicateurs fécaux	**Carbamazépine**	Affluents urbains (50% de CBZ administré et ses métabolites sont excrétés dans les urines et les fèces), 700 Kg de CBZ entrent dans les réseaux d'égouts/année Effluents de STEP (seulement 7%-8,1% de CBZ entrant la STEP est éliminé)	Persistance élevée ($t½$ = 328 j) Dans de l'eau pure, $t½$ = 121,6 j (en présence de la lumière naturelle). $t½$ diminue en présence de nitrate (11,2-69 j) et du carbone organique dissous (8,3-14,4 h) à cause de la génération du radical $HO^{\cdot}$ Dans l'eau douce : $t½ = 82 \pm 11$ j et de 1 j (avec wesimulateur solaire)	Indicateur d'une ancienne contamination fécale Le plus fréquemment détecté dans les effluents des STEP (jusqu'à 6,3 µg/L), dans les eaux de surface (jusqu'à 2,1 µg/L), les eaux souterraines (jusqu'à 0,41 µg/L), l'eau potable (jusqu'à 0,26 µg/L) et dans les sédiments (jusqu'à 49 ng/g) Son adsorption est irréversible et proportionnelle à la teneur en matière organique et en argile et à la salinité (dans le sol), à la force ionique (adsorption à l'albumine). Elle est dépendante de la nature de matière organique dans le sol	(Andreozzi et al., 2002; Clara et al., 2004; Guo & Krasner, 2009; Kolpin et al., 2004; Lam et al., 2004; Lienert et al., 2007; Loffler et al., 2005; Matamoros et al., 2009; Mohle & Metzger, 2001; Stein et al., 2008; Ternes, 1998; Tixier et al., 2003; Williams et al., 2006; Zhang et al., 2010; Zhang, Geissen, et al., 2008)

Tableau 2.6. Analyse de données sur des indicateurs chimiques de la contamination fécale des eaux de surface (suite).

	Indicateurs	Sources	Taux de persistance	Avantages/Limites	Références
Indicateurs fécaux	**Acétaminophène**	Utilisation annuelle de 1310 t (Japon 2002) Ratio d'excrétion du composé parental = 68% Détecté dans les eaux usées	Probablement, rapidement biodégradable dans l'environnement aquatique : 80% biodegradation dans une période de 72 h ($t_{½}$ = 2.1 j) dans l'eau de rivière Son attachement au sédiment et à la matière organique dissoute est peu probable La lumière solaire affecte sa persistance ($t_{½}$ = 0,9 ±0,2 j) : photolyse indirecte par réaction avec le radical $HO^{\cdot}$ Sa biodégradation est plus importante que sa photolyse	Son adsorption est basse sur la silice (- à pH neutre), l'alumine (+ à pH neutre) et sur une matrice synthétique hydrophobe Son devenir dans l'environnement aquatique n'est pas significativement affecté par la sorption Légèrement bioaccumulatif % d'abattement par boues activées (MLSS = 3000 mg/L, 6h) >99,5% et la fraction sorbée = 2,9%	(Lam et al., 2004; Lorphensri et al., 2006b; Yamamoto et al., 2005; Yamamoto et al., 2009)
	Théophylline	Sous-produit de la caféine (par catabolisme chez les humains) Excrétée dans l'urine Se trouve sous forme de trace dans le cocoa, les graines vertes du café (5 mg/Kg) et le thé noir (200-400 mg/Kg poids sec) Production de 1000 à 5000 tonnes/an Ajouté aux boissons (Guarana), aux médicaments et aux produits cosmétiques	Biodégradable par *Pseudomonas putida* CBB5 (oxydase) isolée d'un sol enrichi par la caféine : 1-Méthylxanthine et 3-méthylxanthine sont les deux produits de dégradation Dégradation par photolyse indirecte par réaction avec le radical $HO^{\cdot}$, $t_{½}$ = 20 h Sa persistance dans l'eau est >1 an Son transport dans l'eau (99.98%), dans l'air, le sédiment, le sol et le biote (<0.1%)	La dégradation de la caféine par les bactéries ne produit pas de la théophylline (spécifique aux humains)	(Mohapatra et al., 2006; Organisation for Economic Co-operation and Development (OECD), 2004; Yu, Louie, et al., 2009)

Tableau 2.6. Analyse de données sur des indicateurs chimiques de la contamination fécale des eaux de surface (suite).

	Indicateurs	Sources	Taux de persistance	Avantages/Limites	Références
Indicateurs fécaux	**Sucralose**	Utilisé dans plus de 80 pays : en Europe (2004), aux États-Unis (1999) et au Canada (1991) car il est >600× plus sucré que le sucre de table Le niveau de consommation moyenne quotidienne est de 1,3 mg / kg de poids corporel/ jour 85% du sucralose consommé est excrété sous sa forme parentale La concentration de sucralose mesurée dans les affluents d'eaux usées est >10,8 µg/L	Persistant par suite des processus métaboliques et dans l'environnement Dégradation très lente dans les sols humides, les eaux usées anaérobies, les eaux et les sédiments de lacs : biodégradation partielle par des processus cométaboliques (minéralisation de 55% après 4 semaines) Pas de photolyse après 5 h d'exposition aux UV Hydrolyse très lente (1% après un an) à température élevée (37 °C et 65 °C) et pH acide = 3, A pH neutre, l'hydrolyse n'est pas détectable < 5% de sucralose éliminé par sorption aux particules du sol Adsorption aux sols : *Pahokee* Peat Soil et *Elliott Silt Loam Soil* (K_F = 4,05 (mg/g)(L/mg)$^{1/n}$; 1/n = 1,01 et K_F = 0,421 ; 1/n = 0,113 respectivement).	Possède un spectre de masse unique Non bioaccumulatif (K_{OW} = 0,3) peu ou pas de toxicité signalée à des concentrations dans l'environnement Pas d'abattement de sucralose dans les stations d'épuration des eaux usées, à l'exception d'un traitement avec post-ozonation suivie d'une filtration sur sable (enlèvement jusqu'à 31%) Accumulation potentielle dans les eaux de surface (>1 µg/L) Limite de détection par SPE-LC-MS-MS (RP-LC/ESI-MS-MS) = 10 ng/L (un peu élevée par rapport aux autres indicateurs) Hydrophile, très soluble dans l'eau, n'est pas susceptible de se répartir dans les boues d'épuration et les sols (adsorption très faible) et peut être transporté dans les effluents de stations d'épuration vers les eaux de surface et souterraines. En comparaison avec la caféine, c'est un meilleur indicateur des activités anthropogènes Permet d'indiquer la présence d'autres composés hydrophiles anthropiques à des concentrations inférieures et qui sont plus difficiles à identifier Fournit avec d'autres indicateurs une chronologie de la contamination fécale d'origine humaine	(Grice & Goldsmith, 2000; Hollender et al., 2009; Labare & Alexander, 1993, 1994; Loos et al., 2009; Soh et al., 2011)
	Urobiline	Chez les humains	10% de dégradation après 2j, constant dans le sédiment (en aérobiose et anaérobiose)	Test très sensible	(Miyabara et al., 1994; Piocos & de la Cruz, 2000)

Tableau 2.6. Analyse de données sur des indicateurs chimiques de la contamination fécale des eaux de surface (suite).

	Indicateurs	Sources	Taux de persistance	Avantages/Limites	Références
Indicateurs non fécaux	**Agents fluorescents d'avivage (FWAs)**	Humaine Contamination de fosses septiques Trouvés dans les eaux souterraines peu profondes	Ne sont pas rapidement biodégradables, persistent dans l'environnement, Résilients mais se perdent rapidement dans les sédiments Concentration diminue avec la dilution Comparaison au sein d'un seul bassin hydrologique	S'attachent aux matières particulaires et colloïdales en proportion significative L'indicateur le plus approprié à discriminer entre les sources de contamination fécale (humaine versus animale) en comparaison avec les ratios de stanol (Coprostanol/24-ethylcoprostanol et Coprostanol/épicoprostanol) Grande variabilité de fluorescence de fond naturel d'un bassin à l'autre Coût et temps minimes au laboratoire Long travail sur le terrain	(Gilpin et al., 2002; Gregor et al., 2002)

Tableau 2.7. Mécanismes d'adsorption pour les composés chimiques acides et basiques, A – acide; B – base; H – proton; MO – Matière organique, tiré de (Monteiro & Boxall, 2010).

Composés acides		
pKa > 10	**3 < pKa < 10**	**pKa < 3**
Forme prédominante		
AH	**Ratio A/AH**	**A**
Mécanismes d'adsorption		
Interactions hydrophobes (MO, argile)	**Sols tempérés**	
Van der Waals (MO, argile)	**Répulsion anionique par les adsorbants chargés négativement**	
Liaisons-hydrogène (MO, argile)	**Pontage de cations (MO, argile)**	
	Liaisons-hydrogène	
	Transfert de charges (MO)	
	Van der Waals (MO)	
	Sols tropicaux	
	Échange des anions (Al, Fe, (hydr)oxides)	
	Échange de ligands ((oxi)hydroxides protonés, MO)	
	Pontage de cations (Échange de ligands : H_2O–métal)	
Composés basiques		
pKa > 10 (pKb < 4)	**3 < pKa < 10 (4< pKb <11)**	**pKa < 3 (pKb > 11)**
Forme prédominante		
BH^+ ou B^+	**Ratio BH^+/B ou B^+/B(OH)**	**B ou B(OH)**
Mécanismes d'adsorption		
Échange de cations (MO, argile)	**Partition hydrophobe (MO, argile)**	
Transfert de charge (MO)	**Van der Waals (MO, argile)**	
	Liaisons-hydrogène (MO, argile)	
	Échange de ligands (MO)	
	Transfert de charges (MO)	

Tableau 2.8. Coefficients d'adsorption des composés pharmaceutiques sur le sol, les boues (primaires et secondaires) et le sédiment, tiré et adapté de (Li & Zhang, 2010; Monteiro & Boxall, 2010; Suárez et al., 2008; Suarez et al., 2010; Ternes et al., 2004).

Composé	Coefficient d'adsorption K_d sol (L/Kg) CO ↓ et argile ↑ / CO ↑ et argile ↓	Coefficient d'adsorption corrigé pour la teneur en carbone organique K_{OC} sol (L/Kg) CO ↓ et argile ↑ / CO ↑ et argile ↓	K_d boues (L/Kg) Boues 1aires/Boues 2aires	K_{OC} boues (L/Kg) Boues 1aires/Boues 2aires	K_d (L/Kg) Sédiment
TMP			nd/2,3log		
ROX		62,2±21,6/530±16,9	nd/2,3log-2,6log		
SMX	0,23±0,08/37,6±1,2		nd/2,3log-2,6log		
ERY	164,76		nd/2,2log		
FLX	134,44±0,90-234,83±2,36	2746,33±9,72-7553,34±89,68	176,75±2,06 nd/0,7log	535,59±6,25	
CTL			nd/ 2,0log		
E1		3,14log	402±126 nd/2,4log-2,9log	3,16log	
E2		3,30log	476±192 1468 nd/2,4log-2,8log	3,24log	
EE2		3,35log	584±136 278±3/349±37 2,4log/2,5log-2,8log	3,32log 794±95/860±140	
IBP			453,79 nd/71±2,0 <1,3log/0,9log	nd/21±4	0,18-1,69
NPX	10,13±0,36-252,90±4,77	445,86±47,88-3743,23±184,19	217,20 42,46±2,19	217,20 128,65±6,64	
DCF	0,45±0,03/164,5±6,6	121±8/2310±93	459±32/16±3 2,7log/1,2log	1310±180/47±32	0,55-4,66
CBZ	4,66±0,18-32,78±1,01 0,49±0,01/37±1,6	253,55±9,59-584,61±16,45 132±2,7/521±23	75,33±0,84 25,52 nd/1,2±0,5 <1,3log/0,1log	228,26±2,53 nd/3,5±1,5	0,21-5,32 1,3
HHCB			3,7log/3,3log		
AHTN			3,7log/3,4log		
ADBI			3,7log/3,9log		
DZP			44±26/21±8 1,6log/1,3log	125±75/62±23	3,0
IPM			nd/11±1 <0,7log/1,0log	nd/32±5	
CAF	0,99/0,99	3,89/2,87	< LOQ		

2.3.2.3 Autres indicateurs

L'ADN mitochondrial des cellules eucaryotes

L'ADN mitochondrial ayant plusieurs copies dans la cellule eucaryote de tous les individus (10^6 copies mL^{-1} chez les humains, 10^2 copies mL^{-1} chez les chiens, 10^4 copies mL^{-1} chez les bovins et 10^3 copies mL^{-1} chez les porcs), présente des séquences non conservées différentes d'un hôte à l'autre. Une étude a démontré que le nombre de copies d'ADN mitochondrial par gramme de fèces pouvait atteindre jusqu'à 1,7 x 10^7 (Schill & Mathes, 2008), ce qui est largement suffisant pour l'utiliser comme marqueur de contamination fécale. Il a été utilisé pour différencier des contaminations fécales d'origine humaine et animale (porcine, bovine, ovine, aviaire). La différentiation des origines de la contamination fécale dépend de l'alimentation. Par exemple, la consommation des aliments d'origine animale conduit à l'excrétion de cellules animales par l'homme (Caldwell & Levine, 2009; Kortbaoui et al., 2009; Martellini et al., 2005; Roslev & Bukh, 2011).

2.4 Corrélation entre les indicateurs microbiologiques et chimiques

Il n'y a pas toujours de corrélation entre les indicateurs microbiologiques et alternatifs mais l'utilisation de ces deux types d'indicateurs en combinaison peut être bénéfique et a démontré une plus grande efficacité pour déterminer l'origine de la pollution fécale (Blanch et al., 2006; Field & Samadpour, 2007). Tyagi et al. (2009) ont démontré qu'une forte corrélation, fonction de la saison, existe entre les concentrations de coprostanol et d'*E. coli* (Tyagi et al., 2009). Isobe et al. (2002) ont trouvé que 30-100 ng L^{-1} de coprostanol correspondent à 1000 UFC mL^{-1} de coliformes fécaux (Isobe et al., 2002). Piocos et de la Cruz ont décrit une forte corrélation entre l'urobiline et les coliformes fécaux dans les rivières (Piocos & de la Cruz, 2000). À leur tour, Gilpin et al (2003) ont démontré une contamination fécale humaine avec un ratio de coprostanol/cholestérol > 0.5 et des concentrations élevées de coprostanol, d'épicoprostanol, d'agents fluorescents d'avivage (FWA), de *Bactéroides-Prevotella*, d'*E. coli* et également de *Bifidobacterium adolescentis* avec des ratios élevés de coprostanol/24-ethylcoprostanol et de coprostanol/24-ethylepicoprostanol(Gilpin et al., 2003).

Pour utiliser les indicateurs en combinaison (indicateur générique qui offre une information quantitative concernant la charge fécale dans l'eau et indicateur discriminant qui permet

d'identifier l'origine spécifique de la pollution fécale), il faut tenir compte des facteurs qui affectent l'application des modèles prédictifs en approche DSPM. Parmi ces facteurs, citons entre autres les effets de dilution dans les eaux réceptrices, les différents types de pollution animale, la persistance de l'indicateur dans l'environnement et les mélanges complexes des différentes sources de contamination fécale (Blanch et al., 2006).

Des chercheurs ont suggéré l'utilisation des indicateurs microbiens pour les contaminations récentes et les études de courte durée alors qu'ils ont préféré les indicateurs chimiques (bio-marqueurs lipidiques) pour les investigations de longue durée (Tyagi et al., 2009). Cependant, une étude a utilisé seulement des indicateurs chimiques pour différencier entre les sources de contamination fécale. Parmi les 110 indicateurs utilisés, 35 ont été classifiés comme spécifiques pour les humains et le meilleur indicateur était le coprostanol (Glassmeyer et al., 2005). Young *et al.* (Young et al., 2008b) ont étudié trois composés dans l'eau usée. Ils ont prouvé que l'utilisation de triclosan et de triclocarban ainsi que ces deux derniers combinés à la caféine comme indicateurs chimiques de la contamination fécale était plus efficace que la caféine toute seule. Notons que la caféine est rapidement et complètement dégradable par oxydation sous l'influence de la lumière solaire (UV) (Buerge et al., 2003; Jacobs et al., 2012; Lam et al., 2004). Également, le triclosan et le triclocarban sont biodégradables en présence d'oxygène et stables en conditions anaérobies. Ils sont non volatils et peu solubles dans l'eau à 20 °C. La résistance microbienne augmente en leur présence (Hua et al., 2005). Ils s'attachent aux microorganismes mais l'adsorption du triclosan dépend du pH (Young et al., 2008b).

Le Tableau 2.9 résume la fréquence de choix des indicateurs fécaux utilisés pour détecter la contamination fécale des eaux de surface ainsi que pour retracer les sources de cette contamination. Comme exemples, les virus entériques humains, les *Pseudomonas aeruginosa*, la caféine, l'urobiline et les agents fluorescents d'avivage sont fréquemment utilisés pour détecter les contaminations fécales d'origine humaine.

Tableau 2.9. Choix des indicateurs microbiologiques et chimiques pour le dépistage des sources de contamination fécale dans les eaux de surface (XXX : plus utilisable, XX : moyennement utilisable et X : peu utilisable) (Atherholt et al., 2003; de Victorica & Galvan, 2001; Deller et al., 2006; Edwards et al., 1997; Field & Samadpour, 2007; Gavini et al., 1991; Gilpin et al., 2003; Gilpin et al., 2002; Howard et al., 2004; Kreader, 1995; Long et al., 2002; Long et al., 2003; Lynch et al., 2002; Mara & Oragui, 1981, 1983; Resnick & Levin, 1981; Rhodes & Kator, 1999; Sargeant, 1999; Tyagi et al., 2009).

	Source Humaine	**Source Non Humaine**	**Source Sauvage**	**Source Agriculturelle**	**Source Urbaine ou Mixte**	**Source Ponctuelle**	**Source Diffuse ou Non Ponctuelle**	**Source Récente**
Coliformes Fécaux (*E. coli*)	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XX
Streptococcus bovis	X	XXX	XXX	XXX	XX	XXX	XXX	XX
Bacteroidales	XXX	XXX	XXX	XXX	XXX	XXX		XXX
Bifidobacterium	X	X	X	X	X	XXX	XXX	XXX
Bacteriophages de *B. fragilis*	XXX				XXX	XXX	X	XXX
Rhodococcus coprophilus		XXX		XXX		XXX	XXX	X
Clostridium perfringens	XXX	XXX	XXX	XXX	XXX	XXX		
Coliphages males spécifiques (F^+) à ARN (I-IV) et $^+$ à ADN (FDNA)	X	X	X	X	X	X	X	
Virus entériques humains	XXX				XXX	XXX	XXX	
Pseudomonas aeruginosa	XXX				XXX	XXX		
Stérols et Stanols	XXX	XXX	XXX	XXX	XXX	XXX	X	X
Acides biliaires secondaires : acide lithocolique (LCA) et acide deoxycolique (DOCA)	XXX	XXX	XXX	XXX	XXX	XXX	XXX	
Caféine	XXX				XXX	XX	XX	XXX
Urobiline	XXX							XX
Agents fluorescents d'avivage (FWAs)	XXX				XXX	XXX		

2.5 Méthodes de dépistage des sources de pollution microbienne

La Figure 2.10 résume les différentes méthodes utilisées pour le dépistage des sources de pollution microbienne. Ces méthodes permettent de déterminer l'origine de la pollution fécale dans des environnements aquatiques. Elles sont divisées en deux catégories : méthodes basées sur des indicateurs microbiologiques et méthodes basées sur des indicateurs chimiques.

Les méthodes microbiologiques sont phénotypiques, génotypiques, avec ou sans banque de données (Haznedaroglu et al., 2005; Plummer & Long, 2009). Les méthodes phénotypiques (non moléculaires) sont basées sur la comparaison des caractéristiques cellulaires ou physiologiques (forme, métabolisme et conditions de croissance), utilisant surtout des méthodes traditionnelles de culture. Les méthodes génotypiques comparent les séquences d'ADN au moyen de techniques modernes moléculaires. Les méthodes dépendantes de banques de données utilisent le plus souvent *E. coli* et *Enterococcus* comme microorganismes fécaux indicateurs (Stoeckel et al., 2004). Elles consistent à choisir un microorganisme fécal indicateur d'une source de contamination et à établir une banque de référence des isolats individuels de ce microorganisme (Booth et al., 2003). Par contre, les méthodes indépendantes de banques de données sont fondées sur la détection de marqueurs spécifiques aux hôtes afin d'attribuer la contamination fécale à un hôte humain ou à un animal spécifique. Elles reposent le plus souvent sur l'utilisation de *Bactéroides* sp. (Bernhard & Field, 2000; Field et al., 2003).

Une nouvelle méthode de DSPM indépendante de banque de données a été développée pour identifier les sources de contamination fécale de l'eau d'origine humaine et animale. Elle est basée sur l'ADN mitochondrial de cellules animales (hôtes) entraînées par les matières fécales dans l'intestin. Cette méthode moléculaire est simple et rapide. Elle consiste à utiliser des séquences d'ADN mitochondrial pour créer des amorces de PCR spécifiques pour l'ADN humain et de divers animaux à l'aide de protocoles de PCR nichée en combinaison avec la technique dot blot ou hybridation ADN-ADN (Kortbaoui et al., 2009; Martellini et al., 2005).

Les méthodes chimiques sont fréquemment utilisées pour le dépistage de la pollution humaine. L'analyse des indicateurs pharmaceutiques est effectuée par des méthodes quantitatives, citons entre autres les méthodes colorimétrique et enzymatique, la chromatographie en phase gazeuse ou liquide et la spectrométrie de masse. Récemment, la méthode de l'extraction en phase solide

couplée à la spectrométrie de masse en tandem (on-line SPE-LC–MS/MS) a été automatisée afin d'analyser les contaminants organiques présents sous forme de traces dans les eaux de surface. Cette technique permet de réaliser des purifications et une concentration efficace de l'échantillon et d'analyser des échantillons ayant des concentrations en composés d'intérêt de l'ordre du ng/L (Viglino, Aboulfadl, Prévost, et al., 2008). Les analytes choisis sont des agents anti-infectieux (clarithromycin, sulfaméthoxazole et triméthoprime), un anticonvulsant (carbamazépine) et son produit de dégradation (10,11-dihydrocarbamazépine), l'agent antihypertensif (enalapril), des antinéoplastiques utilisés en chimiothérapie (cyclophosphamide et méthotrexate), des herbicides (atrazine, cyanazine, et simazine) et des produits de transformation de l'atrazine (deséthylatrazine et déisopropylatrazine) ainsi qu'un agent antiseptique (triclocarban). Les limites de détection de la méthode développée sont faibles (entre 0,6 à 6 ng/L). (Garcia-Ac et al., 2009).

Les auteurs ont critiqués les méthodes de DSPM dans plusieurs dizaines d'articles (Field & Samadpour, 2007; Griffith et al., 2003; Meays et al., 2004; Plummer & Long, 2009; Stoeckel et al., 2004). Ils ont conclu qu'il n'existe pas de méthode standard pour le DSPM. Le Tableau 2.10 et le Tableau 2.11 présentent les avantages et les inconvénients de méthodes de dépistage des sources de pollution microbienne. Le choix d'une telle ou telle méthode est basé sur différents facteurs citons entre autres, le type du bassin hydrologique, les sources potentielles de contamination et les indicateurs fécaux choisis, la différenciation entre les sources humaines et animales ou bien entre les différentes sources animales, le temps et le financement disponibles ainsi que le niveau de certitude et de résolution requis.

Globalement, des méthodes indépendantes d'une banque de données (moléculaires et chimiques) ont été sélectionnées pour ce travail pour plusieurs raisons : (i) 60 à 80% du microbiote fécal humain est représenté par des bactéries non cultivables (Suau et al., 1999) ; (ii) l'état de «Bactéries Viables mais Non Cultivables» est souvent rencontré dans les milieux aquatiques naturels ; (iii) l'échappement de l'étape de mise en culture et enfin (iv) la rapidité et la spécificité de ces méthodes.

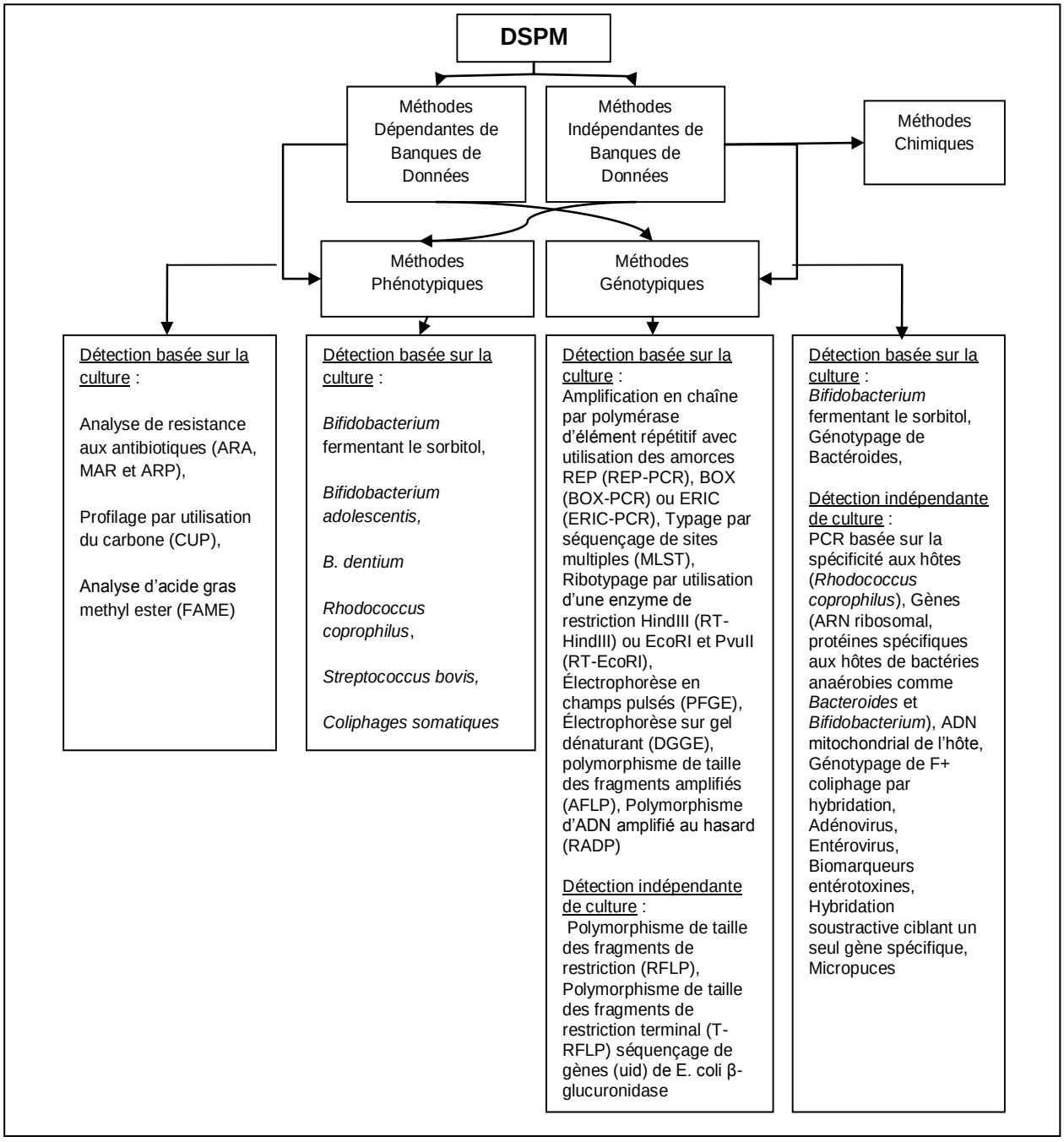

Figure 2.10. Aperçu du dépistage des sources de pollution microbienne (DSPM) (Bernhard & Field, 2000; Booth et al., 2003; Cimenti et al., 2007; Edge & Schaefer, 2006; Field et al., 2003; Field & Samadpour, 2007; Griffith et al., 2003; Haznedaroglu et al., 2005; Meays et al., 2004; Plummer & Long, 2009; Stoeckel et al., 2004).

Tableau 2.10. Avantages et désavantages des méthodes moléculaires de DSPM (Meays et al., 2004; Scott et al., 2002).

Méthodes	Avantages	Inconvénients
Rybotypage	Hautement reproductible, facile et permet la classification d'isolats provenant de sources multiples	Complexe, coûteuse, méthodologie exigeante et variable, demande une banque de données locale, résultats parfois variables
Électrophorèse en champs pulsé (PFGE)	Sensible aux réarrangements chromosomiques des souches et hautement reproductible	Sensibilité très élevée, demande une banque de données, longue manipulation
Électrophorèse sur gradient dénaturant (DGGE)	Utilisé pour des isolats	Demande une banque de données, exigeante du point de vue technique, demande beaucoup de temps, pas bon pour les isolats environnementaux
Amplificationen chaine par polymérase de certaines régions répétitives (Rep-PCR)	Simple et rapide	Préoccupation pour la reproductibilité, demande une culture cellulaire et de grande banque de données, variabilité augmente parallèlement avec la taille de banque de données
Length heterogeneity PCR (LH-PCR)	Ne demande ni culture ni banque de données	Équipements coûteux, méthodologie exigeante
Polymorphisme de longueur des fragments de restriction terminaux (T-RFLP)	Ne demande ni culture ni banque de données	Équipements coûteux, méthodologie exigeante
Marqueur ciblant l'ADNr 16S, spécifique à l'hôte (combinaison de LH-PCR et T-RFLP)	Ne demande ni culture ni banque de données, indicateur de pollution récente (*Bacteroidales*, *Bifidobacterium*)	Seulement testée sur des marqueurs humains et bovins, équipements coûteux, méthodologie exigeante, peu d'informations sur le devenir de *Bacteroides* spp. dans l'environnement
MAR (résistance multiple aux antibiotiques)	Rapide et permet de différencier les sources de contamination animale	Demande une banque de données, variable géographiquement, absence des isolats non résistants aux antibiotiques
BOX-PCR	Rapide et facile	Demande une banque de données locale

Tableau 2.11. Principaux avantages et inconvénients des méthodes de culture/sans culture et des méthodes dépendantes/indépendantes d'une banque de données (Ahmed et al., 2009; Field & Samadpour, 2007; Gilpin et al., 2002; Roslev & Bukh, 2011; Young et al., 2008b).

Méthodes	Avantages	Inconvénients
Avec culture	- Peu coûteuses - Accès technique aisé - Augmentation du nombre de microorganismes cibles pendant la phase de croissance exponentielle - Différenciation entre les contaminations humaines et animales	- Limités à des souches cultivables - Changement drastique de la composition de communautés microbiennes après culture - Semi-quantitative - Souches peu spécifiques de l'hôte - Pas de différenciation entre les espèces animales - Manipulations intensives - Besoin de nombreux échantillons - Faible stabilité géographique de la banque de données - Taille limite de la banque de données

Tableau 2.11. Principaux avantages et inconvénients des méthodes de culture/sans culture et des méthodes dépendantes/indépendantes d'une banque de données (Ahmed et al., 2009; Field & Samadpour, 2007; Gilpin et al., 2002; Roslev & Bukh, 2011; Young et al., 2008b) (suite).

Méthodes	Avantages	Inconvénients
Sans culture	- Détection de bactéries cultivables et non cultivables - Représentation plus large de la population microbienne - Différenciation entre les contaminations humaines et animales - possibilité d'une étape d'enrichissement - Quantitatives (selon le choix) - Simple - Réponse rapide (quelques heures)	- Matériels et équipements onéreux - Peu de corrélation avec les indicateurs classiques et les pathogènes - Persistance des cibles non déterminée - Stabilité géographique non déterminée
Dépendantes d'une banque de données	- Discrimination entre les contaminations humaines et animales - Rapide	- Inexactitude sur le terrain - Classifications erronées - Faux positives - Résultats négatifs - Manque de spécificité à l'hôte (*E. coli* et entérocoques) - Besoin des banques de plus en plus larges et représentatives - Animaux sauvages mal représentés - Banque de données n'est pas toujours cosmopolite - Laborieuses et coûteuses
Indépendantes d'une banque de données	- Pas besoin de banque à chaque site - Discrimination entre les contaminations humaines et animales - Rapide - Quantitative	- Manque de spécificité absolue à l'hôte entre les marqueurs microbiens associés aux humains et aux animaux - Manque de stabilité temporelle de certains marqueurs microbiens dans différents groupes d'hôtes - Transfert horizontal de gène de marqueurs avec des toxines et/ou des gènes virulents - Marqueurs microbiens peu abondants chez certains hôtes et/ou populations - Besoin d'un nombre plus grand de gènes cibles différents (spécifiques) - Faux positifs dépendant de l'alimentation (ex, marqueur mitochondrial bovin retrouvé dans des selles humaines où les individus avaient consommé de la viande bovine)

CHAPITRE 3 OBJECTIFS DE RECHERCHE ET DÉMARCHE EXPÉRIMENTALE

3.1 Objectifs de recherche

3.1.1 Objectif général

L'objectif général est de développer une approche générale pour le dépistage de sources de contamination fécale dans les cours d'eau urbains afin de permettre une meilleure priorisation de sources pour des mesures correctives.

3.1.1.1 Objectifs spécifiques

Les objectifs spécifiques de ce projet sont les suivants :

<u>Article 1</u> :

1. développer et valider une méthode de mesure de dix indicateurs chimiques anthropogènes (acétaminophène (ACE), diclofénac (DIC), carbamazépine (CBZ), aténolol (ATL), caféine (CAF), théophylline (THEO), progestérone (PRO), medroxyprogestérone (MedP), aspartame (APM) et N,N-diéthyl-3-méthylbenzamide (DEET)) dans des matrices complexes telles que les eaux et les sédiments des trop-pleins et des cours d'eau urbains, basée sur la chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS);
2. évaluer l'importance de l'analyse des indicateurs chimiques (ACE, CAF, THEO et CBZ) dans les sédiments par rapport à leur analyse dans l'eau pour dépister les sources de contamination fécale d'origine humaine dans des sites à différents degrés de contamination;

Article 2 :

3. illustrer le rôle de sédiments des trop-pleins dans le devenir et le transport des contaminants émergents (produits pharmaceutiques) lors d'évènements pluvieux importants ou lors d'importantes fontes de neige en étudiant leurs phénomènes de sorption dans des microcosmes (eau : sédiments de trop-plein) par la méthode d'équilibres successifs. Pour ce faire, des objectifs secondaires ont été sélectionnées : 1) développer une méthode permettant l'analyse simultanée du phénomène de sorption d'une sélection de cinq contaminants émergents (ACE, CAF, THEO, CBZ et 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH)) sur la phase particulaire de différentes matrices (SS et StS), 2) mesurer les concentrations de ces contaminants dans les sédiments de trop-pleins, 3) déterminer les coefficients de sorption apparente ($K_{d,app}$) dans des matrices dopées et non dopées, 4) déterminer les cinétiques de ces contaminants dans des matrices naturelles sous différentes conditions (statiques et dynamiques) simulant alors l'addition des eaux pluviales et la forte turbulence dans les systèmes d'égouts et 5) évaluer les contaminants émergents choisis comme traceurs d'eaux usées dans les surverses par rapport à leur dynamique de sorption;

Article 3 :

4. déterminer les indicateurs de contaminations fécales les plus appropriés parmi des indicateurs chimiques et moléculaires spécifiques aux humains afin d'identifier les raccordements inversés tout en considérant leurs concentrations mesurées, leurs limites de détection ainsi que le territoire étudié;
5. évaluer les relations possibles entre les indicateurs de DSPM, microbiologiques (*E. coli* et coliformes fécaux), chimiques (ACE, CAF, THEO et CBZ) et moléculaires (marqueurs ciblant les *Bacteroidales* (HF183) et l'ADN mitochondrial (Hmt) spécifiques aux humains) provenant des sources de contamination fécale dans des bassins versants à activités différentes, par des analyses statistiques comparatives;

6. confirmer la valeur de référence standard de la caféine (400 ng L^{-1}) proposée dans la littérature comme seuil pour la priorisation des secteurs avec des raccordements inversés et de recommander des mesures correctives le cas échéant;
7. proposer des valeurs seuils de référence des indicateurs alternatifs ciblés afin d'établir un indice de contamination fécale d'origine humaine par des raccordements inversés et ainsi de prioriser les travaux de réfection du système de drainage le plus problématique.

3.2 Hypothèses de recherche

Le Tableau 3.1 présente les hypothèses de recherche et leur originalité dans le travail présenté.

Tableau 3.1. Hypothèses de recherche

	Objectifs spécifiques	**Hypothèses**	**Originalité**	**Réfutabilité**
1	Développer et valider une méthode de mesure de dix indicateurs chimiques anthropogènes dans des matrices complexes telles que les eaux et les sédiments des trop-pleins et des cours d'eau urbains, basée sur la chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS).	L'analyse des indicateurs chimiques dans les sédiments apporte davantage de précision en comparaison à la phase aqueuse pour le dépistage des sources de contamination fécale.	Actuellement, l'emphase des plans de dépistage et de protection est mise sur l'analyse de la phase aqueuse. Cependant, en combinant les analyses chimiques de la phase aqueuse et de la phase solide (eau/sédiments), on augmente les possibilités d'interprétation en ce qui concerne les causes et les effets de la contamination fécale des eaux.	L'hypothèse sera réfutée si les concentrations de ces indicateurs dans les sédiments sont négligeables par rapport à leurs concentrations dans la phase aqueuse.
2	Évaluer l'importance de l'analyse des indicateurs chimiques dans les sédiments par rapport à leur analyse dans l'eau pour dépister les sources de contamination fécale d'origine humaine dans des sites à différents degrés de contamination.		Aucune étude n'analyse à ce jour les indicateurs chimiques de la contamination fécale d'origine humaine dans les sédiments de trop-pleins. Ainsi, ses concentrations sont les premières mesures publiées dans ce domaine.	

Tableau 3.1. Hypothèses de recherche (suite).

	Objectifs spécifiques	**Hypothèses**	**Originalité**	**Réfutabilité**
3	illustrer le rôle de sédiments des trop-pleins dans le devenir et le transport des produits pharmaceutiques lors d'évènements pluvieux importants ou lors d'importantes fontes de neige en étudiant leurs phénomènes de sorption dans des microcosmes (eau : sédiments de trop-plein) par la méthode d'équilibres successifs.	Le pic des concentrations de pharmaceutiques au début d'un événement de débordement est directement lié à la désorption des pharmaceutiques dans les dépôts solides dans les égouts (trop-pleins). En d'autres mots, la vitesse de désorption est suffisamment élevée pour minimiser les effets de dilution lors de l'ajout des eaux pluviales	La sorption (désorption) des produits pharmaceutiques n'a jamais été étudiée pour les dépôts solides des égouts. Comparer les vitesses de cinétiques d'adsorption et de désorption des pharmaceutiques dans les trop-pleins. Imiter les conditions réelles des trop-pleins et choisir les meilleurs traceurs d'eaux usées dans les surverses par rapport à leur dynamique de sorption.	L'hypothèse sera réfutée s'il n'y a pas de désorption significative de pharmaceutiques avec un ajout d'eau pluviale.
		La procédure de dopage de sédiments peut influencer leur répartition et par suite les valeurs de $K_{d,app}$.	La comparaison entre les valeurs de $K_{d,app}$ de cinq produits pharmaceutiques dans des matrices dopées et non dopées et l'importance d'utilisation des matrices naturelles dans les études de sorption.	L'hypothèse sera réfutée si les valeurs de $K_{d,app}$ sont semblables.

Tableau 3.1. Hypothèses de recherche (suite).

	Objectifs spécifiques	**Hypothèses**	**Originalité**	**Réfutabilité**
4	Déterminer les indicateurs de contaminations fécales les plus appropriés parmi des indicateurs chimiques et moléculaires spécifiques aux humains afin d'identifier les raccordements inversés tout en considérant leurs concentrations mesurées, leurs limites de détection ainsi que le territoire étudié.	Les indicateurs chimiques spécifiques sont plus sensibles pour le dépistage de contamination fécale que les indicateurs moléculaires spécifiques.	l'utilisation des marqueurs spécifiques en parallèle a permis de s'affranchir d'éventuelles erreurs d'interprétation et ainsi avoir un diagnostic des plus précis.	L'hypothèse sera réfutée si les concentrations des indicateurs dans les différents territoires ne sont pas significativement différentes.
5	Évaluer les relations possibles entre les indicateurs de DSPM, microbiologiques (*E. coli* et coliformes fécaux), chimiques (carbamazépine, caféine, acétaminophène et théophylline) et moléculaires (marqueurs ciblant les *Bacteroidales* et l'ADN mitochondrial spécifiques aux humains) provenant des sources de contamination fécale dans des bassins versants à activités différentes par des analyses statistiques comparatives.	Une corrélation existe entre les marqueurs génétiques de contamination fécale humaine, soit le marqueur *Bacteroides* HF183 et l'ADN mitochondrial et les indicateurs chimiques, soit la caféine, la théophylline, l'acétaminophène et la carbamazépine.	La corrélation entre les marqueurs chimiques et génétiques de contamination fécale humaine n'a jamais été étudiée	L'hypothèse sera réfutée si les corrélations entre les indicateurs classiques et les indicateurs alternatifs, ne sont pas significatives dans les eaux pluviales étudiées.

Tableau 3.1. Hypothèses de recherche (suite).

	Objectifs spécifiques	**Hypothèses**	**Originalité**	**Réfutabilité**
6	Proposer des valeurs seuils de référence des indicateurs alternatifs ciblés afin d'établir un indice de contamination fécale d'origine humaine par des raccordements inversés et ainsi de prioriser les travaux de réfection du système de drainage le plus problématique.	La combinaison de différents marqueurs DSPM permet la résolution des pollutions fécales mixtes.	Développer un indice de contamination sanitaire constitué de plusieurs marqueurs non discriminants (FC et *E. coli*) et discriminants chimique et moléculaires.	L'hypothèse sera réfutée si les niveaux de contamination du territoire étudié ne dépassent pas les limites de détection des marqueurs ciblés.

3.3 Méthodologie

3.3.1 Développement méthodologique

La première étape a consisté de faire la sélection de la méthode utilisée afin d'approfondir la connaissance sur la répartition de dix micropolluants utilisés comme indicateurs de contamination fécale d'origine humaine. La revue de la littérature des méthodes disponibles nous a permis d'identifier les méthodes d'extraction en phase solide et de détection basée sur la spectrométrie en masse en combinaison avec la chromatographie en phase liquide pour leur simplicité et leur capacité à détecter et quantifier ces composés dans des matrices complexes. Le principal défi de ce travail réside dans le traitement des échantillons environnementaux de façon à éliminer les interférents potentiels, isoler et concentrer les analytes d'intérêt (indicateurs chimiques) et fournir enfin un procédé multi-résidus, simple, fiable et reproductible indépendant de la variabilité et de la complexité de la matrice et fait face aux composés sélectionnés de caractéristiques différentes et présents à l'état de trace dans l'environnement.

Les grandes étapes initiales de la méthode appliquée aux sédiments des trop-pleins et des cours d'eau urbains ont été les suivantes:

- séchage de sédiments par lyophilisation;
- extractions successives assistées par ultrasons;
- évaporation de la phase organique surnageante après centrifugation;
- purification et concentration des analytes cibles par extraction en phase solide (SPE-Solid phase extraction) en utilisant une cartouche de type hydrophile-lipophile balance Oasis® HLB;
- quantification des indicateurs chimiques après ionisation chimique à pression atmosphérique en mode positifs en reposant sur une méthode chromatographique en phase liquide LC couplée à la spectrométrie de masse MS-MS avec un mode balayage sélectif d'ions fragments SRM.

La revue de littérature des méthodes de prétraitement et d'analyse des échantillons solides a révélé plusieurs variations dans les prétraitements. Le principal volet de l'optimisation de la

méthode à développer visait à maximiser l'extraction des analytes d'intérêt pouvant être mesurés tout en gardant au minimum l'extraction des interférents. Différentes variations des conditions dans lesquels se produit le prétraitement et l'analyse ont été expérimentées. Visant le mécanisme et les solvants d'extraction, la purification, l'enrichissement et l'analyse des composés, les essais ont porté sur l'influence du volume et du pH de l'échantillon initial, du volume et de la nature du solvant d'extraction, du temps d'extraction, de la température du milieu d'extraction, du nombre de cycles d'extraction, du type de cartouche SPE, du pH d'extrait, du débit de chargement, du volume et de la composition de la solution de lavage, du volume et de la nature du solvant d'élution, de la nature des phases mobile et stationnaire, du mode d'ionisation et de la source de détection sur la capacité de la méthode à générer des concentrations quantifiables d'analytes avec un temps minimum d'analyse. Les résultats obtenus par la meilleure combinaison de conditions figurent en détail dans le Chapitre 4. L'application de la méthode s'est finalement faite dans différentes matrices telles que les eaux et les sédiments des cours d'eau urbains et des trop-pleins.

L'étape suivante était l'application de la méthode développée dans des essais d'adsorption/désorption sur les échantillons solides et liquides. Pour ces essais, on a choisi quatre indicateurs chimiques parmi les dix utilisés dans le développement de la méthode analytique. C'est pour cette raison qu'on a adapté la méthode développée pour son application à quatre des composés déjà utilisés (acétaminophène, carbamazépine, caféine et théophylline) en plus d'un métabolite de carbamazépine. Les études cinétiques de sorption/désorption ont été effectuées à l'aide d'une méthode d'équilibres successifs, en utilisant une méthode en parallèle. Les détails de la partie expérimentale ainsi que les résultats obtenus dans le cadre de ces travaux sont présentés dans le Chapitre 5.

La dernière étape consiste à apprendre la méthode d'amplification en temps réel (qPCR) des marqueurs moléculaires ciblant les *Bactéroidales* et les mitochondries de cellules eucaryotes de l'organisme hôte. Pour y parvenir, nous avons visité les laboratoires de Pr. George Di Giovanni de l'Université A&M du Texas et de Pr. Richard Villemur de l'INRS Institut Armand-Frappier à Laval en été 2010 et 2011. Nous avons pu, par la suite, monter le laboratoire de Niveau II de la Chaire et implanter la méthode semi quantitative de PCR ciblant les *Bactéroidales* dans des échantillons d'eau et de sédiments en 2012. Parallèlement, nous avons implanté une méthode d'extraction et de quantification de coliformes (*E. coli*) dans les sédiments. Pour réduire nos tâches au laboratoire, nous avons décidé de donner les échantillons (eau et extraits de sédiments

provenant du ruisseau US_2 et des bassins versants, MEA et DEN) au groupe de Pr. Richard Villemur afin de faire les analyses des ADN mitochondriaux. Les coliformes fécaux sont également mesurés par le Réseau de surveillance du milieu aquatique de la Ville de Montréal (RSMA). Les détails sur toutes les méthodes analytiques utilisées sont disponibles dans les études menées par (APHA et al., 1998; Besner et al., 2010; Centre d'expertise en analyse environnementale du Québec, 2009; Hajj-Mohamad et al., 2014; Villemur et al., 2015).

3.3.2 Campagne d'échantillonnage

La méthodologie des campagnes d'échantillonnage détaillée dans les trois articles (voir Chapitres 4, 5 et 6) et les études menées par (Guérineau et al., 2014; Madoux-Humery et al., 2013) ne sera pas présentée dans cette section. En bref, les campagnes d'échantillonnage ont eu lieu dans les eaux et les sédiments des deux ruisseaux US_1 et US_2, d'une rivière US_3, et d'un canal d'eau potable. Ce sont des cours d'eau urbains qui ont des contaminations fécales historiques suivant des surverses ou écoulements par temps sec. Plusieurs trop-pleins situés en amont des sites d'échantillonnage de la rivière US_3 ont été sélectionnés à l'aide de la Ville de Laval.

Les échantillonnages de surverses (OA et OB), de leurs bassins de drainage (SA et SB) en amont immédiat des conduites de surverses (par temps sec), d'une seule rive de la rivière US_3 et d'un canal d'eau potable (par temps sec et temps de pluie) ont été faits par nos collègues Anne-Sophie Madoux-Humery (en doctorat) et Hélène Guérineau (en maîtrise).

Dans le but d'aider les municipalités à dépister les raccordements inversés, nous avons contacté la Ville de Montréal qui nous a accompagnés pour échantillonner les bassins versants du ruisseau US_2 (bassin versant MEA) et d'un autre ruisseau canalisé US_4 (bassin versant DEN) dont les réseaux d'assainissement sont séparatifs.

CHAPITRE 4 ARTICLE 1 : WASTEWATER MICROPOLLUTANTS AS TRACERS OF SEWAGE CONTAMINATION: ANALYSIS OF COMBINED SEWER OVERFLOW AND STREAM SEDIMENTS

Ce chapitre présente une méthode de mesure dans les sédiments de 10 micropolluants choisis comme traceurs sanitaires potentiels de contamination fécale d'origine humaine. Dans un premier temps, l'optimisation des procédures expérimentales a été réalisée en compilant et comparant les différents protocoles présents dans la littérature. Cette méthode a été appliquée aux sédiments provenant des trop-pleins et des cours d'eau urbains dont la teneur en carbone organique et la distribution de taille des particules étaient différentes. Les rapports de la concentration moyenne sur la limite de détection (C: LOD) dans les sédiments d'un sous-ensemble des composés ont été comparés aux rapports respectifs dans l'eau. Dans les eaux avec une grande capacité de dilution relative des sources fécales, ce rapport était plus élevé dans les sédiments que dans l'eau. Donc, les programmes de dépistage des sources de contamination fécale utilisant les micropolluants des eaux usées devraient envisager l'échantillonnage des sédiments, en particulier pour les eaux avec des sources de contamination fécale hautement diluées. Ces résultats ont été publiés dans *Environmental Science: Processes & Impacts* en octobre 2014.

WASTEWATER MICROPOLLUTANTS AS TRACERS OF SEWAGE CONTAMINATION: ANALYSIS OF COMBINED SEWER OVERFLOW AND STREAM SEDIMENTS

M. Hajj-Mohamad[a,b], K. Aboulfadl[c], H. Darwano[c], A.-S. Madoux-Humery[b], H. Guérineau[b], S. Sauvé[c*], M. Prévost[b], S. Dorner[a]

[a] Canada Research Chair in Source Water Protection, Department of Civil, Geological and Mining Engineering, Polytechnique Montréal, C.P. 6079, Succursale Centre-ville, Montréal, Qc, Canada H3C 3A7; [b] NSERC Industrial Chair in Drinking Water Treatment, Department of Civil, Geological and Mining Engineering, Polytechnique Montréal, C.P. 6079, Succursale Centre-ville Montréal, Qc, Canada H3C 3A7; [c] Department of Chemistry, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montreal, QC, Canada H3C 3J7; *Corresponding author. Phone: +1 514 343-6749, e-mail: sebastien.sauve@umontreal.ca.

4.1 Abstract

A sensitive method was developed to measure the sediment concentration of 10 wastewater micropollutants selected as potential sanitary tracers of sewage contamination and include: nonsteroidal anti-inflammatory drugs (acetaminophen – ACE and diclofenac – DIC), an anti-epileptic drug (carbamazepine – CBZ), a ß-blocker (atenolol – ATL), a stimulant (caffeine – CAF), a bronchodilator (theophylline – THEO), steroid hormones (progesterone – PRO and medroxyprogesterone – MedP), an artificial sweetener (aspartame – APM) and personal care products (N,N-diethyl-3-methylbenzamide – DEET). Natural sediments (combined sewer overflow and stream sediments) were extracted by ultrasonic-assisted extraction followed by solid-phase extraction. Analyses were performed using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) using atmospheric pressure chemical ionisation in positive mode (APCI+) with a total analysis time of 4.5 min. Method detection limits were in the range of 0.01 to 15 ng g^{-1} dry weight (dw) for the compounds of interest, with recoveries ranging from 75% to 156%. Matrix effects were observed for some compounds, never exceeding |±18%|. All results displayed a good degree of reproducibility and repeatability, with relative standard deviations (RSD) of less than 23% for all compounds. The method was applied to an investigation of stream and combined sewer overflow sediment samples that differed in organic carbon contents and particle size distributions. Acetaminophen, caffeine and theophylline (as confounded with paraxanthine) were ubiquitously detected at 0.13-22 ng g^{-1} dw in stream bed sediment samples and 98-427 ng g^{-1} dw in combined sewer overflow sediment samples. Atenolol (80.5 ng g^{-1} dw) and carbamazepine (54 ng g^{-1} dw) were quantified only in combined sewer overflow sediment samples. The highest concentrations were recorded for DEET (14 ng g^{-1} dw) and progesterone (11.5 ng g^{-1} dw) in stream bed and combined sewer overflow sediment samples, respectively. The ratio of concentration to its limit of detection (C : LOD) in sediments for a subset of compounds were compared to their C : LOD in water. In waters with a large capacity for dilution relative to fecal sources, the C : LOD ranges in sediments were greater than in water. Thus monitoring programs for fecal source tracking using wastewater micropollutants should consider sediment sampling, particularly for waters with highly diluted sources of fecal contamination.

Environmental impact

A sensitive method was developed to measure the sediment concentration of 10 wastewater micropollutants selected as potential sanitary tracers of sewage contamination. This method was applied to an investigation of stream and combined sewer overflow sediment samples that differed in organic carbon contents and particle size distributions. The ratio of the average concentration to the limit of detection (C : LOD) in sediments for a subset of compounds were compared to their C : LOD in water. In waters with a large capacity for dilution relative to fecal sources, the C : LOD in sediments were greater than in water. Thus monitoring programs for fecal source tracking using wastewater micropollutants should consider sediment sampling, particularly for waters with highly diluted sources of fecal contamination.

4.2 Introduction

Wastewater micropollutants (WWMPs) have the potential to produce detrimental effects in the environment (Hernando et al., 2006). Their presence in various matrices such as wastewater influents and effluents, combined sewer overflow (CSO) effluents, surface waters, sources of drinking waters and public water supply has been widely documented (Bahlmann et al., 2009; Benotti & Brownawell, 2007; Benotti et al., 2009; Buerge et al., 2006; Cardoso et al., 2011; Daneshvar et al., 2012; Ellis, 2006; Fatta et al., 2007; Heberer et al., 2002; Phillips & Chalmers, 2009; Viglino, Aboulfadl, Daneshvar, et al., 2008; Wilkison et al., 2002). Recent research has indicated that while some compounds displace easily within the water column, others are hydrophobic and have a tendency to adsorb onto sediments (Chefetz et al., 2008; Durán-Álvarez et al., 2012; Fenet et al., 2012; Mader et al., 1997; Martinez-Hernandez et al., 2013; Stein et al., 2008; Williams et al., 2006; Yu, Fink, et al., 2009; Zhang et al., 2013). Thus, to account for and bring both hydrophylic and hydrophobic compounds into consideration, it is necessary to sample the water column and bed sediments. Water samples and sediments were also analysed to source track fecal sewage pollution in discharges into aquatic environment (Guérineau et al., 2014; Hagedorn & Weisberg, 2009; Hernando et al., 2006). Measured concentrations of WWMPs normally vary from ng/L to µg/L (water samples) or ng/g to µg/g (sediment samples) (Buerge et al., 2003; da Silva et al., 2011; Gracia-Lor et al., 2010; Kolpin et al., 2002; Lahti & Oikari, 2012; Stolker et al., 2004; Ternes, 1998; Tixier et al., 2003; Vanderford et al., 2003; Wille et al., 2010; Wu et al., 2008). However, their concentrations remain unknown in CSO sediments which play a role of vector for WWMPs (Ogrinc et al., 2007) in sewer systems. Such data are needed for

investigating the contribution of storm waters and combined sewer overflows to the accumulation of fines and associated contaminants in the bed sediments of the receiving streams. Such deposited sediments could also contribute to the desorption of sorbed contaminants into receiving waters. The perturbations in contaminant inputs caused by combined sewer overflow (CSO) events can disrupt steady-state conditions and could confound the use of wastewater tracers (Benotti & Brownawell, 2007). Furthermore, knowledge about the concentrations of WWMPs in stream sediments is also necessary to understand the routing, transport and fate of these contaminants in the environment and for estimating their persistence and environmental risks.

There are no standard methods for extraction, elution, concentration and detection for many compounds. To identify and measure WWMPs in sediments, adequate analytical methods with sufficiently low detection limits need to be developed, due to the high sulphur content of anoxic sediments and the potentially high contaminant loadings (Tadeo et al., 2012). LC–MS/MS has become the preferred analytic technique for determining polar environmental pollutants (Kovalczuk et al., 2006; Minten et al., 2011; Primel et al., 2012). Atmospheric pressure chemical ionisation (APCI) is employed in a few studies but its use has increased since it seemed to be less prone to matrix effects than standard electrospray ionization (ESI) (Trufelli et al., 2011; Wu et al., 2010; Zhang et al., 2011). It is commonly used as an interface for the LC–MS analysis of medium and low polarity substances (Souverain et al., 2004; Wick et al., 2010; Zhao & Metcalfe, 2008).

It is nearly impossible to analyse all WWMPs. A preselection of target analytes is therefore crucial in developing an index of human fecal pollution. The standards for preselection that must be considered for WWMPs are: extensive and increased annual use (3–4% by weight per annum) (Daughton, 2004), dissimilar structural and physico-chemical properties, pharmacokinetic behavior, frequent detection in wastewaters, some affinity with solids, potential toxic effect doses/concentrations, relative environmental persistence, usage as anthropogenic waste indicators (AWIs) and presence at trace concentrations in real samples, hence requiring more advanced and laborious analytical tools for their accurate determination.

Given that our focus is to find good tracers of anthropogenic impact on waters and sediments, we have chosen to focus on ten WWMPs belonging to the following groups: ß-blockers, analgesics,

anti-inflammatory drugs, stimulants, diuretics, a sweetener, an antiepileptic drug, personal care products and hormones (see details in the Supplementary material section†[1]).

The objectives of this study were to: (1) develop a sensitive method for the separation and quantification of WWMPs in different types of solids (*e.g.*, sewer and stream sediments) based on an ultra-high-performance liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry (UHPLC–APCI–MS/MS), (2) compare concentrations in sediments with concentrations in water to evaluate the use of sediment sampling for fecal source tracking.

4.3 Methods and materials

4.3.1 Description of study sites

Five study sites were selected for the analysis of sediments and water: two urban streams (US_1 and US_2), a large river (US_3), an urban drinking water supply canal and 1 CSO which is discharged upstream of the sampling site (US_3). The two small ungauged urban streams in the Greater Montréal Area with mean measured dry weather flow rates of less than 0.1 m^3 s^{-1} were selected because of elevated dry weather fecal indicator bacteria (*Escherichia coli* concentrations greater than 400 MPN per 100 mL) potentially contaminated from cross-connected sewers. Both urban streams are upstream of drinking water supplies. The large river with a mean flow rate of 1000 m^3 s^{-1} is used for drinking water supply as described by Madoux-Humery et al. (2013) and receives discharges from storm sewers and combined sewer overflows. The selected CSO receives a mixture of sewage and precipitation primarily from foundation and roof drains. The CSO consists of a 355 mm round pipe draining into a chamber with an overflow structure that channels flow to a 450 mm round pipe draining to a separate storm sewer. The urban canal was selected as a site with very low concentrations of fecal indicator bacteria (dry weather concentrations generally less than 50 MPN per 100 mL) with suspected inputs of sewage from sewer exfiltration. Details regarding the urban canal including WWMP results are available in Guérineau et al. (2014) and are presented in this study for method validation and evaluation of WWMPs in sediments as tracers for field sites with varying degrees of fecal contamination.

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[1] † Electronic Supplementary Information (ESI) is available in Section 4.8 of this chapter.

4.3.2 Apparatus

The system set-up for analysis of WWMPs is described in ESI.†

4.3.3 Procedure

4.3.3.1 Sample collection and pretreatment

Sediment samples were collected from the upper 3 cm of the stream bed in urban streams (US_1) (n = 5), (US_2) (n = 11), (US_3) (n = 5), in canal (n = 15) and CSO (C, n = 3) in the Greater Montréal area. They were taken during dry weather in the summer and fall of 2011 and 2012. Samples were collected in 1-L pre-cleaned amber glass jars and kept cold on ice during transport to the laboratory. Upon arrival, sediment samples were homogenized, divided into several sub-samples, sealed in polypropylene jars, wrapped with aluminium foil, and stored at -20 °C.

Surface water samples were collected under different hydraulic conditions. Samples were taken in clean bottles (capacity 1 L) typically up to 30 cm depth, and filled up to the top to eliminate air bubbles. Forty one surface water samples were collected from urban streams (US_1) (n = 10), (US_2) (n = 11), (US_3) (n = 20) under dry weather conditions and fifty eight samples under wet weather conditions (US_1) (n = 30), (US_2) (n = 6), (US_3) (n = 28). Water samples were filtered and stored at 4 °C after adding formic acid as a preservative (see details in Madoux-Humery et al. (2013)).

Physico-chemical and microbial analysis of the samples (detailed data not shown) revealed a clear sanitary contamination of the waters and sediments.

4.3.3.2 Sediment sample extraction

Preparation and spiking of sediment samples are detailed in ESI.† The final optimized method for simultaneous extraction of 10 selected WWMPs used various extraction solvents. Sediment samples were successively extracted with 4 and 2 mL of the mixture of methanol/water (9:1, v/v, pH 11), followed by two extractions using 2 mL of acetone and ultimately with 4 mL of water with 0.1% formic acid (pH 2.65). There was five operating cycles in the extraction process. In each extraction step, the sample was vigorously vortexed for 1 min, ultrasonicated for 20 min in an ultrasonic bath (frequency 40 KHz, Bransonic 5510, Connecticut, USA) at 30 °C and centrifuged at 4000 *g* for 10 min. The supernatants obtained from each extraction step were

combined, filtered using a 0.2-µm polypropylene syringe filter and concentrated to dryness by evaporation under a nitrogen stream. After the addition of 250 µL of methanol, the extract was diluted to 10 mL with HPLC Grade water (UPW) adjusted to pH 7 with sodium hydroxide 0.5 M and subjected to the SPE procedure. The developed extraction method with previously developed method for stream sediment analysis was also applied to CSO sediments.

4.3.3.3 SPE procedure: Cleanup and preconcentration

To reduce matrix interference, further cleanup of sediment samples is normally required. In this study, specific solid phases were used as a clean-up and preconcentration treatment. Generally, polymeric adsorbents have a higher adsorption capacity than C18 adsorbent for polar analytes (Bossio et al., 2008; Cueva-Mestanza et al., 2008; Dobor et al., 2010; Durán-Alvarez et al., 2009; Edwards et al., 2009; Jelić et al., 2009; Loffler & Ternes, 2003; Martin et al., 2010; Minten et al., 2011; Radjenović et al., 2009; Stein et al., 2008; Varga et al., 2010). As indicated above, SPE cleanup was performed using an Oasis HLB cartridge (30 µm, 60 mg per 3 cc). The cartridge was preconditioned successively with 3 × 1 mL of methanol and UPW prior to sample load. After sample passage, the cartridge was washed with 10 mL of UPW and dried under vacuum for 30 min. The analytes were then eluted successively with 1 mL of methanol and 1 mL of 0.5 M formic acid–methanol mixture. The eluate was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved with the initial mobile phase condition (0.5 mL). Before analysis, 2 µL of instrument internal standard containing 5 isotope-labeled compounds (1 mg L^{-1}) was added to correct for variations in sample recovery and instrumental performance (Buchberger, 2007).

4.3.3.4 Analytical methods

The optimized method was applied to the determination of WWMP concentrations in CSO and stream sediment samples. The four WWMPs (ACE, THEO, CAF and CBZ) selected by Madoux-Humery et al. (2013) were analysed during this study in surface water samples by an on-line solid-phase extraction combined with liquid chromatography electrospray tandem mass spectrometry with positive electrospray ionisation (SPE-LC-ESI-MS/MS) (Madoux-Humery et al., 2013). Detailed information on preservation and analytical methods are published and available (Madoux-Humery et al., 2013; Sauvé et al., 2012). Detection limits were estimated as three times the standard deviation of 5 replicate measurements of a field sample and were 10 ng

L^{-1} for ACE, 6.50 ng L^{-1} for CAF, 6 ng L^{-1} for THEO and 0.52 ng L^{-1} for CBZ. All samples were analyzed in duplicate.

4.4 Results and discussion

4.4.1 Optimization of UHPLC–APCI–SRM/MS analysis and quantification

Atmospheric pressure chemical ionisation tandem mass spectrometry provides high sensitivity and selectivity for the identification and quantitative analysis of selected compounds. The collision gas pressure and the offset energy for the collision quadrupole Q2 were two important factors for determining the major product ion intensity for each compound; they were optimized at 1.5 mTorr and between 13 and 27 eV, respectively (Table 4.7 and Table 4.8, ESI†).

The separation of the ten studied compounds occurred within 3.6 min with good resolution and the total run time was 4.5 min. Detailed description and discussion of the optimization of UHPLC–APCI–SRM/MS method are included in ESI.†

4.4.2 Optimization of extraction and SPE steps

Our study was carried out on the most relevant parameters that affect the recovery of target compounds (*e.g.* extraction solvent, cycle number, contact time and temperature of sonication, sample pH, type of SPE sorbent and volume and type of elution solvent). Detailed description and discussion of the optimization of extraction and SPE steps are included in ESI.†

The developed method (see Methods and materials) was applied to CSO sediment samples. The results of quantitative extraction showed that 63−122% of the total extractable amounts in both sediment samples with the exception of atenolol (46% from stream sediment) and aspartame (53% from CSO sediments) were recovered in the first five cycles (Figure 4.1). In a subsequent extraction step with 2 mL of acidified water, none of the analytes could be detected anymore. The differences in the extraction recoveries between WWMPs and sample matrices are consistent with those reported by Martin et al. (2010).

Table 4.1. Characteristics of the extraction method (USE) described in the literature for the determination of the target compounds in solid environmental samples.

Sample	Compound	Extraction solvents	Total solvent volume (mL)	Ultrasonication cycles	pH extract	Clean-up	Analytical determination	Recoveries (%)	References
Sewage sludge, river and estuary sediments	ATL CBZ MedP	Acetonitrile–water (5:3, v/v)	24	3 x 15 min	7	Oasis HLB	UHPLC–MS/MS (+ESI, MRM)	80–100% excepted for atenolol	(Yu et al., 2011)
Sea sediment	CBZ DIC	Acetone–McIlvaine buffer (1:1,v/v, pH 4)	80	2 x 15 min	–	Evolute ABN and Oasis HLB	UHPLC–MS/MS (+ESI, -ESI, MRM)	60−70% 50−60%	(Minten et al., 2011)
Sewage sludge, compost and sediments	ACE CBZ CAF DIC	Methanol Methanol Acetone	9	3 x 15 min	2	Oasis HLB	HPLC, UV–DAD	10−20% 80−100% 80−100% 60−80%	(Martin et al., 2010)

Table 4.1. Characteristics of the extraction method (USE) described in the literature for the determination of the target compounds in solid environmental samples (cont'd).

Sample	Compound	Extraction solvents	Total solvent volume (mL)	Ultrasonication cycles	pH extract	Clean-up	Analytical determination	Recoveries (%)	References
Primary and excess sludge	ACE DIC CBZ ATL THEO CAF DEET	Methanol–water (1:9,v/v, pH 11)	25	1 x 15 min and 2 x 10 min	–	Oasis HLB	HPLC–MS/MS (API)	80–120% excepted for atenolol (40%)	(Okuda et al., 2009)
Activated and digested sludge	CBZ DIC	Methanol Acetone	10	4 x 5 min	7 2	RP-C_{18}ec Oasis MCX	HPLC–MS/MS (+ESI, -APCI, MRM)	76–85%	(Ternes et al., 2005)

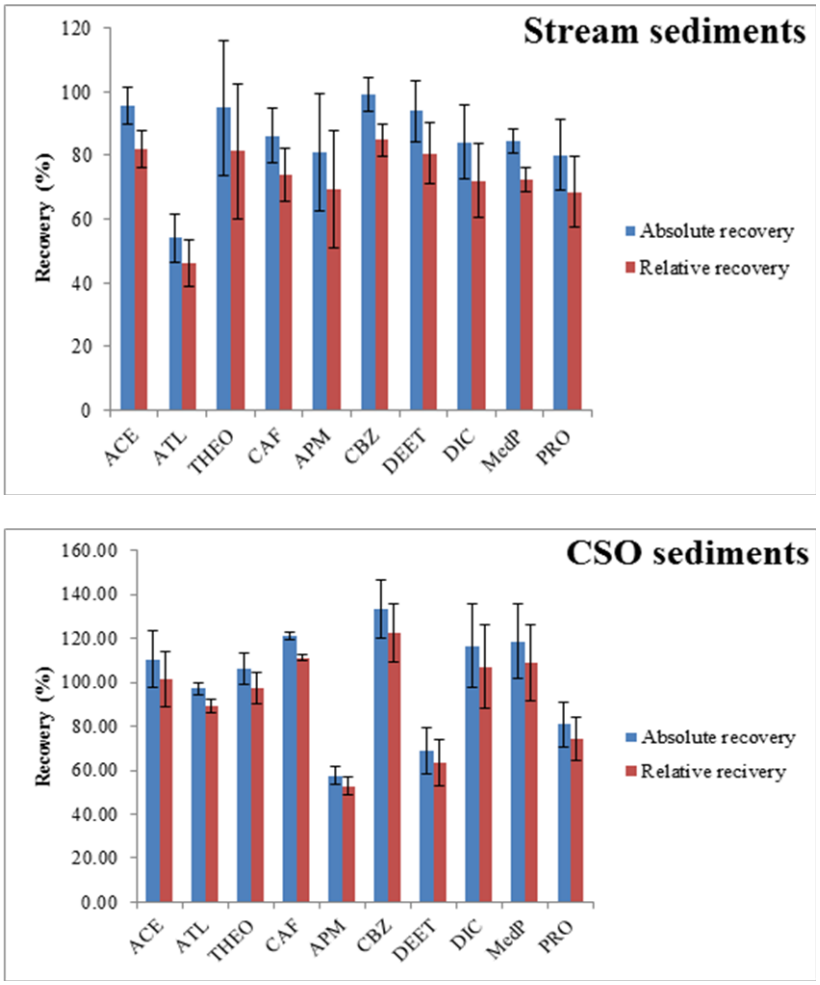

Figure 4.1. Absolute and relative extraction recoveries for selected WWMPs from spiked stream sediment (top) and CSO sediment (bottom). Error bars represent standard deviations (n = 3).

4.4.3 Analytical method validation

In order to assess the performance of the proposed method, the main analytical quality parameters were thoroughly evaluated by determination of recoveries, linearity, precision, repeatability, matrix effects and detection limits. Matrix-matched calibration curves prepared in every type of sample showed good linearity between 0 and 100 µg/L, with a correlation coefficient (R^2) ≥ 0.9946 and ≥ 0.9653 for stream and CSO sediments respectively (Table 4.2). These R^2 values were of the same order of magnitude than those reported by Pérez-Carrera et al. (2010).

Relative recoveries and precision data are also listed in Table 4.2 for stream and CSO sediments. Satisfactory relative recoveries and good repeatability in spiked extract samples illustrated the suitability of the internal standards. Relative recoveries ranged from 75.5% to 156% in stream sediment extracts as well as in CSO sediment extract samples for all analytes, indicating good performance of the proposed method. Our proposed method achieved a significantly better recovery for acetaminophen in comparison with methods developed by Martin et al. and Radjenovic et al. (Martin et al., 2010; Radjenović et al., 2009) for its determination in sample matrices which may have similar properties as CSO sediments.

The repeatability values varied in the range of 2.19–17.4% for stream sediments and 1.80–22.6% for CSO sediments and reproducibility was of 0.04–20.0% for stream sediments and of 5.30–22.9% for CSO sediments. These results did not show apparent differences between stream and CSO sediment samples and are similar to those reported by other studies (Martin et al., 2010; Radjenović et al., 2009; Vazquez-Roig et al., 2010).

Table 4.2 also outlines limits of detection (LODs) for stream and CSO sediment extracts that were in the range of 0.01–0.41 ng/g in stream sediment extracts and 0.21–14.8 ng/g in CSO sediment extracts. The sensitivity for stream sediment samples was better than for CSO sediment samples. The caffeine registered a higher LOD in comparison with the other compounds. Thus, our method is less sensitive to analyse caffeine as compared to the other analytes but given the very high concentrations of caffeine observed, this is a non-issue. LODs for stream sediments were better than reported in previous studies using LC (Loffler & Ternes, 2003; Pérez-Carrera et al., 2010; Vazquez-Roig et al., 2010).

Table 4.2. Recoveries in stream and CSO sediment extracts, repetitivity, reproducibility, linearity (regression coefficient) and detection limit (LOD) for the method[a]

Compound	Recovery (%, n = 2)	Intra-day RSD (%, n = 5)	Inter-day RSD (%, n = 3)	R^2	LOD (ng g^{-1})
ACE	96.6[a]	2.29[a]	4.67[a]		
	101[b]	2.97[b]	6.99[b]	0.9984[e]	0.01[e]
	96.9[c]	2.30[c]	0.72[c]		
	107.6[d]	8.08[d]	6.43[d]	0.9995[f]	3.41[f]
ATL	93.3	5.98	3.33		
	100	6.92	14.45	0.9972	0.17
	88.7	2.67	0.04		
	112	10.51	5.30	0.9653	3.48
THEO	103	3.32	4.05		
	112	8.9	16.32	0.9990	0.33
	100	3.18	4.20		
	156	8.43	5.53	0.9992	3.15
CAF	124	10.00	2.84		
	121	8.60	11.82	0.9975	0.41
	104	5.20	5.31		
	118	6.87	6.13	0.9995	14.79
APM	111	4.00	6.54		
	107	9.00	9.20	0.9971	0.34
	99.0	15.00	3.57		
	99.8	1.80	6.94	0.9991	0.21
CBZ	105	11.94	7.38		
	113	10.99	16.06	0.9972	0.32
	103	2.94	2.40		
	87.6	10.57	17.63	0.9991	3.89
DEET	113	3.38	3.43		
	114	6.68	14.39	0.9995	0.21
	98.8	2.19	10.02		
	81.1	7.50	8.96	0.9991	0.90
DIC	82.6	17.42	6.10		
	85.0	17.37	19.98	0.9988	0.25
	75.5	9.11	2.59		
	104	5.96	18.68	0.9988	1.50
MedP	106	6.77	7.49		
	104	4.08	15.34	0.9946	0.13
	91.8	8.18	6.05		
	85.7	22.62	22.90	0.9991	0.84
PRO	109	15.8	10.24		
	85.6	10.37	3.53	0.9998	0.23
	88.7	7.43	0.18		
	94.9	6.05	11.87	0.9995	2.32

[a] a, b and c represent respectively 10, 20 and 30 µg L^{-1} as nominal concentrations of analytes doped into stream sediment extract and d represents a concentration of 100 µg L^{-1} in CSO sediment extract. The linearity is ranged between 0 and 30 µg L^{-1} in stream sediment extract (e) and between 0 and 100 µg L^{-1} in CSO sediment extract (f). This nomenclature is applied to the other rows of the table according to a logical follow-up.

4.4.4 Matrix effects

We successfully reduced matrix effects by using selective extraction and cleanup procedures and using an APCI interface as the ionisation source instead of the more standard ESI interface (see details in ESI†).

4.4.5 Method applicability in sediment samples and comparison with water samples

After the optimization and validation, the developed method was applied to real samples with different matrices, to evaluate its applicability in the determination of the investigated WWMPs in stream and CSO sediments. To the best of our knowledge, this is the first time that such compounds were measured in CSO sediments.

With the exception of medroxyprogesterone, almost all of the target compounds were detected in sieved sediment samples (80 mesh) with concentrations ranging from 0.13 to 427 ng g^{-1} dw and the most abundant compound was theophylline/paraxanthine (Table 4.3). Comparison among the concentrations of compounds in combined sewer overflow sediments (CSO) and sediments from the river downstream of CSOs (US_3) show that the majority of compounds are effectively removed by natural attenuation (*e.g.* dilution, hydrolysis, sorption, biotransformation and phototransformation).

Table 4.4 presents mean concentrations of 4 compounds measured in surface water samples during dry and wet weather conditions. Our results were compared with data, reported by Madoux-Humery et al. (2013) and Guérineau et al. (2014), in order to compare the water and sediment samples from sites having different degrees of human fecal contamination relative to the available dilution (CSOs > US_2 = US_1 > canal > US_3).

Figure 4.2 shows the ratio between the average concentrations of WWMPs measured in water and sediment samples for each of the sites and their limits of detection. This ratio indicates the range over which a particular compound (ACE, THEO, CAF or CBZ) could be useful as a wastewater tracer similar to the recommendation of Benotti and Brownawell for comparing various WWMPs as tracers of wastewater contamination using dynamic ranges of the WWMPs (Benotti & Brownawell, 2007) see Table 4.9. (ESI†).

Table 4.3. WWMP content of real sediment samples (CSO, urban streams and canal) under dry weather conditions

Compound	Mean concentration (ng/g)														
	CSO			US_1			US_2			US_3			Canal[b]		
ACE	98	±	17	0.87	±	0.03	2.5	±	1.1	0.13	±	0.05	8.2	±	4.6
ATL	80	±	9	nd			Trace			nd			Na		
THEO/PX	427	±	13	0.69	±	0.05	0.4	±	0.04	Trace			1.4	±	0.8
CAF	297	±	16	22	±	0.72	1.6	±	1.2	1.6	±	0.6	1.3	±	0.4
APM	nd[a]			nd[a]			Trace			nd			Na		
CBZ	54	±	6.8	Trace			Trace			nd			nd		
DEET	2.30	±	1.17	0.7	±	0.01	14	±	11	nd			Na		
DIC	Trace			nd			nd			nd			Na		
MedP	nd			nd			nd			nd			Na		
PRO	11.5	±	1.05	nd			nd			0.6	±	0.1	Na		

[a] Na and nd represent not analysed and not detected respectively; trace refers to cases where an MS transition peak was observed, but it was below the LOD; [b] refers to results (n = 15) reported during the study of Guérineau et al. (2014).

In contrast to the dynamic range of Benotti and Brownawell (Benotti & Brownawell, 2007), the concentration : LOD ratio (C : LOD) is defined here with average concentrations rather than the maximum concentration measured in order to reduce the bias from extreme values that are not representative of environmental conditions:

$$\text{C:LOD} = \frac{\text{Average measured concentration in environmental sample}}{\text{LOD}} \qquad (1)$$

For highly concentrated sampling locations such as sewers (SA and SB) in dry weather and one of the CSOs in wet weather (OA, the overflow of sewer SA), the C : LOD was greater (from 5 to 43 times higher for THEO and CAF, respectively) in water than in sediments for all WWMPs. In general, as the degree of dilution increased, the C : LOD in sediments increased relative to the C : LOD in water. For example, OB, the overflow from combined sewer SB is highly diluted because it receives runoff from a large impermeable surface (Madoux-Humery et al., 2013). For all WWMPs in OB except for CAF, the C : LOD of CSO sediments (C) was greater than in water. For ACE, the C : LOD in sediments was always greater than in water with the exception of the highly concentrated sewage samples (SA, SB, and OA). In contrast, the C : LOD for CBZ in water was higher than in sediments with the exception of site OB because of the higher LOD for CBZ in sediments. In CSO sediments, the WWMP with the highest C : LOD was THEO followed by ACE. For urban stream and canal sediments, ACE was the best tracer of fecal contamination followed by CAF and THEO. For water, CAF appeared most frequently as the WWMP with the highest C : LOD followed by ACE. However, ACE was below the detection limit in water samples from the most highly diluted urban streams.

Benotti and Brownawell (Benotti & Brownawell, 2007) have shown the potential of using some of the selected compounds (acetaminophen, caffeine, paraxanthine and carbamazepine) as wastewater tracers of a highly sewage-impacted estuary bay. By determining the dynamic range of LC-ToF-MS analysis in waters, they found that caffeine and paraxanthine were some of the compounds with the largest dynamic ranges. Unique to our study, was the analysis and comparison of WWMPs concentrations in sediment samples with their concentrations in water samples.

The dynamic range as defined by Benotti and Brownawell (Benotti & Brownawell, 2007) or the C : LOD depend strongly on the method used for measurements. The comparison of the dynamic

ranges or C : LOD of WWMPs in water versus sediments strongly supports the use of sediment sampling in addition to water sampling for fecal source tracking. Because the C : LOD of some WWMPs (specifically ACE in our study) in sediments can be higher than the C : LOD in water, sediment sampling can be more useful for establishing gradients related to contaminant sources, particularly for highly diluted water sources with wastewater sources that are intermittent (Guérineau et al., 2014). The disadvantages of sediment sampling include the greater heterogeneity of the samples with regards to particle size, natural organic matter, etc. that can influence WWMP sorption (Khunjar & Love, 2011; Pan et al., 2009; Sun et al., 2012; Sun et al., 2006; Yu, Fink, et al., 2009) and the smaller volumes of samples collected. Advantages of sediment sampling include the lower mobility of WWMPs in sediments at specific locations, particularly in relation to wastewater discharges that are intermittent such as CSOs, cross-connections or spills that are difficult to monitor in water because of their highly dynamic nature. Many factors influence the concentrations of WWMPs in water and sediments, including human consumption patterns and excretion rates, discharge patterns, sorption processes, degradation rates and dilution processes (Loffler et al., 2005; Luo et al., 2014; Monteiro & Boxall, 2010). The relative importance of these factors for a given system, in addition to the LOD of WWMPs will determine the most appropriate WWMP tracers to select and whether they should be measured in both the aqueous and sediment phases. Results of this study showed that even WWMPs considered to have a relatively low sorption potential, such as ACE (Lin et al., 2010) can serve as useful tracers in sediments because they had a relatively high C : LOD ratio. Dilution processes often dominate when travel times are short, discharges are intermittent or highly variable, and flow rates are low compared to the receiving water. In these cases, where the C : LOD is low, water sampling alone will not likely provide meaningful results and sediment sampling should be considered.

Table 4.4. WWMP content of water samples (CSOs, urban streams and canal) under dry and wet weather conditions

Compound	Mean concentration (ng L^{-1})					
	Dry weather (DW)					
	SA[a]	**SB**[a]	**US_1**	**US_2**	**US_3**	**Canal**[b]
ACE	8460 ± 5270	3280 ± 1683	170 ± 126	508 ± 243	nd	nd
THEO/PX	4460 ± 1613	3200 ± 3049	80.7 ± 31.9	130 ± 58.4	22.6 ± 15.1	14.1 ± 3.38
CAF	7740 ± 5561	810 ± 504	92.2 ± 26.9	318 ± 51.1	21.3 ± 5.30	55.3 ± 24.0
CBZ	310 ± 239	101 ± 136	6.61 ± 1.29	0.97 ± 0.16	2.12 ± 0.65	1.65 ± 0.17
	Wet weather (WW)					
	OA[a]	**OB**[a]	**US_1**	**US_2**	**US_3**	**Canal**[b]
ACE	6420 ± 6808	1 ± 0	124 ± 20	72.6 ± 36.9	nd	nd
THEO/PX	3630 ± 3203	128 ± 190	53.6 ± 15.6	nd	20 ± 8.74	11.7 ± 4.48
CAF	5520 ± 4968	336 ± 190	165 ± 24.5	36.2 ± 5.35	26.6 ± 24.7	35.1 ± 5.53
CBZ	207 ± 204	8.79 ± 14	3.68 ± 2.56	1.32 ± 0.53	2.12 ± 0.62	1.83 ± 0.13

[a] nd represents not detected. [b] Refer to data and results reported by Madoux-Humery et al. (2013) and Guérineau et al. (2014) respectively.

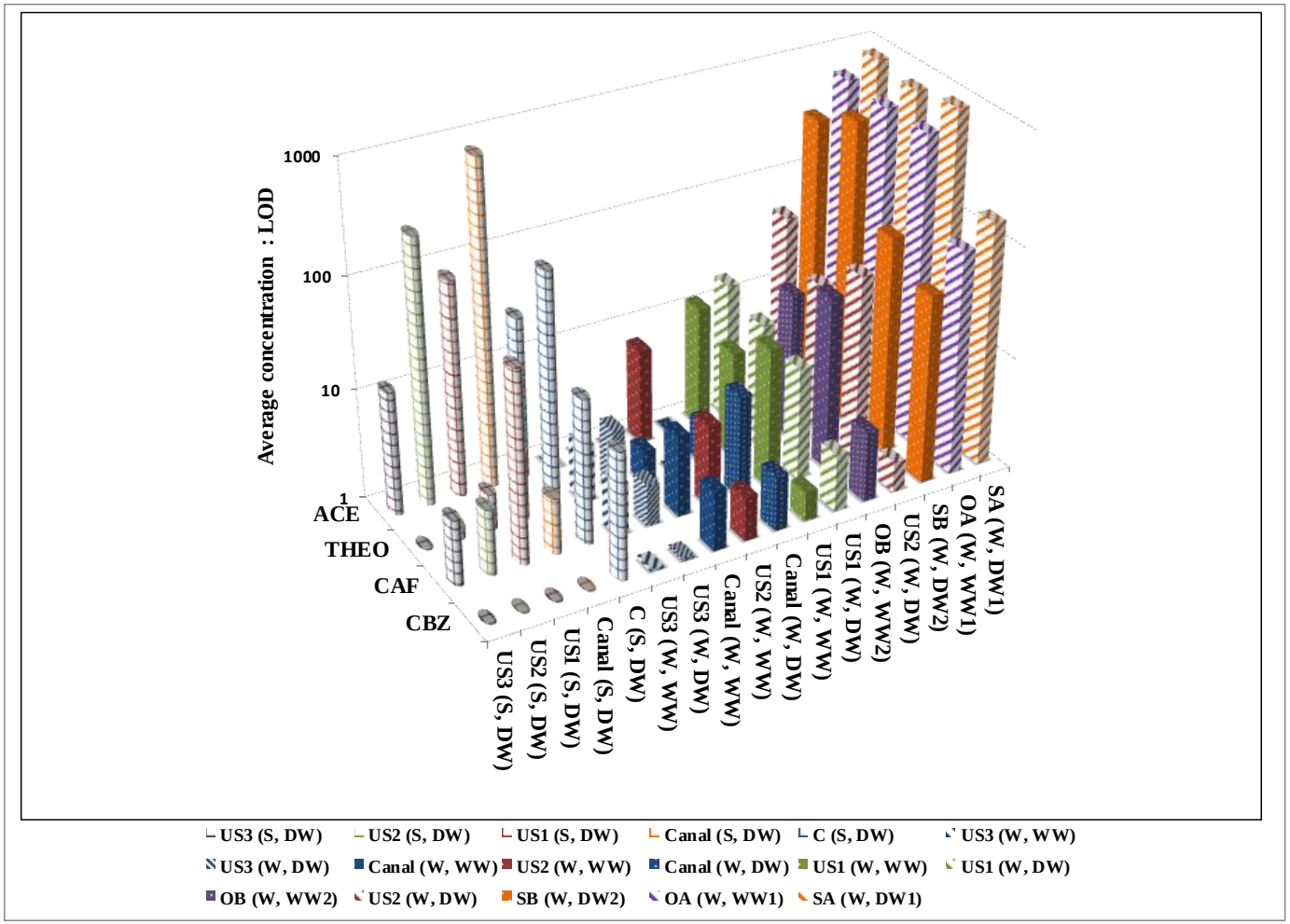

Figure 4.2. Average concentration to LOD ratios of WWMPs in CSOs (C, SA, SB, OA and OB), urban streams (US_1, US_2 and US_3) and canal sediment (S) and water (W) samples under dry (DW) and wet (WW) weather conditions.

4.5 Conclusions

A method using ultrasonication–assisted extraction and UHPLC–APCI–MS/MS detection was successfully developed for the simultaneous analysis of 10 WWMPs from a diverse group of markers of sanitary contamination, including pharmaceuticals, hormones and personal care products. Our work helps to demonstrate the versatility of USE methods to target a more diverse range of compounds. Optimisation of the ultrasonic extraction, SPE and analytical parameters are required for more efficient and reproducible extractions, purifications and analyses. Ultimately, the choice of the extraction, cleanup and analytical methods was dependent on the effectiveness, capital cost, operating cost, simplicity of operation, and waste production. The optimized ultrasonic extraction and cleanup procedures were found to extract WWMPs at ng g^{-1} levels from stream and CSO sediments with recoveries greater than 70% for most analytes. All selected compounds were eluted within a 3.6 minute period; with a short chromatography run (4.5 min). It allows for significant improvement over previously published works in terms of matrix effect for the analysis of the target compounds in sediments (especially acetaminophen). The method has been used in our laboratory to extract WWMPs from field-collected sediments from rivers, creeks and combined sewer overflows. The range of WWMPs extracted and its complete separation obtained by the optimized method was found to be very useful in the application of fingerprinting and source determination of human fecal contaminated samples. Chromatographic separation of theophylline and paraxanthine was necessary for accurate quantitation of the theophylline used as a medication on one hand and of the primary metabolite of caffeine on the other hand. This method has excellent extraction efficiency, precision and recovery of WWMPs. In addition, when combined with easy sample preparation, it makes it an ideal technique for laboratories engaged in analyzing a large number of sediment samples. Based on the optimized conditions, the level of selected WWMPs in the sediment samples ($\leq$ 80 μm) collected in the Greater Montreal area was found to be between 0.13 and 427 ng g^{-1} dw. Our results support the hypothesis of Madoux-Humery et al. (2013) who suggested that internal sewer sediments were the source of WWMPs that were remobilized with the increase of flow rate associated with rain events. Sediment sampling of WWMPs should accompany water sampling for fecal source tracking for systems where dilution rates are high and the C : LOD is low. Thus confirmation of concentrations in

sediments is necessary to understand their environmental fate and potential ecological effects, in addition to their use as tracers of sewage contamination.

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4.8 Electronic supplementary information

4.8.1 Introduction

4.8.1.1 Descriptions of compounds analysed

Selected PPCPs typically have properties favoring their use as wastewater tracers. Information on the proposed function as a wastewater marker are summarized in Table 4.5. Their physico-chemical properties suggest that they are relatively water-soluble and non-volatile. Additionally, these compounds are present, albeit at trace concentrations, in aquatic environment receiving wastewater effluents. The present section reviews some data present in the literature about these compounds.

Atenolol, a beta-blocker discovered in 1958, has received massive clinical attention due to its effectiveness in treating hypertension and heart diseases, and ranks 34th in the top-selling drugs in the world (Cardoso et al., 2011). Atenolol was found to be present in rivers at levels exceeding 100 ng L^{-1}. This was expected due to its higher dispension (over 2300 kg/year) and its high excretion rates as an unchanged drug (50%) (Kasprzyk-Hordern et al., 2008). The removal efficiency for atenolol in wastewater treatment plants is as low as 10% (Paxeus, 2004) up to 19% (Kim et al., 2012). This compound is resistant to biodegradation and once released into the aquatic environment, it can bind to dissolved organic matter (DOM) which enhances its transport in the environment; however it is reported to be moderately accumulated into aquatic sediments

(Yamamoto et al., 2005), persistent and ubiquitous in the aqueous environment (Kasprzyk-Hordern et al., 2008).

Diclofenac is an anti-inflammatory drug developed in the 1960's, and is a highly consumed drug showing analgesic, antipyretic and anti-inflammatory properties. It tends to be relatively persistent in the environment (Groning et al., 2007) and detected pharmaceutical in the water cycle of Europe (Sun et al., 2008) and North America (Metcalfe et al., 2003). The concentration of diclofenac detected in sewage treatment plant (STP) effluents has been reported to reach up to 5.5 µg L^{-1} (Andreozzi et al., 2003; Letzel et al., 2009; Stulten et al., 2008) and decreases by substantial degradation when gathered in river sediments (Groning et al., 2007). It seems eliminated from lake waters mainly by phototransformation (Tixier et al., 2003). Regarding ecotoxicological effects, previous studies reported that 1 mg $L^{-1}$of diclofenac is the lowest concentration which causes an observed effect on zooplankton (Fent et al., 2006); however, effects on microorganisms were also reported at much lower concentrations (Lawrence et al., 2007; Paje et al., 2002).

The analgesic **acetaminophen** (paracetamol) is commonly used as Over-The-Counter (OTC) medications. Approximately 96% of acetaminophen is excreted as metabolites by sulfate and glucuronide conjugation. It has been found at low part per billion (or µg L^{-1}) concentration in wastewater and surface water. Previously published methods for the analysis of acetaminophen in environmental samples commonly use gas or liquid chromatography coupled to mass spectrometry (Ahrer et al., 2001; Choi et al., 2008; Fatta et al., 2007; Hilton & Thomas, 2003; Kolpin et al., 2002; Ternes, 1998). Recently, levels of acetaminophen detected by LC-MS/MS in STPs influent in Korea were very high (75 µg L^{-1}) compared to the effluent concentrations (0.023 µg L^{-1}) (Kim et al., 2012). It was detected eventually in the hospital effluents with a relatively high concentration of around 16 µg L^{-1} (Gómez et al., 2006). However, it was not detected in any of the STPs effluent or receiving water samples taken during the study of Roberts and Thomas (Roberts & Thomas, 2006). The highest reported concentration for acetaminophen was 10 mg L^{-1} in stream water (Kolpin et al., 2002). Acetaminophen has a predicted no effect concentration of 9.2 µg L^{-1} and a hazard coefficient (Predicted environmental concentrations (PEC)/predicted no-effect concentration (PNEC) ratio) of 1.8, thus demonstrating some risks for producing potential adverse effects in the environment (Bedner & MacCrehan, 2006; Jones et al., 2002; Kim et al.,

2007). It was estimated that acetaminophen was neither accumulative in both DOM and sediments but slightly bioaccumulative, whereas it was highly biodegradable when discharged into the aquatic environment (Yamamoto et al., 2005). Results obtained by Gros et al. (2006) showed that anti-inflammatories and analgesics were the major groups detected in WWTP and among them acetaminophen, diclofenac and atenolol were the most abundant, with concentrations in high ng L^{-1} or low µg L^{-1} levels (Gros et al., 2006).

Caffeine is widely distributed in many plant species and produced in high volume around the world. Being a major human dietary component, it can be found in common food and beverage products such as coffee, tea, colas and chocolates. It is associated with theophylline in tea and with theobromine in chocolate. In pharmaceuticals, caffeine is generally used as a mild neurological, cardiac and respiratory stimulant, psychoactive drugs, and analgesic enhancer in cold, cough and headache medicines (Daly, 2007; Renner et al., 2007). Only 0.5% to 2% of ingested caffeine is excreted as such in the urine due to 98% tubular reabsorption; caffeine is metabolized in the liver to dimethylxanthines (paraxanthine (PX) is the main metabolite of caffeine, theobromine and theophylline) (Arnaud, 1993). Discarding unconsumed caffeine-containing beverages (Seiler et al., 1999) and combined sewer overflows (Buerge et al., 2006) have been reported as the main sources of caffeine in surface water. Caffeine showed excellent elimination rates (>99%) throw the wastewater treatment process (Buerge et al., 2006; Kim et al., 2012), while it has high detection frequency and been reported to reach concentrations in streams of 6.0 mg L^{-1}, with a median concentration of 0.1mg L^{-1} (Kolpin et al., 2002). It has nutrient-like effects on microbial community development (Lawrence et al., 2005), having a no effect concentration of 182 µg L^{-1} (Santos et al., 2007). Even the short mean half-life of caffeine (1.5 days) (Lam et al., 2004), it can act as a persistent chemical in the aquatic environment (Thomas & Foster, 2005). Caffeine has been suggested as an anthropogenic marker for wastewater pollution entering the aquatic system (Buerge, I. J., Poiger, T., Muller, M. D., & Buser, H.-R., 2003a; Hillebrand, Nodler, Licha, Sauter, & Geyer, 2012). In correlation with caffeine, theophylline can qualitatively be used to support the specificity of caffeine for wastewater.

Theophylline is among the primary monodemethylated metabolites of caffeine in humans (Lelo et al., 1986). It can also be administered directly as drugs for different pharmaceutical applications including their use as diuretics, bronchodilators, asthma control and for relief of

bronchial spasms. For the most part, theophylline enters the environment via liquid effluents and domestic wastes and originates from coffee consumption. Given that the majority of consumed caffeine is excreted as metabolites, it is pertinent to see whether measuring the metabolites would provide better predictive models than caffeine itself. By comparing the concentration of theophylline in a wastewaster treatment plant (WWTP), the influent (4.2 µg L^{-1}) and effluent STP (0.023 µg L^{-1}) shows excellent elimination rates during wastewater processing (Kim et al., 2012).

Carbamazepine is often used to control seizures, epilepsy, bipolar disorders and pain (Thacker, 2005). Once ingested, 2 to 3% of this drug is excreted unchanged in the urine (Clara et al., 2004). Carbamazepine is a persistent contaminant commonly detected in sewage treatment effluents and surface waters, and 7% of it is removed upon sewage treatment (Möhle & Metzger Jörg, 2001; Ternes, 1998). The half-lives of this pharmaceutical compound have been reported between 82 and 100, at 328 and 495 d in aqueous matrices, sediments and soils respectively (Andreozzi et al., 2003; Lam et al., 2004; Loffler et al., 2005; Walters et al., 2010), thus raising concerns over its potential accumulation and persistence in aquatic environment. It has been found at low part per billion levels in wastewater and surface water (Andreozzi et al., 2003; Clara et al., 2004; Ferrari et al., 2004; Heberer et al., 2002; Metcalfe et al., 2003; Ternes, 1998). In U.S. rivers, average levels were 60 ng L^{-1} in water and 4.2 ng mg^{-1} in sediments (Thacker, 2005). Furthermore, the growth of Daphnia and midges was inhibited at 12.7 and 9.2 mg L^{-1} respectively; however, they were killed when carbamazepine was present at 17.2 mg L^{-1} and midges at 34.4 mg L^{-1}. There were no significant toxic effects to either *C. tentans* or *H. azteca* at concentrations as high as 56 mg kg^{-1} dry weight of spiked sediments (Dussault et al., 2008). Carbamazepine, acetaminophen, caffeine, diclofenac (Daughton & Ternes, 1999 ; Fent et al., 2006; Heberer, 2002a; Klavarioti et al., 2009; Nikolaou et al., 2007) and atenolol (Fent et al., 2006; Klavarioti et al., 2009) were among the most studied pharmaceutical compounds in the aquatic environment according to recent reviews. Atenolol, diclofenac, acetaminophen and carbamazepine can be used as chemical indicators of human faecal contamination because they were found in surface water with 100% frequency at the sampling points located below WWTPs (Kasprzyk-Hordern et al., 2008). Acetaminophen, caffeine, theophylline and carbamazepine have been frequently observed in wastewater in the Greater Montreal Area (Viglino, Aboulfadl, Daneshvar, et al., 2008).

DEET - N,N-diethyl-3-methylbenzamide is widely used as an insect repellent for humans and may be applied in agriculture; it also is the most important analytes within this group (Buchberger, 2011; Riha et al., 1991). Annual usage of DEET have been estimated to be approximately 1.81 million kg per year in the US (Agency, 1998). Excretion of DEET may be a minor route(Selim et al., 1995). It enters aquatic environments mainly through communal WWTP effluents (Aronson et al., 2012) where it is frequently detected at concentrations ranging between 51 and 773.9 ng L^{-1} (Bartelt-Hunt et al., 2009; Chen et al., 2012; Ryu et al., 2011; Trenholm et al., 2008; Trenholm et al., 2006). Although DEET is considered to be neither persistent, bioaccumulative, nor toxic (Weeks et al., 2012).

Aspartame (methyl ester of a dipeptide) is a popular artificial sweetener used in diet soft drinks. It is a substance 180 to 200 times sweeter than sugar, not heat-stable and degradable over a long period of time. Aspartame was assumed to be quickly biodegraded in WWTPs (Buerge et al., 2009). In the literature, aspartame is the most controversially discussed artificial sweetener regarding health aspects causing adverse effects such as neurological disturbances (Shaywitz et al., 1994; Simintzi et al., 2007; Tsakiris et al., 2006) or even cancer in rats (Soffritti et al., 2006; Soffritti et al., 2007). Nevertheless, Health Canada, the Scientific Committee for Food of the European Community, and the Joint Expert Committee on Food Additives (JECFA) of the United Nations Food and Agriculture Organization and World Health Organization consider this substance to be safe based on toxicological and clinical studies (Canada, 2005). Aspartame was not detected in wastewater and surface water samples analysed by Scheurer *et al.* (Scheurer et al., 2009).

Natural (**progesterone)** and synthetic (**medroxyprogesterone**) steroids have been regarded as the most important members of endocrine disrupting chemicals (EDCs) which may cause dangerous effects on aquatic organisms at a low ng L^{-1} level (Besse & Garric, 2009; Sumpter, 2005). Progesterone was detected in wastewater influent, surface water and river sediment at concentrations up to 6.1 ng L^{-1}, 2.5 ng L^{-1} and 6.82 ng g^{-1} respectively (Liu et al., 2011; López de Alda et al., 2002), while medroxyprogesterone was detected in wastewater effluent at concentration up to 15 ng L^{-1}. This compound was not rapidly attenuated from an effluent-receiving engineered treatment wetland and shallow groundwater wells (Kolodziej et al., 2003).

4.8.1.2 Extraction methods

Most methods developed to determine WWMPs in sediments usually include an extraction protocol followed by chromatographic analysis. Various extraction techniques used in the past include : ultrasonic-assisted extraction (USE) (Buchberger, 2007; Martin et al., 2010), pressurized liquid extraction (PLE) (Björklund et al., 2000; Vazquez-Roig et al., 2010) and microwave extraction (MAE) (Brown & Kinney, 2011; Sparr Eskilsson & Björklund, 2000). Depending on effectiveness, capital cost, operating cost, simplicity of operation, and waste production, USE is a robust method for extracting organic contaminants from solid matrices comparable to other commonly used methods such as Soxhlet, PLE, and MAE (Bossio et al., 2008; Buchberger, 2011; Tadeo et al., 2012). During the USE process, cavitation bubbles that are produced may initially have extreme internal temperature and pressure, which collapse afterwards causing the extraction solvent to propagate outwards with a high velocity on a collision course with matrix particles. These collisions separate the matrix and produce smaller particles and expose more surface area to the extraction solvent (Capelo & Mota, 2005). Since extractions are carried out at room temperature instead of high temperatures of around 100 °C, USE reduces the risks of degrading the target compounds; while a higher precision is also reported than with using MAE methods (Martin et al., 2010). It can be effectively applied to extract organic contaminants that differ significantly in their physico-chemical properties (Bossio et al., 2008; Capelo & Mota, 2005). Sample extracts obtained from solid matrices contain other components which may affect the signal of target analytes; therefore, it is necessary to introduce an additional clean-up step before chromatographic analysis (Champion et al., 2004). Solid phase extraction (SPE) is the best method providing both cleanup and preconcentration at the same time (Buchberger, 2007).

Table 4.5. Reported functions of selected test pharmaceuticals in the literature.

Compound/abbreviation	Specific functions	References
Acetaminophen/ACE	Tracer of raw or insufficiently treated wastewater/ Indicator of WWTP malfunction or CSOs	(Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2008, 2009)
Aspartame/APM	Potential marker of domestic wastewater in groundwater	(Buerge et al., 2009)
Atenolol/ATL	Indicator of human faecal contamination	(Kasprzyk-Hordern et al., 2008)
Caffeine/CAF	Indicator of recent and cumulative wastewater contamination of natural waters (surface waters and stormwater outfalls)	(Buerge et al., 2003; Daneshvar et al., 2012; Glassmeyer et al., 2005; Hillebrand et al., 2012; Madoux-Humery et al., 2013; Peeler et al., 2006; Sankararamakrishnan & Guo, 2005; Sauvé et al., 2012; Viglino, Aboulfadl, Daneshvar, et al., 2008; Young et al., 2008b)
Carbamazepine/CBZ	Indicator of cumulative wastewater discharges	(Clara et al., 2004; Daneshvar et al., 2012; Gasser et al., 2011; Gasser et al., 2010; Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2008; Madoux-Humery et al., 2013; Viglino, Aboulfadl, Daneshvar, et al., 2008)

Table 4.5. Reported functions of selected test pharmaceuticals in the literature (cont'd).

Compound/abbreviation	Specific functions	References
Diclofenac/DIC	Indicator of human faecal contamination	(Kasprzyk-Hordern et al., 2008)
Medroxyprogesterone/MedP	Indicator of municipal wastewater discharges	(Kolodziej et al., 2003)
N,N-diethyl-3-methylbenzamide /DEET	Indicator of human fecal contamination to identify human sewage contamination in water bodies	(Aronson et al., 2012; Glassmeyer et al., 2005)
Progesterone/PRO	Indicator of fecal contamination in drinking water sources	(Daneshvar et al., 2012)
Theophylline/THEO	Indicator of human faecal contamination	(Madoux-Humery et al., 2013)

4.8.2 Methods and materials

4.8.2.1 Chemicals and reagents

High purity (> 97%) analytical standards of caffeine, carbamazepine, theophylline, acetaminophen, atenolol, aspartame, progesterone, medroxyprogesterone, diclofenac and N,N-diethyl-3-methylbenzamide were provided by Sigma-Aldrich Canada (Oakville, ON, Canada). Some of the important parameters of the compounds are summarized in Table 4.6. The surrogate (carbamazepine-d_{10}; 98%) and internal standard ([$^{13}C_3$]-atrazine; 99%, [$^{13}C_3$]-caffeine; 99%, [$^{13}C_2$]-acetaminophen; 99%, progesterone-d_9; 98% and diclofenac-d_4; 98%) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

Selected compounds have low vapor pressure and medium to high water solubility. They remain stable under normal operating conditions and variable time of storage (Aboulfadl et al., 2010). Individual stock standard solutions were prepared by dissolving each compound in methanol at a concentration of 1000 mg L^{-1} for the analytes and at 1 mg L^{-1} for the isotopically-labeled standards. A standard mixture solution containing all the analytes was prepared weekly in methanol at a concentration of 50 mg L^{-1}.

All organic solvents and water used were HPLC grade and obtained from Fisher Scientific (Whitby, ON, Canada) and all the other reagents were of analytical grade. The SPE with the following adsorbents was evaluated: Strata C18-E, 52 µm, 500 mg per 6 cc (silica-based, reversed phase absorbent with a hydrocarbon and aromatic functional group) and Oasis HLB, 30 µm, 60 mg per 3 cc (lipophilic divinylbenzene + hydrophilic N-vinyl pyrrolidone) (Samaras et al., 2011). The adsorbents were obtained respectively from Phenomenex (Torrance, California, USA) and Waters (Milford, MA, USA). Polypropylene syringe filters (hydrophobic, 0.22 µm, 30 mm diameter) were obtained from Sterlitech (Kent, WA, USA).

4.8.2.2 Apparatus

Liquid chromatography with mass spectrometric detection was performed using a thermostated autosampler (CTC HTS PAL analytics AG, Switzerland) fitted with a cooled sample holder at 10 °C, a 100 µL syringe, a 25-µL loop, six-port switching valves, an additional solvent reservoir and an Accela 1250 quaternary pump from Thermo Fisher Scientific (San Jose, CA, USA).

Chromatographic separation was carried out in a reversed phase Hypersil GOLD C18 UPLC column (50 x 2.1 mm, 1.9 µm) from Thermo Fisher Scientific, preceded by a security guard column (0.2 µm, 2.1 mm) of the same packing material from the same manufacturer. It was used to prevent particles from clogging the column and to prolong column life. Polyetheretherketone (PEEK) capillary tubing (1/16 in O.D x 0.005 in I.D., Thermo Scientific) was used to minimize the void volume of the system. Mass spectrometry was performed on a Thermo Scientific TSQ Quantum Ultra AM Mass Spectrometer equipped with an atmospheric pressure chemical ionisation (APCI) interface. Analytical instrument control, data acquisition and treatment were performed by Thermo Fisher Scientific Xcalibur 1.2 software (Waltham, MA, USA).

4.8.2.3 Preparation and spiking of sediment samples

The frozen sediment samples were lyophilized and sieved through an 80-µm screen in order to optimize the mechanism of sorption of analytes to solids and then stored in a closed flask at -20 °C. To optimize the extraction procedure, fractions of the sample (1 and 0.1 g of stream and CSO sediments respectively) were placed in different 15-mL conical polypropylene centrifuge tubes, covered with acetone (800 µL) and spiked with a mixture of standards (10 and 100 ng g^{-1} for stream and CSO sediments respectively). The samples were shaken at 100 rpm in a refrigerated incubator shaker Innova 4230 (New Brunswick Scientific Edison, NJ. USA) and allowed to equilibrate overnight in the dark before extraction, in order to obtain a dry and homogenous material.

4.8.2.4 UHPLC–APCI–MS/MS

A sample of 20 µL was injected through a 25-µL sample loop. Compounds were separated and measured by ultra-high performance liquid chromatography (UHPLC) using a Hypersil GOLD C18 UPLC column (50 x 2.1 mm, 1.9 µm). The analytical column was previously chosen and analysis was successfully done in our laboratory for the majority of the studied compounds (Viglino, Aboulfadl, Daneshvar, et al., 2008; Viglino, Aboulfadl, Prévost, et al., 2008). The column temperature was set to 45°C. We used a binary gradient of mobile phases A (H_2O) and B (acetonitrile) both contained 0.1% formic acid and operated at a constant flow rate of 0.4 mL min^{-1}. An HPLC gradient program was applied as follow: hold at 5% (B) over 0.7 min, increased from 5% to 30% (B) over 0.1 min, increased from 30 % to 35 % (B) over 1 min then increased linearly to 95 % over 0.1 min and hold on for 0.6 min and then decreased to 5 % (B). The

composition was held at 5 % (B) for a further 1.7 min for re-equilibration, giving 4.5 min of total run time for each sample (Figure 4.3).

The APCI probe with a corona discharge used pneumatic nebulization to vaporize the solvents and analytes. The vaporizer was operated at a temperature of 450 °C with the heated capillary temperature set at 350 °C. Samples were ionized by reacting with solvent reactant ions produced by the corona discharge (3 µA) in the chemical ionization (CI) mode. The pressures were 45 and 5 arbitrary units for the nitrogen sheath gas and the auxiliary gas respectively. Mass spectrometry detection was performed under the time-scheduled selected reaction monitoring (SRM) conditions shown in Table 4.7, by using APCI interface operating in the positive ion (PI) mode. The parent ions $[M + H]^+$ monitored under the optimized MS conditions are listed in Table 4.8. Collision induced dissociation (CID) was performed by introducing argon at 1.5 mTorr in the Q2 chamber. Collision offset energy was appropriately optimized for each compound transition. The daughter scan width was set at 1.0 amu, and the total scan time was 0.02 s. Compounds were identified on the basis of their relative retention time, which is the ratio of the retention time of the analyte to that of the internal standard. Quantification was performed using internal standard method. The peak areas of analytes were normalized to those of the internal standard.

4.8.2.5 Method performance

Extraction and SPE conditions

Our preliminary choice of evaluated extraction solvents and extract pH was based on other previous studies (see Table 4.1). In preliminary tests with the help of the Strata C-18E absorbent, the extraction procedure was optimized for extraction solvents, extraction time, and extraction temperature using samples of treated stream sediments spiked with analytes. Different extraction organic solvents and aqueous buffers (water, water with 0.1% formic acid, methanol, acetone, 2-propanol, acetonitrile, dimethyl sulfoxide, water/methanol (9:1, v/v, pH 11 and 5:5, v/v, pH 4), water with 0.1% formic acid /methanol (5:5 and 4:6, v/v), water/acetone (2.5:7.5, v/v), water mixture contains 0.1% formic acid /acetone (6:4, v/v) and methanol/acetone (5:5 and 6:4, v/v)) and different time (20, 30 and 45 min) and temperature of sonication (30 and 50 °C) were applied. Once the optimal extraction conditions were found, multiple sequential extractions (up to 6 cycles) of the same sediment samples were conducted to ensure quantitative extraction during USE. The extracts were collected individually and analyzed.

In order to ascertain the extraction recovery of analytes at different pH values, extract samples diluted with 10 mL of UPW and were spiked with the analytes at a concentration of 1 µg/L and adjusted to the desired pH values (non-adjusted, 4, 7 and 11) using formic acid (95%) and NaOH (0.5 M). With the use of SPE cartridge (Strata C-18E), the extraction recovery rates of the analytes in interest were identified.

Prior to the SPE extraction, conditioning, washing and elution procedures were implemented on the selected cartridges (Strata C-18E and Oasis HLB). We tried different volumes and ratios of mixture washing buffers (0, 5 and 10 mL of water/methanol (100:0 and 95:5, v/v)), different volumes of the loading solution (10 and 250 mL) and different volumes and proportions of elution solvents (methanol followed by 0.5 M formic acid–methanol mixture, 1:0, 1:1 and 2:1 ratios with 5, 2 and 1.5 mL as total volume respectively).

In order to compare the SPE step with Strata C-18E and Oasis HLB cartridges, ultra-pure water (UPW) samples (n=3) with 10 mL volume and adjusted pH to7 were spiked with analytes at concentration of 1 µg/L. SPE recovery was checked through comparing the samples spiked before and after SPE. To confirm the results obtained, we repeated the same test with the extract sediment samples instead of water and compared the shape of the chromatographic peaks of each compound.

Extraction efficiency was evaluated an absolute recovery determined in relation to a non-enriched standard solution and calculated as follows: 6 replicate sediment samples for the extraction method were prepared. Three samples were added with mixture of standards after extraction (BL) and the other 3 samples were added with a mixture of standards before extraction (ADD). The recoveries were determined by comparing the measured concentrations in BL with the measured concentrations in ADD using the following Eq. (1):

$$\text{Absolute recovery} = \frac{\mathbf{ADD}}{\mathbf{BL}} * 100 \qquad (1)$$

In order to distinguish between the extraction efficiency of WWMPs sorbed to the sediments and the recovery of the clean-up method, the following experiment was performed: first, 3 sediment samples were spiked with 100 µL of the non-deuterated standard solution at 0.1 mg/L. Second, 3 sample extracts were spiked prior to the clean-up step. Finally, 3 samples were spiked after the clean-up step. The differences in mean recoveries between these three sets of samples were then

used for the calculation of the recoveries for each step. Carbamazepine-d_{10} was added as a surrogate standard, at concentration levels of 5 and 50 ng/g for stream and CSO sediments respectively, to all samples (before the extraction step, prior to cleanup or after cleanup) to correct for losses during sample extraction or cleanup (Ternes et al., 2005). Relative recoveries (relative to the recovery of Carbamazepine-d_{10}) were then calculated using the following Eq. (2):

$$\text{Relative recovery} = \frac{\text{Absolute recovery of the analyte}}{\text{Absolute recovery of the surrogate standard}} \qquad (2)$$

Analytical parameters: calibration, validation and matrix effects

To optimize the gradient separation and the conditions in the MS–MS detector, we tested water different pH values (2.65, 6 and 8) and two organic solvents (methanol and acetonitrile). The full-scan mass spectra and the MS/MS spectra of the selected compounds were obtained from the direct injection of a 2 mg/L standard of each compound at a flow-rate of 0.4 mL/min using both ionization modes (positive and negative). The highest intensity was selected for further study. Optimisation of the compound-dependent parameters such as the collision energy and tube lens for individual analytes were adjusted by syringe pump infusion, as described above. Mass spectrometer parameters were also optimized by continuous infusion of standards in order to find the best method to detect all compounds with the best possible signal for the compound of interest. Fragmentation voltage and collision energy were tested in order to select the transitions in the selective reaction monitoring (SRM) mode.

The analytical method was validated for each type of matrix through the estimation of the recovery, linearity, repeatability, reproducibility, sensitivity and matrix effects. Method trueness and precision were evaluated by recovery studies which were calculated as the percentage of agreement between the method results and the nominal amount of compound added in sediment extracts. Linearity was studied using standard solutions and matrix-matched calibrations by analyzing in triplicate six concentration levels, between 0 and 100 µg/L in the final extract. Experimental data fitted a linear mode, $y = a + bx$ in the concentration range studied (0, 1, 5, 10, 20 and 30 µg/L for stream sediment and 0, 5, 10, 20, 60 and 100 µg/L for CSO sediment). Calibration curves were built with the response ratio (area of the analyte standard divided by area of the internal standard) as a function of the analyte concentration. [$^{13}C_3$]-caffeine was used as an internal standard to improve quantitation of caffeine, theophylline and aspartame. [$^{13}C_2$]-

acetaminophen was used as internal standard to normalize acetaminophen and atenolol peak area variations. Diclofenac-d_4 was used as internal standard for diclofenac, carbamazepine, carbamazepine-d_{10} and N,N-diethyl-3-methylbenzamide, while progesterone-d_9 was used for progesterone and medroxyprogesterone. Because unspiked sample extracts already contained some of the compounds, a calibration curve was constructed by subtracting the level concentration for these analytes in this matrix from the spiked concentration. This procedure was also carried out for the quantitative determination of the analyte recoveries in real samples. The precision of the method was determined by the repeated intra-day (n = 5) and inter-day (3 different operating days) analysis of a spiked sample extracts at concentrations levels of 10, 20 and 30 µg/L for stream sediments and 100 µg/L for CSO sediments. The precision of the method was expressed as the relative standard deviation (RSD) of replicate measurements. Precision was deemed acceptable when RSDs were lower than 15%. Limit of detection (LOD) was defined as 3.707 times the standard deviation (n = 5) of analyte measurements in stream and CSO sediment extracts at 1 and 5 µg/L respectively (Glaser et al., 1981; Viglino, Aboulfadl, Daneshvar, et al., 2008).

Matrix effect (ME) can affect reproducibility and efficiency of the developed method. This phenomenon was evaluated at 10, 20 and 30 µg/L for stream sediment extracts and 20, 60 and 100 µg/L for CSO sediment extracts, by comparing the MS/MS response of known amounts of standards spiked in mobile phase with those measured in sediment extracts. ME was calculated as the following Eq. (3):

$$\text{ME } \% = \left(\frac{S1-S2}{S2}\right) * 100 \qquad (3)$$

where S_1 = slope of the curve obtained by injection of the analytical solutions of pharmaceuticals prepared in the extract obtained after the SPE step and S_2 = slope of the curve obtained by injection of pharmaceuticals prepared with the initial mobile phase condition. ME=0 means no matrix interference while ME>0 and ME<0 represent signal suppression and enhancement, respectively. Matrix effects were considered low for a range of signal suppression/enhancement −20% < C% < +20%, medium for the ranges −50% < C% <−20% or +20% < C% < +50% and high for the ranges C% <−50% or C% > +50% (Economou et al., 2009).

Table 4.6. Physicochemical properties of selected test pharmaceuticals (SRC, 2006).

Compound	Molecular weight (g)	$LogK_{OW}$[a]	pKa[b]	LogS (mg/L)[c]
ACE	151.17	0.46	9.3	4.15
APM	294.30	0.07	4.5–6.0	10,000
ATL	266.34	0.16	9.6	4.11
CAF	194.19	-0.07	10.0	4.33
CBZ	236.27	2.7	13.9	1.25
DIC	296.16	4.51	4.14	0.37
MedP	344.50	3.50	–	–
DEET	191.26	2.18	–	912
PRO	314.4	3.87	–	8.81
THEO	180.16	-0.02	8.81	3.87
PX	180.17	-0.63	8.5	-1.30

[a]$LogK_{OW}$, octanol-water partition coefficient; [b]pKa, acid constant; [c]LogS, solubility at a temperature of 20–25 °C.

Table 4.7. Details of the selected reaction monitoring (SRM) parameters of selected compounds in APCI–MS/MS under positive ionization mode.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision Energy (eV)	Tube lens (V)
ACE	152.10	110.14	13	67
APM	295.10	120.20	27	90
ATL	267.16	190.10	16	90
CAF	195.10	138.10	17	70
CBZ	237.10	194.10	18	67
DIC	296.02	215.10	20	66
MedP	345.16	123.10	22	127
DEET	192.15	119.10	16	111
PRO	315.15	109.10	26	100
THEO/PX	181.10	124.10	19	67

Table 4.8. MS parameters.

Ionization mode	$APCI^+$
Discharge current	3 µA
Vaporizer temperature	450 °C
Capillary temperature	350 °C
Sheath gas pressure	45 arb units
Aux. gas pressure	5 arb units
Collision gaz pressure	1.5 mTorr
Scan time	0.02 s

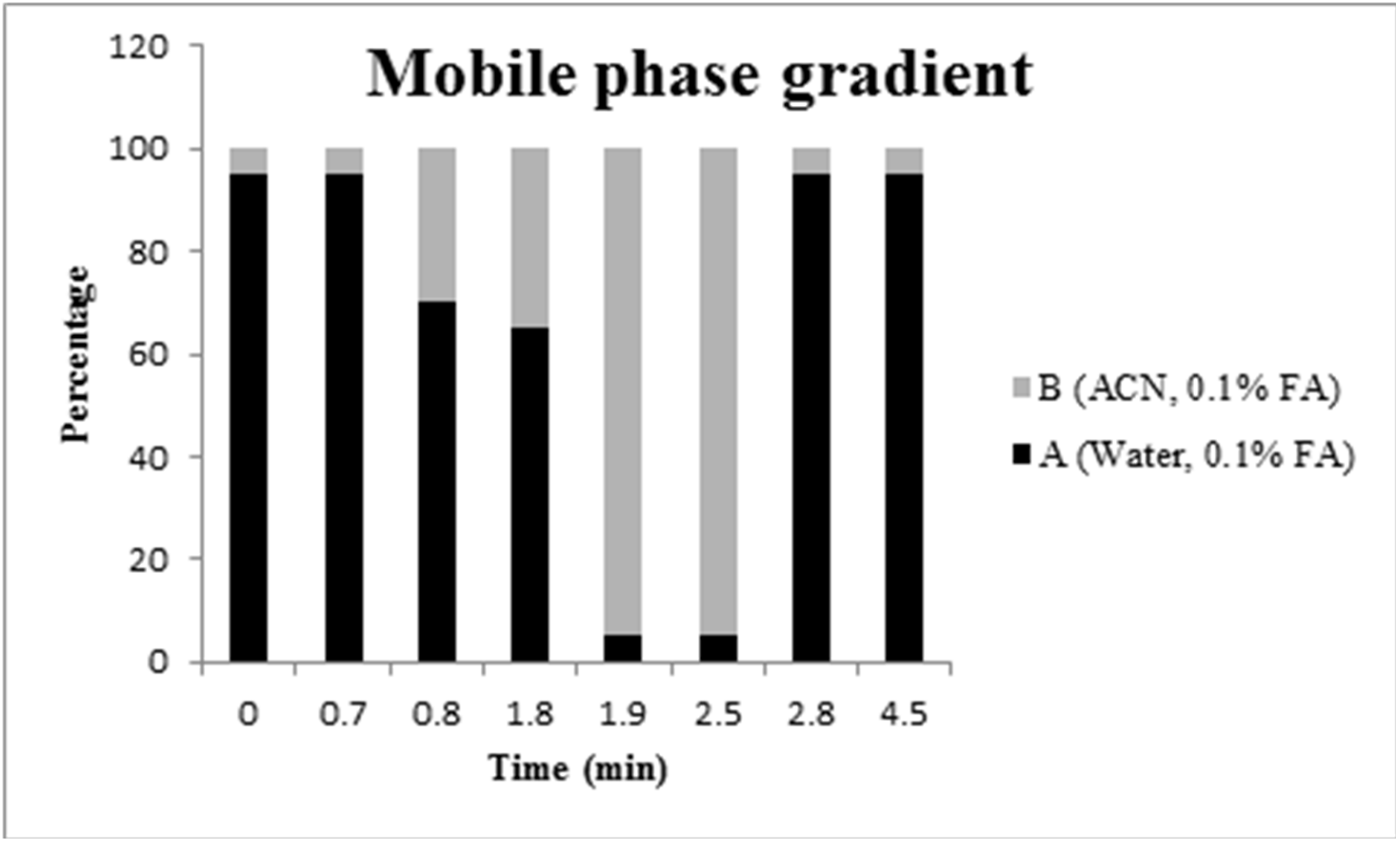

Figure 4.3. Binary gradient of mobile phase A (water/0.1% Formic acid) and mobile phase B (acetonitrile/0.1% Formic acid) by using LC Pump Accela 1250.

4.8.3 Results and discussion

4.8.3.1 Optimization of UHPLC–APCI–SRM/MS analysis and quantification

Other studies have previously studied the product ions of selected compounds using positive ESI or APCI. Transitions are in agreement with those reported for caffeine, carbamazepine, medroxyprogesterone, progesterone, diclofenac, atenolol, acetaminophen, and aspartame (Cardoso et al., 2011; Galletti et al., 1996; Vazquez-Roig et al., 2010; Viglino, Aboulfadl, Daneshvar, et al., 2008; Viglino, Aboulfadl, Prévost, et al., 2008; Yu et al., 2011). The monitoring of theophylline using positive ion conditions is unusual, as the compound is acidic in nature (Batt et al., 2008). The intensities of pseudo molecular ion peaks were higher in positive mode than negative mode, therefore (+) APCI was chosen for further study.

The most commonly used chromatography phase is C18, regardless of the compounds to be determined (Primel et al., 2012). Good chromatographic separation and MS efficiency were achieved by using Hypersil GOLD C18 UPLC column (1.9 µm, 50 x 2.1 mm, i.d.) preceded by a guard column (0.2 µm, 2 x 2 mm, i.d.) maintained at 45 °C. It provided sharp peaks and short retention times in previous studies (Viglino, Aboulfadl, Daneshvar, et al., 2008; Viglino, Aboulfadl, Prévost, et al., 2008). A chromatographic column based on 1.9 µm material reduced the analysis time. It is commonly accepted that the presence of the acid facilitates the protonation of analytes with basic functional groups in positive-ion mode (Wu et al., 2004). Formic acid improved the sensitivity of APCI (+) sources which were selected for all standards. Therefore, the best conditions for obtaining a good separation and symmetric peaks were found with acidic water (0.1% formic acid) and acetonitrile (0.1% formic acid). Sample chromatography gradients are shown in Figure 4.3 and allowed sufficient chromatographic separation of all analytes. The interference of paraxanthine (PX, the primary metabolite of caffeine in humans) to theophylline is overlooked for many LC/MS/MS methods including our own - theophylline and paraxanthine are isobaric and have the same MS/MS transition.

4.8.3.2 Optimization of extraction and SPE steps

The extraction efficiency of different solvents for different periods and temperatures of sonication was tested using spiked stream sediments. We used a Strata C-18E absorbent cartridge. Three solvents (methanol/water (1/9,v/v, pH 11), acetone and water with formic acid

0.1%) were chosen because they provided satisfactory extraction efficiencies in the range of 70−120% for all compounds excepted for atenolol, caffeine and aspartame. Acetaminophen was extracted in the first three cycles while atenolol and aspartame were extracted in the first and last cycles. The remaining compounds (caffeine, carbamazepine, theophylline, DEET, diclofenac, progesterone and medroxyprogesterone) were progressively extracted throughout the cycles. In the last cycle, we used water with formic acid 0.1% to enhance the recovery of aspartame which has good stability at pH values between 3.4 and 5 (Berset & Ochsenbein, 2012).

We observed a slight increase in extraction efficiency when the extraction time was extended from 20 to 30, and 45 min. Recoveries of some compounds (e.g., aspartame, caffeine and atenolol) declined. Other extraction parameter that affected extraction efficiency is the ultrasonic extraction temperature. Increasing the temperature from 30 to 50 °C generated relatively low recoveries for all compounds. Therefore, a sonication of 20 min at 30 °C was set for sample treatment.

The choice of extract pH prior to SPE is a key point in solid-phase extraction, especially when we have compounds with different physicochemical properties. Extraction efficiencies for clean-up with SPE were also determined with a procedure similar to that described for extraction. The optimum pH was determined for the recovery of the selected compounds prior to the SPE of extract samples. The lowest recovery for theophylline/paraxanthine was found at pH 11, which is why we rejected the SPE method at pH 11. The highest intensity and sharp peak for theophylline/paraxanthine and best recovery for caffeine occurred at pH 7. The recovery for carbamazepine at pH 4 was very close to its recovery at pH 7 whereas the recovery for diclofenac was highest at pH 4 but remains above 70% at pH 7. In the light of these results, the optimum pH for the simultaneous extraction of all selected compounds was chosen as pH 7. Similar results have been shown in other studies: the recoveries for basic and neutral pharmaceuticals with acidification of the purification extract was between 5% and 20% lower than those obtained without acidification, whereas recoveries for acidic compounds were very similar (Vazquez-Roig et al., 2010). The effect of pH on the SPE efficiency was also studied by Gómez et al. (2006) by increasing the pH value of the sample from 2 to 4 and 7. The results showed that the extraction recoveries for many of target compounds (e. g. carbamazepine, atenolol, acetaminophen and diclofenac) from spiking hospital effluent wastewater samples was higher at pH 7 and ranged from 88.1 to 114%. Weigel, Kallenborn, et al. (2004) obtained good recoveries, ranging from 97

to 102%, for caffeine, carbamazepine and DEET with Oasis HLB sorbent and sample pH at 7. Acetaminophen showed low recovery in the same conditions (Weigel, Kallenborn, et al., 2004). Similar recoveries were obtained for acetaminophen (20−40%) when the Oasis HLB sorbent was used and the sample pH prior to SPE was 2 and 7. The influence of sample pH on the extraction efficiency of diclofenac, carbamazepine and caffeine was significant. The best recoveries for these compounds were obtained under acid conditions when the pH sample prior to SPE was 2 (Camacho-Muñoz et al., 2009). Pichon et al. (1996) showed that the removal of humic and fulvic acid interferences from water samples was performed at neutral SPE pH by using polymeric sorbents for the simultaneous extraction of polar acidic, neutral and basic components. The co-extracted interferences directly affect the quantitation of analytes. The effect of signal suppression decreased with increasing SPE pH up to 8.5 (Pichon et al., 1996).

We compared the SPE recovery rates achieved in UPW without the sediment matrix by Strata C-18E and Oasis HLB cartridges. The SPE recovery rates for acetaminophen and atenolol were very low with Strata C-18E cartridge, (respectively; 15.21 and 0.27%) while Oasis HLB showed a good retention for all analytes. Recoveries were greater than 90%, with the exception for aspartame and progesterone, which showed recoveries of 61.1% and 75.2% respectively. Moreover, the use of an Oasis HLB cartridge has produced a peak for acetaminophen with symmetrical (Gaussian) shape and straight line spikes in which no broadening occurred (Figure 4.4). Therefore, the Oasis HLB cartridge (at pH 7) was selected for all compounds. Several papers reported on the evaluation of a number of stationary phases for solid phase extraction (SPE) of the selected WWMPs (Ahrer et al., 2001; Camacho-Muñoz et al., 2009; Hilton & Thomas, 2003; Mutavdzic Pavlovic et al., 2010; Vazquez-Roig et al., 2010; Weigel, Kallenborn, et al., 2004), however in many situations, the optimal SPE material is variable and highly dependent on target analytes and experimental conditions. For example, for diclofenac, some authors indicated that C18 silica sorbents give results superior than polymeric sorbents (Ahrer et al., 2001; Samaras et al., 2011), while other reported higher recoveries with the polymeric Oasis HLB cartridges (Marchese et al., 2003; Quintana & Reemtsma, 2004). The comparison of different types of polymeric SPE sorbents (Bakerbond SDB-1, Lichrolut EN, Isolute Env+, Chromabond HR-P, Chromabond EASY, Abselut Nexus and Oasis HLB) demonstrated that the best performance was achieved using Oasis HLB giving highest recoveries for all tested compounds (caffeine, 97%; DEET, 100%; carbamazepine, 101% and diclofenac,102%) except

for acetaminophen (14%) (Weigel, Kallenborn, et al., 2004). Another study compared Oasis HLB and Oasis MCX sorbents and showed that acetaminophen, carbamazepine and caffeine were more efficiently recovered when Oasis HLB sorbent was used, while the recovery for diclofenac was best using an Oasis MCX sorbent (Camacho-Muñoz et al., 2009).

The choice of elution solvent and volume is dependent of the target compounds and the SPE material. The most common elution solvents of Oasis HLB are regarded as methanol, ethyl acetate and acetone (Camacho-Muñoz et al., 2009; Ollers et al., 2001; Rodil et al., 2009; Weigel, Kallenborn, et al., 2004). In our study, the best recoveries for selected analytes were obtained with elution by 1 mL of methanol and 1 mL of acidified methanol (with 0.5 M formic acid), successively.

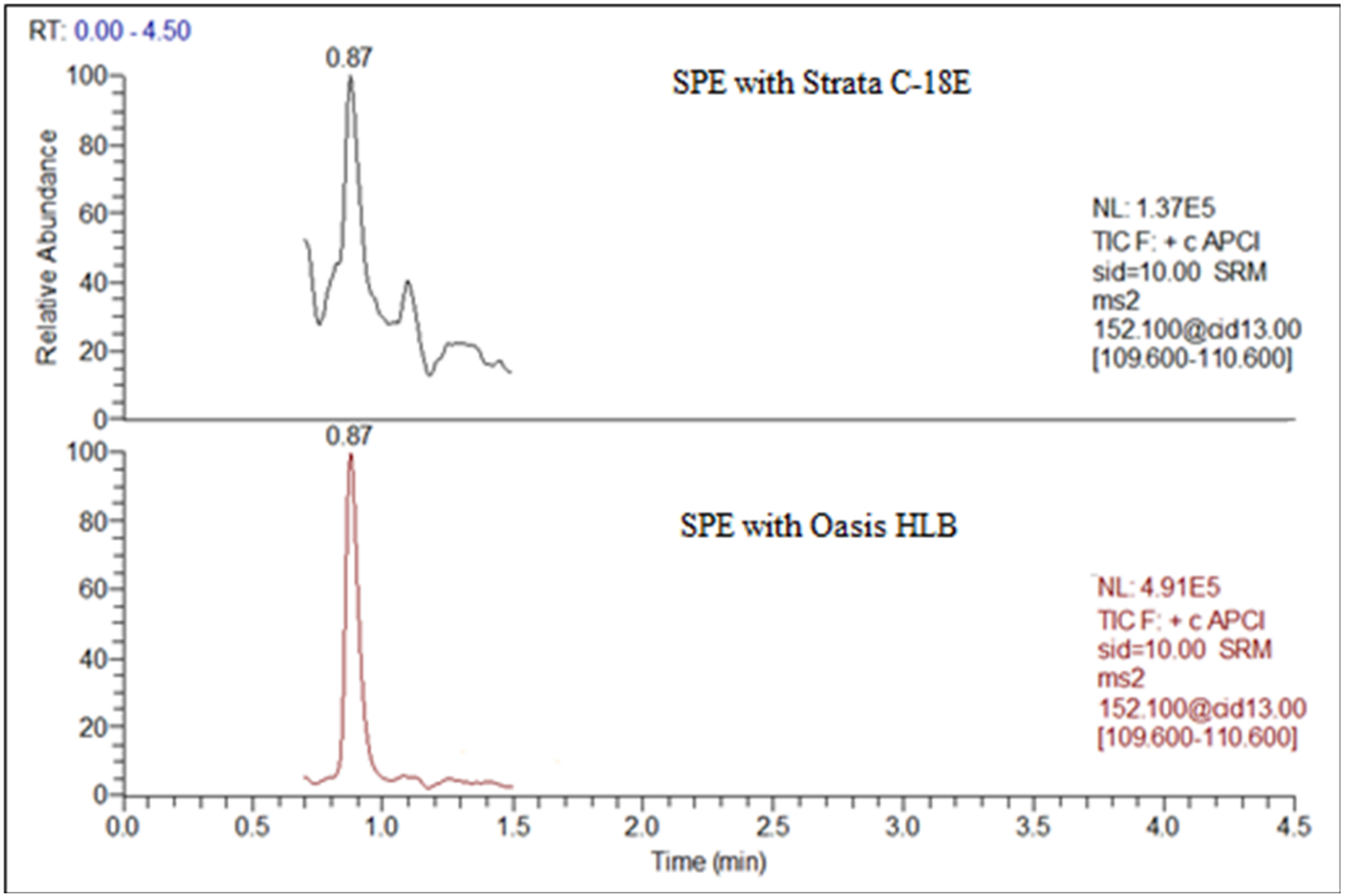

Figure 4.4. Shape and intensity of peak for acetaminophen after extraction from stream sediment and purification with Strata C-18E (top) and Oasis HLB (bottom).

4.8.3.3 Matrix effects

Internal standards have been used to further correct for residual matrix effects and compensate for signal suppression/enhancement. At the concentrations studied, the matrix effects were low for all analytes, demonstrating the effectiveness of the sample pre-treatment. Among the compounds analyzed in APCI positive mode, diclofenac (pKa = 4.14), a weak proton acceptor, was particularly sensitive to matrix effects. Matrix effects ranged from -18% and 18% as shown in Figure 4.5. Wick et al. (2010) studied matrix effects in different sample matrices (activated sludge, raw and treated wastewater, and surface water) using ESI and APCI. Similarly to our results, APCI was generally less susceptible to ion suppression than ESI but partially susceptible to ion enhancement of up to a factor of 10. In addition, Zhao and Metcalfe (Zhao & Metcalfe, 2008) observed a signal enhancement in wastewater extracts analyzed for neutral pharmaceuticals (e. g. caffeine and carbamazepine) as a result of interferences from the sample matrix.

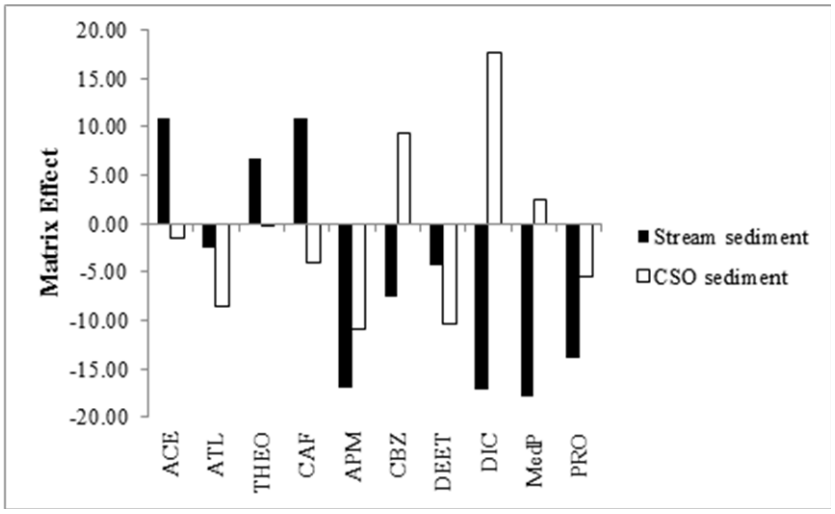

Figure 4.5. Percentage of matrix effect (ME) for the technique using ultrasonication-assisted extraction and LC-APCI-MS/MS for the determination of WWMPs in stream and CSO sediments.

4.8.3.4 Method applicability in sediment samples and comparison with water samples

Table 4.9. Classification of WWMPs depending on their average dynamic ranges in sediment (S) and water (w) under dry (DW) and wet weather (WW) conditions.

	The highest range ⟶ **The lowest range**			
C $_{(S, DW)}$	**THEO**	**ACE**	**CAF**	**CBZ**
US1 $_{(S, DW)}$	**ACE**	**CAF**	**THEO**	**CBZ**
US$_2$ $_{(S, DW)}$	**ACE**	**CAF**	**THEO**	**CBZ**
US$_3$ $_{(S, DW)}$	**ACE**	**CAF**	**THEO**	**CBZ**
Canal $_{(S, DW)}$	**ACE**	**THEO**	**CAF**	**CBZ**
SA $_{(W, DW1)}$	**CAF**	**ACE**	**THEO**	**CBZ**
SB $_{(W, DW2)}$	**THEO**	**ACE**	**CAF**	**CBZ**
OA $_{(W, WW1)}$	**ACE**	**CAF**	**THEO**	**CBZ**
OB $_{(W, WW2)}$	**CAF**	**THEO**	**CBZ**	**ACE**
US$_1$ $_{(W, DW)}$	**ACE**	**THEO**	**CAF**	**CBZ**
US$_1$ $_{(W, WW)}$	**CAF**	**ACE**	**THEO**	**CBZ**
US$_2$ $_{(W, DW)}$	**ACE**	**CAF**	**THEO**	**CBZ**
US$_2$ $_{(W, WW)}$	**ACE**	**CAF**	**CBZ**	**THEO**
US$_3$ $_{(W, DW)}$	**THEO**	**CAF**	**CBZ**	**ACE**
US$_3$ $_{(W, WW)}$	**THEO**	**CAF**	**CBZ**	**ACE**
Canal $_{(W, DW)}$	**CAF**	**CBZ**	**THEO**	**ACE**
Canal $_{(W, WW)}$	**CAF**	**CBZ**	**THEO**	**ACE**

C (CSO); S (sediment); W (water); DW (dry weather); WW (wet weather); C, SA, SB and OA (high degree of human fecal contamination); OB, US_1, US_2 and US_3 (medium degree of human fecal contamination); Canal (low degree of human fecal contamination); Data about SA and SB (samples collected in the two sewersheds A (SA) and B (SB), immediately upstream of the CSO outfall during dry weather conditions), the two CSO outfalls (OA and OB), and canal were presented in Madoux-Humery et al. (2013) and Guérineau et al. (2014).

4.8.4 References

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CHAPITRE 5 ARTICLE 2 : THE KINETICS AND DYNAMICS OF SORPTION AND DESORPTION OF MOBILE PHARMACEUTICALS AND CAFFEINE TO COMBINED SEWER SEDIMENTS

L'article suivant décrit les différents comportements de sorption (adsorption et désorption) qui ont été observés pour cinq composés étudiés (acétaminophène (ACE), caféine (CAF), théophylline (THEO), carbamazépine (CBZ) et 10,11-dihydro-10,11-dihydroxycarbamazépine (CBZ-DiOH)) dans deux matrices différentes (SS et StS) provenant d'un trop-plein (TP). La chromatographie liquide couplée à la spectrométrie de masse a été utilisée pour la quantification des analytes dans les deux phases, la phase solide et la phase liquide. Deux approches ont été utilisées pour imiter la remise en suspension des contaminants par temps sec et lors d'épisodes de pluie ou fonte des neiges, une approche statique et une autre dynamique avec l'étude de l'équilibre en batch. Les deux approches ont montré clairement l'influence de rinçages successifs sur les concentrations des contaminants dans les rejets des trop-pleins. Elles apportent également des informations sur la dynamique de sorption des contaminants étudiés sur des sédiments dopés et non dopés ainsi que sur leurs cinétiques d'adsorption et de désorption. Ces résultats ont été soumis à *Water Research* en février 2016.

THE KINETICS AND DYNAMICS OF SORPTION AND DESORPTION OF MOBILE PHARMACEUTICALS AND CAFFEINE TO COMBINED SEWER SEDIMENTS

M. Hajj-Mohamad[a,b*], H. Darwano[c], S. Vo Duy[c], S. Sauvé[c], M. Prévost[b], H. P. H. Arp[d], S. Dorner[a]

[a] Canada Research Chair in Source Water Protection, Department of Civil, Geological and Mining Engineering, Polytechnique Montréal, C.P. 6079, Succursale Centre-ville, Montréal, Qc, Canada H3C 3A7; [b] NSERC Industrial Chair in Drinking Water Treatment, Department of Civil, Geological and Mining Engineering, Polytechnique Montréal, C.P. 6079, Succursale Centre-ville Montréal, Qc, Canada H3C 3A7; [c] Department of Chemistry, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montreal, QC, Canada H3C 3J7; [d] Norwegian Geotechnical Institute (NGI), P.O. Box 3930 Ullevål Stadion, N-0806 Oslo, Norway; *Corresponding author. Phone: +1 514 340-4711 ext. 2983, e-mail: mariamhajjm@gmail.com

5.1 Abstract

Pharmaceuticals are discharged to the environment from wastewater resource recovery facilities, sewer overflows, and illicit sewer connections, amongst other sources. Pharmaceuticals can be used as tracers of fecal and sewage contamination. However, to understand the risk of this contamination there is a need to better understand the sorption dynamics of pharmaceuticals to suspended sediments (SS) and settled sediments (StS) in sewer systems. In this study, such sorption dynamics to both SS and StS were assessed using a batch equilibrium method under both static and dynamic conditions. Experiments were performed with naturally occurring and spiked sewer pharmaceuticals (acetaminophen, theophylline, carbamazepine, and a metabolite of carbamazepine) and caffeine. Differences in sorption coefficients, $K_{d,app}$, between SS and StS were related to differences in their organic carbon (OC) content, and spiked versus naturally occurring pharmaceuticals, with $K_{d,app}$ values of spiked contaminants and high OC sediments being substantially higher. Pseudo-second order desorption rates for these mobile compounds were also quantified. Successive flushing events to simulate the addition of stormwater to sewer networks revealed that aqueous concentrations will not necessarily decrease, because the added water will rapidly return to equilibrium concentrations with the sediments. Sorption and desorption kinetics must be considered in addition to dilution, to prevent the underestimation of the influence of dilution.

Keywords

Emerging contaminants; anthropogenic tracers; suspended solids; settled sediment; storm water flushing; batch equilibrium

5.2 Introduction

Many urban regions around the world have combined sewer systems (CSS) that handle both sanitary and stormwater flows within the same network (Even et al., 2007). In addition, "illicit CSS" via sanitary sewer connections to storm sewer networks are frequent (Burian et al., 2000; Hoes et al., 2009). Large quantities of pollutants that settle and accumulate in sewer networks will be resuspended during wet weather events, which can lead to pulses of contaminant discharge to receiving waters (Wang et al., 2011), resulting in the degradation of their water quality and aquatic habitats (Blumensaat et al., 2012). Accordingly, Combined Sewer Overflow

(CSO) outfalls are an important point source of wastewater micropollutants such as mobile pharmaceuticals in aquatic environments (Buerge et al., 2006; Gromaire et al., 2001; Phillips & Chalmers, 2009; Stewart et al., 2014; Zhou & Broodbank, 2014). CSOs have been shown to contribute approximately 10 % of the fine particles found in urban river bed sediments (David et al., 2013). Large variations of mobile pharmaceutical concentrations during CSOs have been reported (270–3248 ng caffeine L^{-1}, 4.1–184 ng carbamazepine L^{-1}, non-detected–3591 ng acetaminophen L^{-1}, 57.3–2381 ng theophylline L^{-1}) (Madoux-Humery et al., 2013).

Sorption is one of the key factors controlling the input, transport, and transformation of pharmaceuticals in an aquatic environment, including within CSS (Scheytt et al., 2005). In sewer networks, suspended sediments settle out during periods of dry weather, but then are subsequently resuspended when flow rates increase due to precipitation or snowmelt (Madoux-Humery et al., 2013). Resuspension of sewer sediments during wet weather contribute up to 75% of the suspended solids, 10-70% of the *E. coli* and 40-80% of the intestinal enterococci in urban rivers (e.g. the Seine (Passerat et al., 2011)). The degree to which pharmaceuticals are released from resuspended CSS sediments is not clear since both stormwater runoff and wastewater can influence sediment resuspension and their pharmaceutical sorption behaviour. Previously, we investigated the occurrence of pharmaceuticals in CSS sediments and their role as both a source and sink of pharmaceuticals in urban water systems (Hajj-Mohamad et al., 2014). Other than this, the few investigations on the occurrence of pharmaceuticals in CSS focused solely on the water column and have seldom considered interactions with sediments (Benotti & Brownawell, 2007; Madoux-Humery et al., 2013; Stewart et al., 2014).

The extent of pharmaceutical sorption to environmental solids is highly variable, partly because pharmaceuticals include a wide variety of different substances that can exert different mobility in water and different sorption interactions with environmental solids. Several recent reports describe a variety of different sorption-desorption behaviours of pharmaceuticals in soils, sediments and sludges (Chefetz et al., 2008; Drillia et al., 2005; Jones et al., 2006; Lin et al., 2010; Navon et al., 2011; Stein et al., 2008; Ternes et al., 2004; Williams et al., 2006; Yamamoto et al., 2005; Yu, Fink, et al., 2009). In many of these aforementioned studies, these environmental media were artificially spiked in the laboratory with pharmaceuticals at the µg L^{-1} range or even higher, which is not representative of either the means nor concentration of which pharmaceuticals come in contact with sediments. A concern is that the sorption behaviour of

spiked contaminants is not the same as native contaminants, which is a recognized issue with hydrophobic organic contaminants (Arp et al., 2009), but has not been explored with pharmaceuticals.

In this study, the general objective was to illustrate the role of CSS sediments on the fate and transport of pharmaceuticals during wet weather events, using a simple laboratory test system. These tests were done to assess the extent of sorption dynamics for native and spiked mobile pharmaceuticals in shaken and still batch systems, to simulate changes during water dilution and resuspension events. All experiments were done using actual CSS sediments, to more closely reflect real-world conditions. The specific goals were: (1) to quantify the solid-water distribution coefficient (K_d) and the sorption/desorption kinetics of pharmaceuticals in static and dynamic sewer conditions, simulating the addition of storm water flushing; (2) assess the difference between native and spiked contaminated systems; (3) to consider our findings in the context of initial resuspension of settled sewer sediments and their implications for the fate of the targeted compounds during CSOs; and (4) to evaluate selected pharmaceuticals as wastewater tracers in relation to their sorption dynamics.

As such, we selected four mobile pharmaceuticals (acetaminophen, theophylline, carbamazepine, and a metabolite of carbamazepine) and caffeine. To the best of our knowledge, this is the first study demonstrating the sorption and desorption behaviour of the selected pharmaceuticals in actual CSS sediments.

5.3 Experimental methods

5.3.1 Chemicals

Acetaminophen (ACE), caffeine (CAF), carbamazepine (CBZ)), theophylline (THEO) and the metabolite 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH) were selected as target analytes based on several criteria: (1) their consumption, pharmacokinetic behaviour and occurrence in the environment (Daughton & Ternes, 1999 ; Heberer, 2002a; Viglino, Aboulfadl, Daneshvar, et al., 2008); (2) their classification in various groups to cover a wider range of properties and functions; (3) the proposed use of ACE, CAF and CBZ as anthropogenic tracers (*e.g.*, (Benotti & Brownawell, 2007; Daneshvar et al., 2012; Wu et al., 2008); (4) the higher detection of metabolite CBZ-DiOH in wastewater compared to its parent compounds (Hummel et

al., 2006; Miao & Metcalfe, 2003); and (5) the importance of CSOs as a primary source of pharmaceuticals including CAF (Musolff et al., 2009).

Table 5.1 and the ESI† (including Section 5.8.1 and Table 5.5) contain a detailed description of selected pharmaceuticals, including literature sorption measurements. High purity (> 97%) analytical standards of acetaminophen, caffeine, theophylline, carbamazepine and 10,11-dihydro-10,11-dihydroxycarbamazepine were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Stock solutions (1 mg mL^{-1}) of the standards were prepared by dissolving each compound in methanol and stored at -20 °C. Internal standards of acetaminophen [$^{13}C_2$]-acetaminophen, caffeine (trimethyl-$^{13}C_3$, 99%) and carbamazepine (carbamazepine-d_{10}; 98%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). All solvents, HPLC grade water (H_2O), 0.1% formic acid in H_2O (0.1% FA in H_2O) were purchased from Fisher Scientific (Whitby, ON, Canada). HPLC grade formic acid (FA) (98% pure) was purchased from Sigma-Aldrich (Oakville, ON, Canada). GF-75 glass fiber membrane filters (0.3 µm, 47 mm diameter) were obtained from Sterlitech (Kent, WA, USA).

5.3.2 CSS sampling and pre-treatment

CSS sediments from Greater Montréal were collected manually with a stainless steel trowel from surface sediment deposits in a CSO collection system (See Figure 5.5 in the ESI). Collected sediments were placed in pre-cleaned glass bottles and covered with aluminum foil to avoid photodegradation. CSS water samples were collected at the same time of sediment sampling in a pre-cleaned 1-L polypropylene bottle at approximately 0.3 m below the water surface, and stored cool in an insulated cooler chest. In the lab both sediment and water samples were immediately sterilized using gamma radiation (30 kGy, 5.2 h) and then stored at 4 °C. Sediment/water sterilization was confirmed by testing the total concentration of aerobic and anaerobic bacteria after gamma irradiation, using tryptone soybean agar (TSA) at temperature of 30 °C for up to 10 days (Vieira & Nahas, 2005). Prior to the batch experiments, sub-samples were pooled to form composites, homogenized and wet sieved (< 1.25 mm) with CSS water (see the next paragraph) in order to remove debris. The pH of the aqueous phase from the first and last sampling intervals was measured for each sediment type in separate replicates to avoid cross contamination of samples.

CSS sediment samples were divided into Suspended Sediments (SS) and Settled Sediments (StS) after decanting the CSS sediments following 5 days of storage in the dark at 4 °C. The SS and StS were separately used in batch reactors. Sediment properties such as moisture content, total solid concentrations, fraction of organic carbon (f_{OC}) and sediment pH were analysed using standard methods (Table 5.6 in ESI). Important differences were that f_{OC} by dry weight was much higher in the SS (51.7%) than StS (6.3%), though because the water content in the SS is much larger, the OC content per wet volume was similar for SS (13.7 g L^{-1}) and StS (9.9 g L^{-1}).

Table 5.1. Abbreviations and characteristics of acetaminophen caffeine, theophylline, carbamazepine and 10,11-dihydro-10,11-dihydroxycarbamazepine. Note that all compounds are neutral at the pH range considered in this study (7.05 – 7.35).

Substance (abbreviation)	**Formula and MW**	**Solubility[a] (mg/L)**	**pKa**	**Log K_{ow}**	**Log K_d in sludge (L Kg^{-1})**	**Log K_{oc} in sludge (L Kg_{oc}^{-1})**
Acetaminophen (ACE)	$C_8H_9NO_2$	14000	9.46[b]/9.4[c]	0.46[c]/0.49[f]	1.3[g]	
Analgesic	151.16				-0.4[c]	1.8[c]
Caffeine (CAF)	$C_8H_{10}N_4O_2$	21600	10.4[d]	-0.07	1.1[g]	1.7[g]
Stimulant	194.08				0.8; 0.9[h]	
Theophylline (THEO)	$C_7H_8N_4O_2$	7360	8.8	-0.02		
Bronchodilator, also metabolite of caffeine	180.166					
Carbamazepine (CBZ)	$C_{15}H_{12}N_2O$	17.7	13.9[c]	2.25[c]	1.4[c]	1.9[c]
Antiepileptic	236.1			2.67 ± 0.38[j]	1.6[g] 1.0[h] -1.0[k]	2.1[g]
10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH)	$C_{15}H_{12}N_2O_2$	103.9	12.84[e]	0.13 ±0.41[j]	0.3[i] (river sediment), -0.5[l] (creek sediment)	1.5[l] (creek sediment)
Metabolite of carbamazepine	270.1					

[a] from PubChem (https://pubchem.ncbi.nlm.nih.gov, accessed January 2016), except CBZ-DiOH, from EPI-Suite v4.11 (http://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface - accessed January 2016), [b] (Stevens-Garmon et al., 2011), [c] (Jones et al., 2002), [d]

(Karnjanapiboonwong et al., 2010), [e] (Lee et al., 2011), [f] (Stuer-Lauridsen et al., 2000), [g] (Barron et al., 2009), [h] (Morissette et al., 2015), [i] data for a river sediment (Stein et al., 2008), [j] (Miao et al., 2005), [k] (Ternes et al., 2004), [l] data for a creek sediment (Loffler et al., 2005).

5.3.3 Analysis of Suspended and Settled Sediment samples

Sediment samples (SS and StS) were extracted in methanol:acetone by ultrasonic-assisted extraction based on the method by Darwano et al. (2014), and then cleaned-up using online solid phase extraction, followed by Liquid Chromatography Atmospheric Pressure Chemical Ionization tandem Mass Spectroscopy (LC-APCI-tandem MS) detection for quantification Additional details are provided in the ESI (Sections 5.8.2.3, 5.8.2.4, and 5.8.2.5).

5.3.4 Analysis of water samples

Fifteen microliters of labeled internal standards (600 ng mL^{-1} of [$^{13}C_2$]-acetaminophen, 500 ng mL^{-1} of trimethyl-$^{13}C_3$ and 500 ng mL^{-1} of carbamazepine-d_{10}) were spiked to 1.5 mL of particle-free water (filtrated using a 0.3-µm glass fiber membrane filter and acidified with LCMS grade formic acid, 0.5%) withdrawn from centrifuged batch reactors. The water was directly injected into the LC-APCI-tandem MS in positive mode. Details are given in the ESI (Section 5.8.2.4).

5.3.5 Sorption and desorption experiments

The sorption and desorption batch experiments were designed to focus on the following key parameters: the influence of particle type (SS or StS), the influence of spiking contaminants (to compare differences in natural and spiked systems), the influence of shaking (to simulate sediment resuspension), and the influence of sequential dilution (to simulate additional storm water events). The influence of these variables was investigated by systematically varying each of these parameters, resulting in 7 different types of batch experiments.

Several conditions were common throughout the batch experiments. They generally followed the OECD guideline 106 (Organisation for Economic Cooperation and Development, 2000), with some modifications. Sediment concentrations for SS and StS (CSS and Csed) or aqueous concentration (Cw,SS and Cw,Sed) were quantified in sacrificed tubes at planned time intervals. For the SS batch experiments, 5 mL of SS (solid/liquid, 1:40, w/v) were pre-equilibrated overnight in pre-weighed conical polypropylene centrifuge tube under constant agitation on a

horizontal auto shaker at 100 rpm and 20 °C. For the parallel StS experiments, 1 g of wet StS was mixed with 5 mL of irradiated CSS water (adjusted solid/liquid, 1:7, w/v) and similarly pre-equilibrated. Irradiated combined sewer water was used in order to have sufficient water supply of exactly the same composition for all sorption experiments. Mass balances were conducted in all batch experiments, to account for potential mass loss artefacts. This was done by analysing the mass of pharmaceutical in the batch system in the liquid, solid and filter phases at specified time intervals, the sum of which was compared to the total mass (mass balance (%) = 100 x (mass in water + mass in sediment + mass in filters)/(spiked mass + initial mass in water + initial mass in sediment + initial mass in filters)). No important losses of test compounds were observed during the 72 h from biological degradation, sorption to the container, or from volatilization (see Figure 5.10).

The differences in the 7 types of batch experiments are summarised in the ESI Table 5.9 and described below:

Batch Experiment A: Uptake of spiked analytes while shaking: Target analytes dissolved in methanol (0.1 volume % of total solution volume) at final concentrations of 250 μg L^{-1} ACE, 50 μg L^{-1} CAF, 250 μg L^{-1} THEO, 50 μg L^{-1} CBZ, and 2.5 μg L^{-1} CBZ-DiOH were spiked in the batch tubes. The reactors were then shaken at 120 rpm at 20 °C. Immediately (0h), 12 h and 24 h after spiking, replicate batch reactors were centrifuged (3 min at 6000 rpm); the supernatants were filtered using 0.3-μm glass fiber membrane filter, acidified with formic acid (0.5%) and stored at 4 °C. Preliminary tests showed that from 98 to 100% of adsorption occurred within 5 min, similar to observations in other pharmaceutical batch systems (Morissette et al., 2015).

Batch Experiment B - Desorption of spiked analytes while shaking: After spiking as described Batch A, the sample was centrifuged and the supernatant water in the tubes were replaced with tap water to study desorption of spiked analyte while shaking (120 rpm, 20 °C). Water samples in replicated, sacrificed tubes were analysed at various intervals up to 383 h, sediments less frequently (see Table 5.9), and filters at 120 h.

Batch Experiment C - Desorption of native analytes while shaking: This batch was identical to Batch B, but with the absence of spiking, to measure the desorption of native analytes at various time intervals up to 71 h.

Batch Experiment D - Desorption of native analytes under static conditions: This was identical to Batch Experiment C, but in the absence of shaking.

Batch Experiment E - Desorption of native analytes under static conditions and double dilution: This was identical to Batch Experiment D, but with twice the volume of tap water.

Batch Experiment F – Sequential desorption of native analytes with shaking: This was identical to Batch Experiment E, except the centrifugation and water replacement occurred at three times (at 0h, 2h, and 8h), to mimic various suspension and washout events (to enhance depletion of analytes).

Batch Experiment G - Sequential desorption of native analytes without shaking: This was identical to Batch Experiment D, except the centrifugation and water replacement occurred four times (at 0h, 12h, 24h, and 48h), to mimic the effect of dilution with no major sediment resuspension.

5.3.6 Statistical analysis

The data obtained were analyzed using statistical software (STATISTICA 12 - Ultimate Academic Bundle, StatSoft Inc.). Nonparametric Kruskal-Wallis and one way ANOVA-tests were used to ascertain the significant differences among treatments. A 5% confidence level ($p \leq 0.05$) was applied to assess significance.

5.3.7 Quality Assurance and Control

Average percent extraction recovery from sediments was 98 ± 8.4, 63 ± 5.0, 93 ± 19, 80 ± 5.0 and 84 ± 9.3 % for ACT, CAF, THEO, CBZ and CBZ-DiOH, respectively (see Figure 5.9 in ESI). No corrections to sediment concentration were made to account for extraction recoveries. The LOD of the analytical method for all analytes ranged from 0.33 to 1.21 µg L^{-1} for aqueous samples and from 1 to 2 µg Kg^{-1} for sediment samples (see Table 5.10 in ESI).

5.4 Results and discussion

5.4.1 Occurrence of the native contaminants in CSS

All of the selected pharmaceuticals were found to natively occur in both the water and sediments of the collected SS and StS samples. Figure 5.1a shows the total concentrations, $C_{total} = C_{ss}$ (or C_{sed}) + C_w. In general, concentrations were comparable or higher than previously reported concentrations in wastewater influents or in untreated biosolids (Blair et al., 2013; Miao et al., 2005) (see ESI, Section 5.8.1.2). Note that the collected samples are discharged directly to the environment with little subsequent treatment; thereby they represent a real-world pollution source.

Figure 5.1b compares C_{ss} and C_{sed}, and shows that the compounds are more abundant per gram (dry weight) in the SS, likely because the SS had a much higher f_{OC} than the StS (51.7% vs 6.3%) (see Table 5.6). The compounds in this study are present mainly in the neutral form at the intrinsic pH of the tested batch systems (pH = 7.05 – 7.35); theophylline has the pKa closest to the test system (pKa 8.8Table 5.1), indicating that only a minor fraction would be negatively charged. Therefore ionic interactions would be negligible and sediment-water sorption interactions would be dominated by specific (polar) and non-specific (apolar) van-der Waals interactions.

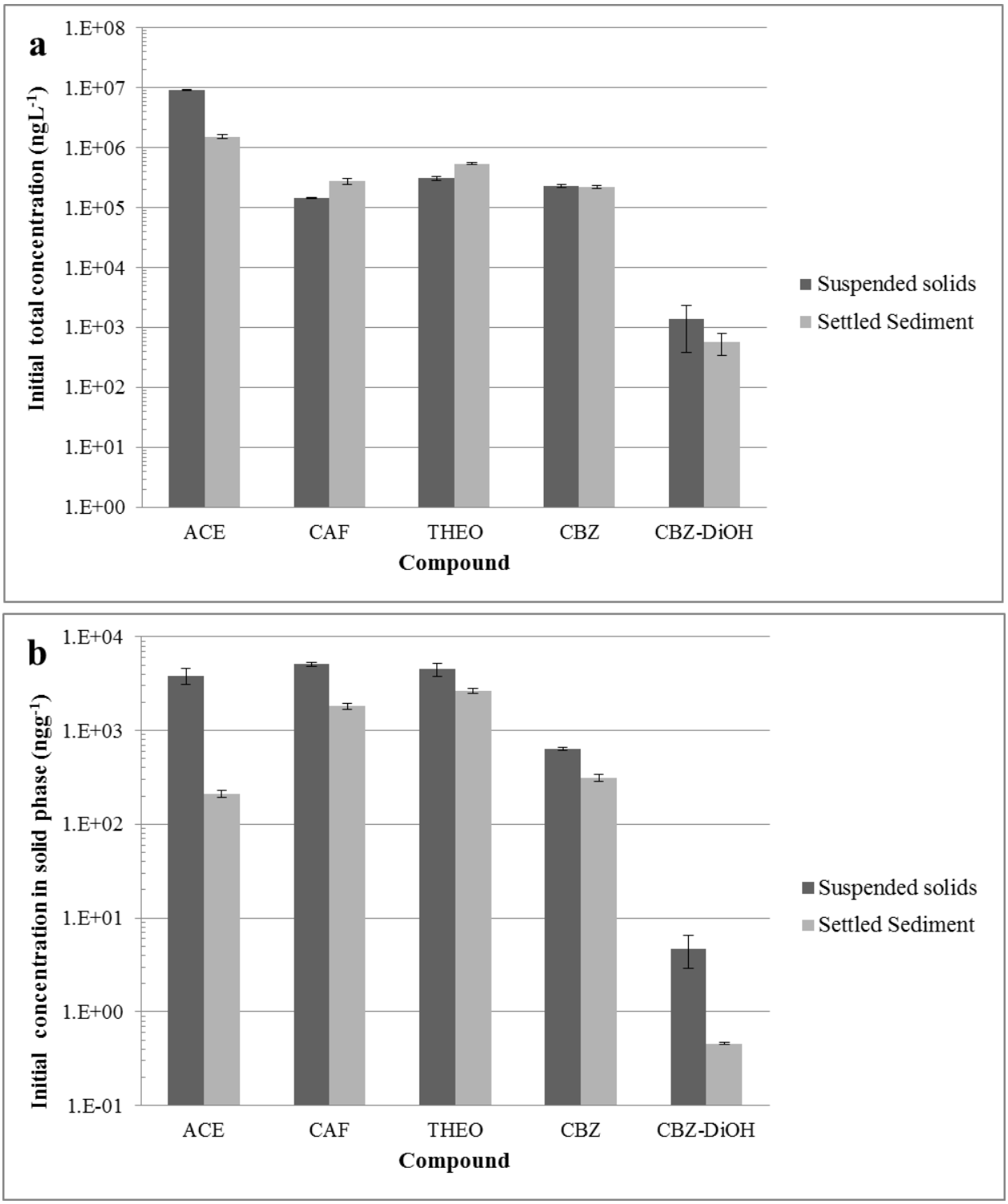

Figure 5.1**.** Bar charts comparing (a) initial total **(**water and solid) (ng L^{-1}) and b) solid concentrations (ng g^{-1}dry weight) of pharmaceuticals and caffeine in the mixture of native combined sewer suspended (SS) and settled sediments (StS), error bars indicating standard deviations (n=2).

5.4.2 Sorption behaviour of pharmaceuticals to SS and StS

5.4.2.1 Mass balances

For all sorption and desorption measurements, analytes were quantified in both the aqueous and the solid phases, allowing for mass balance calculations as described in 5.3.5. Mass balances demonstrated that there were no significant analyte losses or gains (see Figure 5.10 of the ESI for Reactors A and B). Coefficients of variability of mass measurements in Reactors A- E ranged from 0.009 to 0.640 (both for CBZ-DiOH). CBZ-DiOH showed the greatest variability in measurements because its native concentrations were closest to detection limits.

5.4.2.2 Solid-water distribution coefficients of spiked and native pharmaceuticals

The mobility and transport of pharmaceuticals in the environment depends on their sorption behaviour, which is best quantified by an experimentally determined soil-water distribution coefficient (K_d). The apparent solid water distribution coefficient ($K_{d,app}$) is defined as the ratio of compound concentration in the solid phase (C_s, µg $kg_{dw\ sediment}^{-1}$) to the compound concentration in the aqueous phase (C_w, µg L^{-1}) in a system that has not reached phase equilibrium:

$$K_{d,app} = C_s / C_w \qquad (1)$$

When $K_{d,app}$ is normalized to the organic carbon content of the sediments f_{OC} (%), the organic carbon normalized sorption coefficient $K_{OC,ap}$ is obtained :

$$K_{OC,app}, \text{ L Kg}^{-1} = K_{d,app} / f_{OC} \times 100 \qquad (2)$$

Polyparameter Linear Free Energy Relationships (pp-LFERs) have been proposed to describe the partitioning behavior of organic compounds (Abraham & Acree Jr, 2004; Abraham et al., 2009; Abraham et al., 2008; Bronner & Goss, 2011; Endo et al., 2009; Tülp et al., 2008). The partition coefficient of substance between the two phases (soil and water) was calculated by the following equation:

$$\text{Log } K_{OC} = c + Ee + Ss + Aa + Bb + Vv \qquad (3)$$

The five terms quantify the molecular interactions that govern the partitioning process: Van der Waals interactions (e, E), polar interactions (s, S), H-bond donor (b, A) and acceptor (a, B) interactions and cavity formation in the case of bulk media (v, V).

Sorption coefficient values ($K_{d,app}$ and $K_{OC,app}$) derived from the spiked systems (Reactors A and B) as well as non-spiked system (average from Reactors C, D and E) are reported in Table 5.2, Figure 5.2, and Figure 5.11. More comparisons of K values can be found in the ESI Table 5.11. Figure 5.2 also shows the difference for spiked and non-spiked K_{OC} in relation to the Kow. $K_{OC,app}$ values were significantly higher ($p < 0.05$) for the spiked (Reactors A and B) versus non-spiked reactors (Reactors C, D, and E).

The result of seeing higher K values in spiked than non-spiked systems is the opposite of what is typically observed for hydrophobic aromatic compounds, where lower K values are seen in spiked system due to lack of equilibrium or lack of aging with the strong sorbing sediment particles (*e.g.*, PAH, PCB) (Arp et al., 2009), or possibly the presence of a solvent used in the spike (Schwarzenbach et al., 2003). Further, the spiked, higher concentration system exhibiting larger K values than the non-spiked, lower concentration system is also different than the general trend of decreasing sorption with increasing aqueous concentration, following a Freundlich type isotherm (Schwarzenbach et al., 2003). One potential explanation for this is matrix effects in the aqueous phase due to the presence of dissolved organic carbon (DOC) and colloids. If DOC/colloids preferentially sorbed the native pharmaceuticals compared to sediments, but were slower to reach equilibrium with the spiked pharmaceuticals than the sediments, or if the DOC/colloids were already saturated with pharmaceuticals, higher K values in the spiked system would occur, because the spiked chemicals would not be as substantially in the aqueous phase sorbed to DOC/colloids. These results demonstrate the importance of using native compounds in sorption experiments and modifying matrices as minimally as possible.

$K_{d,app}$ values from Reactors A, B (at 125 hours) (Table 5.2), C, D, and E (at 72 hours) (Table 5.11) are generally higher in SS than in StS, likely because of the higher organic carbon fraction in SS as compared to StS. Results from direct measurements show that CAF has the highest sorption $K_{d,app}$ on both sediment types (SS and StS). Based on log K_{ow} values (Table 5.1, Figure 5.2) one would expect that caffeine would exhibit the least sorption. The sorption of the selected compounds does not seem to be proportional to K_{ow}, indicating that the sorption-system here is more complex than octanol-water. In such CSS sediments, phases like coffee grounds could be especially adept at sorbing caffeine.

$K_{OC,app}$ values were similar magnitude for CBZ ($p > 0.05$) for both types SS and StS in Reactors A, whereas for ACE, CAF, THEO, and CBZ-DiOH $K_{OC,app}$ values were significantly lower in SS ($p < 0.05$). The $K_{OC,app}$ values calculated for ACE and CBZ-DiOH were lower than those observed in the literature (Table 5.5). $K_{OC,app}$ values for CAF and CBZ were comparable to those observed by (Barron et al., 2009) and (Jones et al., 2002), respectively.

Table 5.2**.** Solid water distribution coefficients log $K_{OC,\ app}$ and log $K_{d,\ app}$ (L Kg^{-1}) of selected pharmaceuticals for the sorption of selected pharmaceuticals on solid phases SS and StS in spiked batch reactors with shaking (Reactors A and B) and non-spiked batch reactors (Reactors C, D, and E).

Compound	**Log $K_{OC,app}$**				**Log $K_{d,app}$**				**Log K_d**
	Spiked[a]		Native (Non-Spiked)[b]		Spiked[a]		Native (Non-Spiked) [b]		Literature (range)[c]
	SS	StS	SS	StS	SS	StS	SS	StS	
ACE	0.8 ± 0.1	1.3 ± 0.1	-1.9 ± 0.0	-1.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.1	-2.2 ± 0.0	-2.4 ± 0.0	-0.4 - 1.3
CAF	1.9 ± 0.1	2.2 ± 0.1	0.6 ± 0.2	1.2 ± 0.1	1.6 ± 0.1	1.0 ± 0.1	0.3 ± 0.2	0.0 ± 0.1	0.8 - 1.1
THEO	1.3 ± 0.2	1.8 ± 0.1	-0.6 ± 0.2	0.2 ± 0.1	1.1 ± 0.2	0.6 ± 0.1	-0.9 ± 0.2	-0.1 ± 0.1	
CBZ	1.8 ± 0.2	1.9 ± 0.3	0.4 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	0.7 ± 0.3	0.1 ± 0.1	-0.1 ± 0.1	-1.0 - 1.6
CBZ-DiOH	1.2 ± 0.4	1.3 ± 0.4	-2.3 ± 0.4	-1.0 ± 0.1	1.0 ± 0.4	0.1 ± 0.4	-2.6 ± 0.4	-2.2 ± 0.1	-0.7 - 0.3

[a] Average of solid water distribution coefficient (Log $K_{OC,app}$ or Log $K_{d(ads),\ app}$) values from adsorption (Reactors A at 0, 12, and 22 h) and desorption (Reactors B at 120 h), [b] Average of solid water distribution coefficient (Log $K_{OC,app}$ or Log $K_{d(ads),\ app}$) values from desorption at 72 h (Reactors C, D, and E), [c] min and max values from Table 5.1.

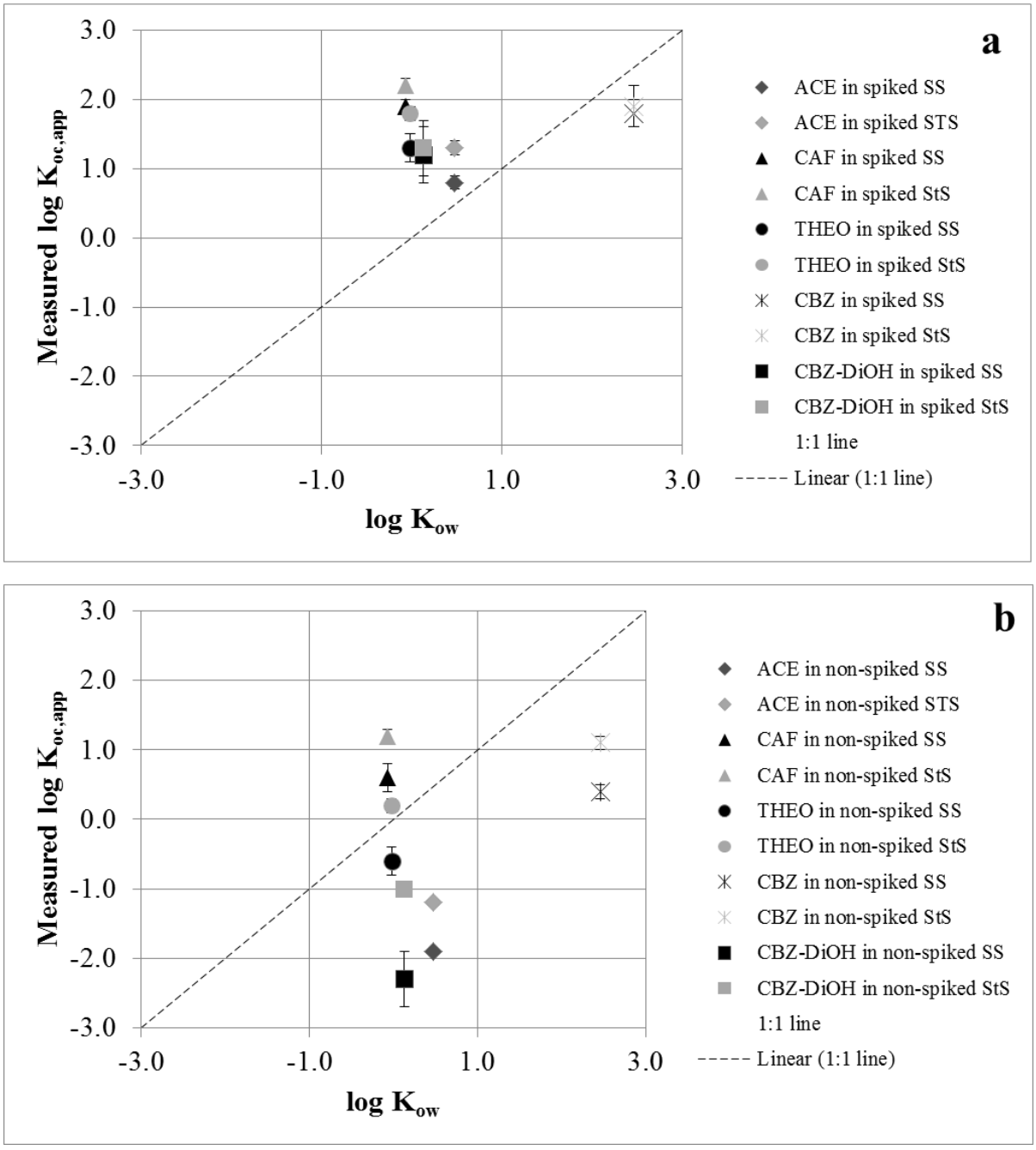

Figure 5.2**.** Average log $K_{OC,\ app}$ values (L Kg^{-1}) of pharmaceuticals and caffeine in both spiked (a) and non-spiked (b) SS and StS matrices as a function of log K_{OW}, error bars indicate standard deviations (n=2).

Effects of shaking and dilution on concentrations and solid-water distribution coefficients

The changes in the aqueous (ng L^{-1}) and solid concentrations (ng g^{-1}) of native contaminants from the initiation of the batch experiments (i.e. when supernatant water is replaced with tap water) are presented here using C/C_0 ratios, where C and C_0 correspond to concentrations at a defined time and 0 h of desorption, respectively. Figure 5.3 presents changes in C/C_0 over time in the aqueous and solid phases for natively occurring contaminants undergoing shaking at 120 rpm (Batch Reactor C). Figure 5.12 in ESI shows changes in the C/C_0 ratio under static conditions (Batch Reactor D) and static conditions with extra dilution (Batch Reactor E). CBZ-DiOH concentrations were much higher in the aqueous phase and the solid phase as compared to the other compounds. They did not fit on the same scale as the other compounds and are therefore shown separately in ESI Figure 5.12.

Despite different initial total concentrations in SS and StS (Figure 5.1), desorption as observed by the increase in the C/C_0 ratio in the aqueous phase (Figure 5.3a and 3c) generally followed the same trend (CAF > THEO > ACE > CBZ). CAF was initially found in lower concentrations as compared to ACE and THEO in the liquid phase, yet exhibited the highest C/C_0 ratio at T_{71h}. Although desorption increases concentrations in the aqueous phase, the relative effects on solid phase concentrations were relatively negligible, due to the higher relative concentrations in the solid phase throughout the experiment (i.e. the solids were not substantially depleted), see Figure 5.3c and 3d.

The changes in the $K_{d,app}$ over time after the initiation of batch reactions are quantified with $K_{d,appT}/K_{d,appT0}$ ratio, where $K_{d,appT}$ and $K_{d,appT0}$ correspond to the apparent distribution coefficients ($K_{d,app}$) at defined time (T) and 0h (T_0) of desorption, respectively. The non-parametric Kruskal-Wallis test (see Table 5.12 in ESI) was used, based on $K_{d,app}$ data presented in Table 5.11, to evaluate the differences between the distributions of $K_{d,appT}/K_{d,appT0}$ ratios of pharmaceuticals considering shaking or dilution (Reactors C and E) as compared to the control experiment (no shaking and no dilution in Reactors D). The small positive effect of shaking on desorption was not statistically significant ($p > 0.05$) in SS and StS except for ACE ($p = 0.0446$) and CBZ-DiOH ($p = 0.0258$) in SS. With regards to dilution, the differences were significant only for CBZ-DiOH and ACE in SS and StS, respectively ($p = 0.0039$).

An ANOVA-test was used to determine if variations of $K_{d,app}$ within reactors at different times were significant (from T_{0h} until T_{71h}). In general, $K_{d,app}$ values decrease over time. The results from the 3 conditions (with shaking, without shaking and with dilution) are available in Table 5.13. The variations of $K_{d,app}$ values were significant for all compounds ($p < 0.05$) except for CBZ with shaking in SS and CBZ-DiOH without shaking in both SS and StS. The variation of $K_{d,app}$ starting at 2h increased the frequency of non-significant variations of $K_{d,app}$ suggesting that the greatest variation in $K_{d,app}$ values occurs within the first two hours, particularly for shaking in SS.

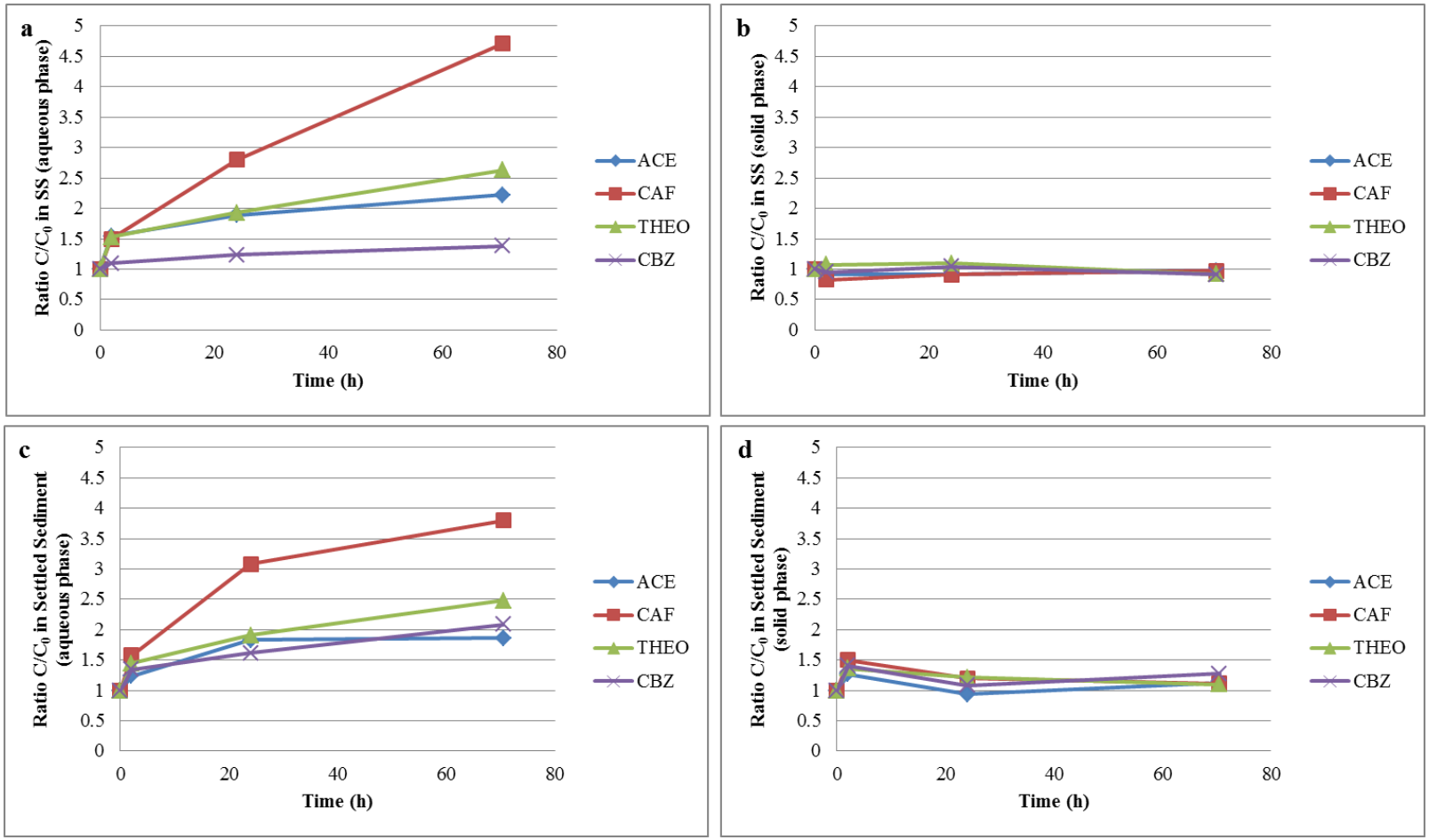

Figure 5.3. Desorption as a function of time without spiking and with shaking (Reactors C) expressed in C/C_0 ratio of the selected pharmaceuticals except CBZ-DiOH in aqueous phase of SS (a) and StS (c) and in solid phase of SS (b) and StS (d) C and C_0 correspond to the concentrations in aqueous phase (ng L^{-1}) or in solid phase (ng g^{-1}) at defined time and 0h of desorption respectively.

Effects of successive dilution on concentrations and solid-water distribution coefficients

Changes in native concentrations while flushing/dilution events occur, both with and without shaking (Reactors F and G), are presented in Figure 5.4. The first addition of tap water (at T = 0 h) with shaking played the role of the first flush (defined here exclusively as the initial addition of stormwater to a sewer system rather than the operational definition from an increased hydraulic loading and resuspension of sewer sediments (Bertrand-Krajewski et al., 1998)). Figure 5.4 shows that flushing events do not necessarily lead to a direct dilution of contaminants. The SS and settled sediments can act as a reservoir that can either increase the aqueous concentration (*e.g.*, observed initially in Figure 5.4a and 4c for ACE and THEO without shaking), or more commonly restore (pseudo-)equilibrium and lead to negligible changes in concentration despite the added volume of water over several flushing events.

Without shaking, the concentration of ACE increased in the aqueous phase following the first flush (Figure 5.4). Following this, the second flush led to a dilution, followed by a steady-state for the third and fourth flushes, possibly indicating that the first flush released a portion of ACE that was not in equilibrium with the sediment. For the other compounds, CAF, THEO and CBZ, similar patterns are observed. In comparing the concentrations of the compounds before and after the second flush (between 2 h and 8 h with shaking and between 12 h and 24 h without shaking), it can be observed that the second flush enabled a larger reduction of the concentration of ACE as compared to the other three compounds (CAF, THEO, and CBZ). CAF appeared to be the least influenced by the second flush (with and without shaking) followed by CBZ and then THEO. All compounds reached a steady-state concentration after multiple flushing.

Statistical significance was tested using the Kruskal-Wallis test (Table 5.14). Boxplots of $K_{d,app}$ following dilution steps are presented in Figure 5.13. (ESI). In general, successive dilution increased $K_{d,app}$ in both SS (ranged between -0.2 and 2.6 log units) and StS (ranged between -1.0 and 2.4 log units). $K_{d,app}$ values generally showed significant differences ($p < 0.05$) in SS and StS before and after dilutions. This could be explained by pharmaceutical rich colloids being removed by the first flush, or by the removal of weakly sorbed pharmaceuticals in the sediment. Only CAF in SS showed significant decrease in $K_{d,app}$ after the first flush, possibly related to the removal of stronger-sorbing coffee grounds from the sediments.

The Kruskal-Wallis test was also used to compare $K_{d,app}$ shaking and double dilution with no shaking and a single dilution after the first flush. There was a significant difference among the $K_{d,app}$ in SS and StS for all compounds (Boxplots are available in Figure 5.14 in ESI) with higher $K_{d,app}$ values observed for shaking with double dilution as compared to no shaking with a single dilution. Thus, extra dilution and shaking, like multiple flushing, will lead to an increase in $K_{d,app}$. CAF and THEO had the lowest $K_{d,app}$ values observed in SS and StS after double dilution and flushing respectively (Figure 5.15 in ESI).

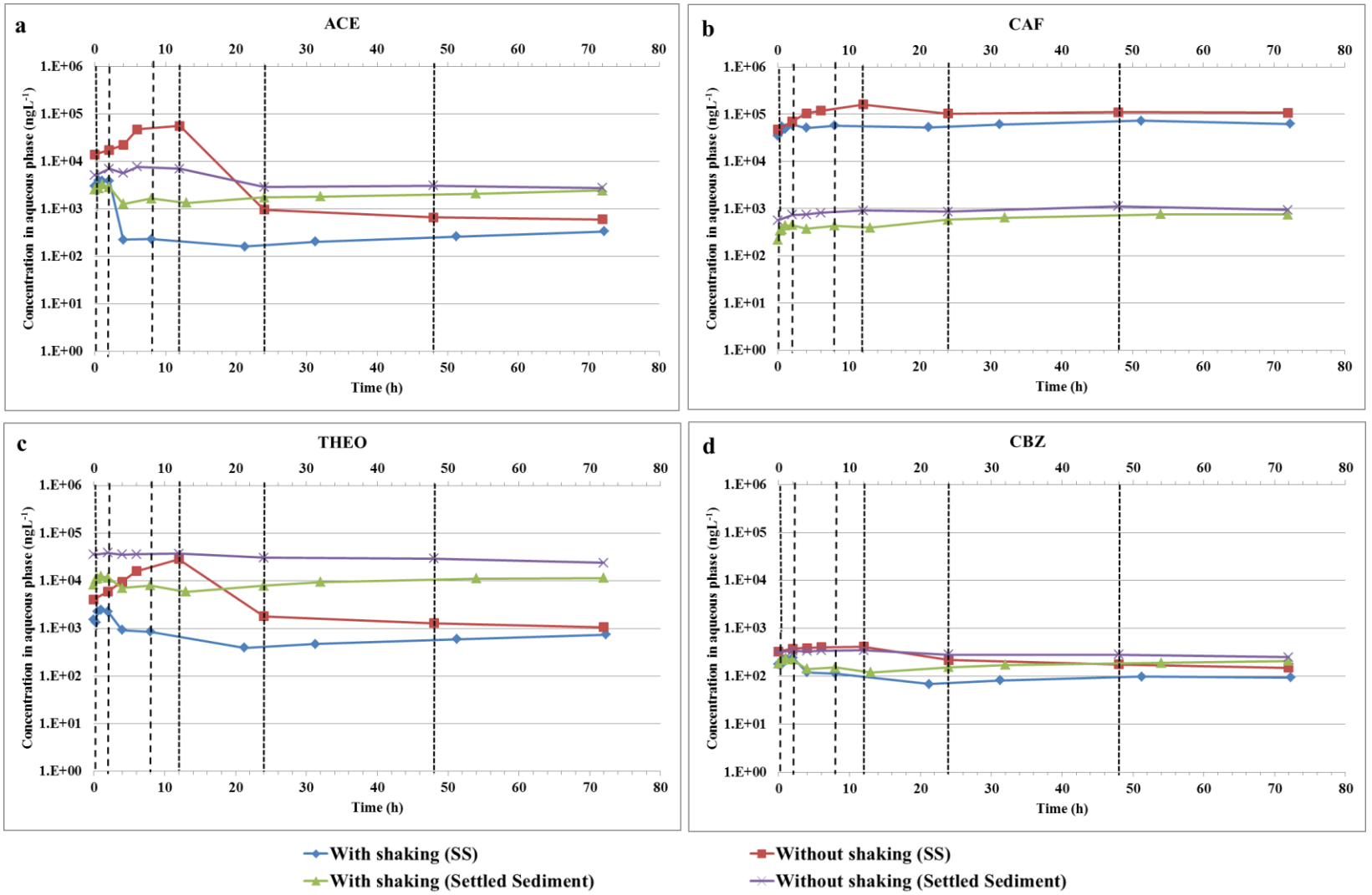

Figure 5.4**.** Desorption of pharmaceuticals (Reactors F and G) in SS and StS samples as a function of time and flushing (indicated by dash and square dot vertical lines for experiments with shaking and without shaking, respectively).

5.4.2.3 Uptake and dissipation kinetics

The sorption of the selected pharmaceuticals onto the solid phase occurred nearly instantaneously (with the exception of CBZ, Figure 5.16), possibly within the first 3−5 minutes following spiking the SS and the StS. The same near instantaneous sorption of CAF and CBZ was observed in Morissette et al. (2015). Loffler et al. (2005) assumed that ACE was transformed immediately upon contact with the sediments (Loffler et al., 2005), possibly the result of strong binding to the sediment matrix (*e.g.*, by covalent binding). Figure 5.17 shows the changes in the aqueous concentration of pharmaceuticals to the spiked SS and StS that occurred in Reactors B. All compounds demonstrated desorption following the initial spiking, where there was an increase in the C_w and a decrease in the solid phase until experiments ended at 120 h (Figure 5.17).

Pharmaceutical desorption kinetics on the CSS sediment samples were examined with a pseudo-second order (PSO) kinetics model to compare desorption capacities and desorption kinetics rates on different CSS under different conditions (i.e. with and without spiking, with and without shaking, and with dilution). The PSO kinetic model is chosen as it is appropriate for low contaminant concentration with a high solids content (Ho, 2006).

Equation 4 was used to describe the sorption kinetics (Wu et al., 2009):

$$\frac{dq_t}{dt} = k_2 (q_e - q_t)^2 \qquad (4)$$

where q_t is the desorption capacity (ng L^{-1}) at time t (min), *qe* is the equilibrium desorption capacity (ng L^{-1}), and k_2 (in L ng^{-1} min^{-1}) is the pseudo second-order rate constant. Integrating Eq. (4) and applying the initial conditions, we have:

$$\frac{t}{q_t} = \left(\frac{1}{k_2 q_e^2}\right) + \left(\frac{1}{q_e}\right) t \qquad (5)$$

PSO modeling allowed evaluating the desorption capacity at equilibrium (i.e. q_e) and the kinetics constant (i.e. k_2). The parameters k_2 and q_e can be obtained from the intercept and slope of the plot of (t/q_t) against t.

Experimental results from Reactors B, C, D and E followed the trend of this linearized equation for pseudo second order kinetics ($R^2 > 0.84$). Values for the pseudo second order rate constant,

k_2, for each of the compounds are available in Table 5.3. Also, q_e (cal) values were close to the final experimental concentrations, suggesting equilibrium was attained.

Desorption rates are plotted against molecular weight in Figure 5.18 of the ESI, CBZ and CBZ-DiOH demonstrated the highest desorption rate constants, and caffeine amongst the lowest. The metabolite CBZ-DiOH may more easily desorb as it is more likely to be just at the surface of particles, where more metabolization takes place; whereas caffeine may reside deep in the matrix of coffee grounds. Similar, it is possible that some of the native contaminants are within the sorption matrix in an ionic state, and must first be dissolved in the surrounding water before dissolution.

Table 5.3**.** Pseudo second-order desorption rate constants for spiked (Reactor B) and native (Reactors C, D, E) pharmaceuticals in SS and StS.

Rate Constant	**Pseudo second-order rate constant k_2 (L ng^{-1} min^{-1})**							
Batch Reactor	B		C		D		E	
Matrix	SS	StS	SS	StS	SS	StS	SS	StS
Compound								
ACE	1.E-09	3.E-07	1.E-07	1.E-06	1.E-07	6.E-07	2.E-07	1.E-06
CAF	5.E-07	1.E-06	7.E-07	3.E-06	3.E-07	6.E-06	4.E-06	1.E-05
THEO	2.E-07	5.E-08	2.E-07	2.E-07	2.E-07	3.E-07	1.E-06	5.E-07
CBZ	2.E-06	4.E-06	3.E-05	1.E-05	2.E-05	1.E-04	1.E-04	2.E-05
CBZ-DiOH	1.E-03	1.E-04	4.E-06	1.E-04	4.E-05	3.E-04	-3.E-03	3.E-04

5.4.3 Chemical markers

The most hydrophilic compound had the highest $K_{OC,app}$ value ($K_{OC,app}$ for CAF > $K_{OC,app}$ for THEO > $K_{OC,app}$ for CBZ-DiOH). In comparison to the parent compound CBZ, the doubly hydroxylated and thus more polar metabolite showed a drastically reduced affinity for the sediments, which is generally the case for metabolites of increased polarity (Loffler et al., 2005). Thus, CBZ-DiOH is potentially an interesting chemical marker that is rather insensitive to sediments' contribution and releases. The compounds studied, notably CAF, and THEO are good tracers of environmental contamination by combined sewer overflows during the initial washoff of sewer sediments, although increasing CAF concentrations would be partly related to the desorption from coffee grounds rather than fecal contaminants. When considering the similarity of sorption behaviour with dilution in stormwater, ACE or CBZ would be the best tracers among the pharmaceuticals tested. ACE is also present in the highest relative concentrations, which is an important characteristic for a tracer. Other considerations for selecting tracers, such as their transformation rates, were not investigated in this study.

5.5 Conclusions

The general aim of the water/sediment study to investigate the sorption/desorption of pharmaceuticals under controlled laboratory conditions representative of combined sewers. The conclusions on the environmental behaviour of pharmaceuticals and the associated risks therein are as following:

1. Adsorption equilibrium after spiking contaminants was nearly instantaneous for the tested pharmaceuticals except for CBZ; however, desorption kinetics showed that equilibrium was attained either rapidly (on the order of hours) for CBZ and its metabolite CBZ-DiOH or slowly over the course of days for CAF and THEO.

2. $K_{d,app}$ values in native and spiked sediments were significantly different (native sediments $K_{d,app}$ < spiked sediments $K_{d,app}$) demonstrated that spiking procedures could affect partitioning behavior and that native compounds best represent partitioning behavior in real systems.

3. Lab-scale results suggest that when storm waters are added to wastewaters, there will be a shift in equilibrium and a desorption of pharmaceuticals from the solid to the aqueous phase. The results confirm what has been observed at the full-scale in combined sewer overflows by Madoux-Humery et al. (2013), that is, the observation of an increase of dissolved concentrations of pharmaceuticals at the beginning of stormwater events despite the greater dilution from stormwaters. The first flush (i.e. the first addition of water) had the greatest influence on the desorption of pharmaceuticals as compared to mixing (representing sewer turbulence) and subsequent dilution. Pharmaceutical loads from untreated CSOs will be higher than expected from a calculation as a dilution of dry weather concentrations because of the mobilization of pharmaceuticals from the particulate phase to the dissolved phase. Monitoring programs focusing exclusively on the dissolved phase are missing a large fraction of total pharmaceutical concentrations.
4. CBZ-DiOH, CBZ, and CAF were the most rapidly desorbing compounds among those tested and they would therefore be useful tracers to study the dynamics of stormwater influenced sewer systems.

† Electronic Supplementary Information (ESI) available: 11 tables, 14 figures, including a literature review of properties and occurrence of selected pharmaceuticals, K_d and K_{OC} values of analytes and description of extraction and analytical methods. See DOI:

5.6 Acknowledgments

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5.8 Supplementary Information

5.8.1 Introduction

Sorption is strongly influenced by the physicochemical properties of molecules such as the polarity of functional groups (e.g., -OH, -C-O-C-), acid dissociation constant (Ka), partitioning coefficients such as K_{OW}, K_{OC} in addition to media properties such as temperature, pH, quantity and quality of both soil organic matter and minerals, surface reactivity and specific surface area and other factors such as ionic strength (cation bridging, cation exchange, hydrogen bonding) (Drillia et al., 2005; Goss & Schwarzenbach, 2001; Jones et al., 2006).

Non-electrostatic interactions (non-ionic van der Waals interactions between chemicals and the sediment surface) govern sorption of compound in the neutral hydrophobic form. The aromatic content and polarity of the organic compound being sorbed can influence partitioning and sorption can be related to the organic carbon content of sediments. The most important mechanisms involved in pharmaceutical sorption are sorption to organic matter, surface adsorption to mineral constituents, ion exchange, complex formation with metal ions, and hydrogen bonding (Díaz-Cruz et al., 2003; Navon et al., 2011). For soils/sediments containing high amounts of organic matter (OM), the sorption of pharmaceuticals is often controlled by the organic carbon content of the medium due to the hydrophobicity of OM (Karnjanapiboonwong et al., 2010; Navon et al., 2011; Pan et al., 2008; Pan et al., 2009; Williams et al., 2006). The fine particles in soils/sediments are more important than the coarse size particles in the sorption and transport of pharmaceuticals as their suspended organic matter is more abundant, more humified and more hydrophobic (Sun et al., 2012). Otherwise, the decrease in the sorption rate constant occurs with increasing molecular size and hydrophobicity (Pignatello & Xing, 1995). Therefore, the potential for flushing pharmaceuticals from granular deposits in sewers is limited whereas pharmaceuticals sorbed to fine particles will move in suspension (Williams, Tait, et al., 2009).

5.8.1.1 Properties of selected pharmaceuticals

Acetaminophen (ACE) is a moderately water- and lipid-soluble weak organic acid. It is extensively metabolized in the human body and excreted with urine as parent compound (2−5%) and metabolites (about 90%) (Prescott, 1980; Whirl-Carrillo et al., 2012). By using activated sludge as microbial inocula, ACE showed fast biotransformation rates and nearly complete

biotransformation following 14 days of incubation (Yu et al., 2006). It had relatively slow biodegradation (i.e., half-life>24h) and moderate stability against sunlight in batch biodegradation and sunlight photolysis experiments using river water (Yamamoto et al., 2009). In terms of biodegradation, microbes may be more important than photolysis for ACE (Lam et al., 2004). It was easily biodegraded; 50% of the initial concentration of ACE was removed after 72 h (Yamamoto et al., 2009). ACE displayed a low persistence against sorption and transformation with 50% and 90% dissipation time values in the water/sediment system of 3.1 ± 0.2 d and 10 ± 1 d respectively. Biotic conversion was predominant for ACE in water/sediment system. Rogers (1996) provided a general rule of thumb for applying K_{OW} for the estimation of sorption potential: $\log K_{ow} < 2.5$ indicates low sorption potential, $2.5 < \log K_{ow} < 4$ indicates medium sorption potential, and $\log K_{ow} > 4$ indicates high sorption potential. Sorption of nonionic solutes has been accurately expressed by the octanol-carbon distribution coefficient normalized to the organic-carbon content (Schwarzenbach et al., 1993), that is: $K_d = f_{OC} K_{OC}$ where K_d is in L kg_{solid}^{-1}, K_{OC} is the organic-carbon distribution coefficient (L kg_{OC}^{-1}), and f_{OC} is the fraction of organic carbon present on the solid (kg_{OC} kg_{solid}^{-1}). The rapid movement of ACE into the sediment cannot be explained by its lipophilicity or ionic interactions ($\log K_{OW} = 0.49$, *p*Ka = 9.5) (Stuer-Lauridsen et al., 2000). The creek sediment played a key role in the elimination of ACE due to the rapid and extensive formation of nonextractable residues (64−53% of ACE) by strong covalent binding (Loffler et al., 2005). Sorption alone was responsible only for a 30% loss of aqueous-phase ACE after 15 days (Lin et al., 2010). ACE partitioned more readily to the sludge than the sediment, likely because of the former's higher organic carbon content (Jones et al., 2006). However, the coefficients (K(d) values) were higher for sediment/soil with higher organic content. The organic carbon-based sorption coefficient (logK(oc)) showed a poor linear correlation with the octanol-water distribution coefficient (logD(ow)) at neutral pH. These results suggest the importance of sorption mechanisms other than hydrophobic interactions between soil organics and ACE at neutral pH (such as electrochemical affinity by ionization of some functional groups) (Yamamoto et al., 2009). Because of ACE amphiphilic characteristic, they can interact with both polar and nonpolar surfaces (Suntisukaseam et al., 2007). Therefore, the sorption of ACE was negligible for aquifer sand, silica, and alumina (Lorphensri et al., 2006a).

Caffeine (CAF) is absorbed after oral ingestion, distributed to various tissues, and broken down to metabolites which are then excreted. Slightly more than 80% of administered CAF is

metabolized to paraxanthine, and about 16% is converted to theobromine and theophylline. Metabolites are recovered in the urine, but little (less than 3%) or none of the ingested CAF is found in urine (Mandel, 2002). More than 98% of CAF is degraded in wastewater treatment plants (WWTPs) (Buerge et al., 2003). In receiving water bodies, untreated wastewaters have been reported as the primary sources of CAF in wet weather (Musolff et al., 2009). CAF has been used as an anthropogenic marker for the quantification of discharges of untreated wastewater from combined sewer overflows (CSOs) (Buerge et al., 2006). Photolysis of CAF studied in sunlit surface water was an important loss process in limiting its persistence in the aquatic environment with an average half-life ($t_{(1/2)}$) of about 1.5 d (Lam et al., 2004). It exhibited high adsorption to sludge (Blair et al., 2013). Both biodegradation and sorption were important; $t_{(1/2)}$ for combined sorption-biodegradation for CAF was 1.5 d. It was be difficult to predict the sorption behavior of CAF based simply on sorbent type since CAF has very high water solubility (low K_{ow}) and was present in the cation form in tested soils. There was a large difference between Freundlich constant (K_f) and sorption coefficient (K_d). Desorption experiments revealed that the sorption process was mostly irreversible. A high value was found for K_d for caffeine (250 L kg^{-1}), which explained its greater tendency for sorption onto sediments as compared to acetaminophen (K_d = 5.0 L kg^{-1}) (Lin et al., 2010). The sorption capacity for CAF was directly related to organic carbon content. The type of clay was played an important role in the sorption of CAF. It was strongly and moderately sorbed in sandy loam and silt loam soils respectively. According to an additional classification system relating sorption to chemical mobility (Swann et al., 1983), CAF would have low mobility in silt loam soil. Desorption tests over 24 h indicated that CAF had the greatest desorption capacity (>15%) in sandy loam soil. (Karnjanapiboonwong et al., 2010).

There is a little information on the Theophylline (THEO). The interference of paraxanthine to theophylline is overlooked for many LC–MS/MS methods including our own. They are isobaric and have the same MS/MS transition (Hajj-Mohamad et al., 2014). Kim et al. (2009) have studied on the UV and UV/H_2O_2 degradations of a THEO. It was observed that H_2O_2 addition could improve degradation rates of THEO which is highly resistant for UV treatment (Kim et al., 2009). To the best of our knowledge, there are currently no studies on THEO adsorption/desorption kinetics.

Carbamazepine (CBZ) is an anti-epileptical drug used widely (Cunningham et al., 2010). Oral administration of ^{14}C- CBZ indicates that 72% was found in urine while 28% was found in feces.

Urinary radioactivity was composed of metabolites while 3% was unchanged and the feces contained a little over half as many metabolites and the rest as the unchanged drug that was not absorbed by the body (Faigle & Feldmann, 1975). The CBZ molecule is uncharged and polar, but has been found to create weak, non-specific interactions with soils and minerals (moderate lipophilicity (log P_{OW}= 2.25)) (Jones et al., 2002; Kosjek et al., 2009; Scheytt et al., 2005; Ternes, 1998). It is found to be highly persistent within the environment (Cunningham et al., 2010; Heberer, 2002a; Heberer, 2002b; Zhang, Geissen, et al., 2008). Therefore, it used as a marker for groundwater contamination through sewer exfiltration (Fenz et al., 2005). It is also recommended for the detection and quantification of domestic wastewater mixed into surface waters (Kahle et al., 2009). The results of batch biodegradation and sunlight photolysis experiments using river water suggested relatively slow biodegradation (i.e., half-life>24h) and high stability against sunlight (Andreozzi et al., 2003; Yamamoto et al., 2009). Photodegradation was important in limiting the persistence of ACE, CAF and CBZ, and biodegradation was not an important loss process in surface water over the duration of the study of Lam et al. (2004). ACE and CAF were less persistent than CBZ, with average half-lives of about 1 d for ACE and CAF and 82 d for CBZ (Lam et al., 2004). Nitrate and humic acid have opposite effects on its photodegradation, the first promoting the second inhibiting (Andreozzi et al., 2002). It shows high resistance to degradation (90% dissipation time value > 365 d) and a moderate to low adsorption to mineral soils (Loffler et al., 2005; Scheytt et al., 2006; Yu, Fink, et al., 2009). More recent studies showed that CBZ persisted in biosolids-soil mixtures for 495 d (Walters et al., 2010) and in soils, biosolids, and soil-biosolids mixtures for a period of 60 d without observable degradation (Monteiro & Boxall, 2009). In water/sediment system, CBZ was more persistent than ACE (Loffler et al., 2005). In sandy sediments with low organic content and different grain size distributions (Medium sand with fine-grained sand and little fines (LP) and fine sand with medium sand and little fines (ST), the adsorption of CBZ was significantly higher on LP than on ST. On both sediment types, the sorption was almost linear. The normalized distribution coefficient with respect to the organic content OC (%) of the solid matrix (CBZ K_{OC}) values were remarkably lower for sediment ST (Scheytt et al., 2005). No significant amount of CBZ was removed with sand under aerobic and anaerobic conditions, showing CBZ's low adsorption properties and high persistence with non-adapted organisms (Ternes et al., 2002). All sorption studies confirmed that CBZ has a low sorption affinity to soils, which was depending on the

organic carbon contents. With increasing the fraction of organic carbon, the sorption of the CBZ increased continuously (Yu, Fink, et al., 2009). The predominant mechanism of sorption of CBZ was the hydrophobic interactions between the organic contents of sediments/soil (Scheytt et al., 2005; Yamamoto et al., 2009). K_{OC} of the adsorption step of CBZ is 4 times greater in soil with high organic content than in soil with low organic content. The adsorption of CBZ on soils of the low and high organic carbon was not dependent only on the organic content of the matrix but also on the other matrix properties and the dissociation degree of CBZ. A hysteresis phenomenon occurred in soil with high organic content, indicating irreversible interactions between CBZ and the soil particles (Drillia et al., 2005). Stein et al. (2008) studied the sorption of CBZ on two natural river sediments that differed in organic carbon contents and particle size distributions. Sorption isotherms to both sediment types showed pronounced nonlinearity in the form of decreasing affinities with increasing analyte concentrations. Sorption of CBZ was reversible on the sediment with lower organic and clay contents. It was clearly concentration independent hysteretic on sediment with higher organic and clay content (Stein et al., 2008). The adsorption of CBZ in the upper soil layers (0-25 cm) in infiltration basins of a soil aquifer treatment system was correlated to the higher organic matter content. The soluble and adsorbed fractions of CBZ obtained from the two upper soil layers comprised 45% of the total CBZ content in the entire soil profile (Arye et al., 2011). The removal efficiency of CBZ from treatment in sewage treatment plants had an efficiency of only 9% (Bendz et al., 2005; Ternes, 1998). CBZ was not sorbed to an appreciable degree to primary and secondary sludges (Ternes et al., 2004). Leclercq et al. (2009) compared the removal of CBZ in three different WWTPs (conventional activated sludge, trickling filter, and stabilization ponds); removal rates of 0%, 30% and 73% were observed for the three WWTPs respectively. The substantial elimination of CBZ in the WWTP lagoon type has been assigned to the hydraulic retention time of significant (> 30 days) and a probable contribution of sorption (Leclercq et al., 2009). Effluent concentrations of CBZ occasionally exceed influent concentrations, which may be due to fluctuations in concentrations that are not accounted for in short term studies, or may be caused by processes in the treatment plant that convert some metabolites back into CBZ (Zhang, Geissen, et al., 2008). The most successful method for the removal of CBZ was UV treatment (Kosjek et al., 2009). During drinking water treatment, CBZ is effectively removed by ozonation; 0.5 mg/L ozone was shown to reduce CBZ by more than 90%.

Compared to the parent compound CBZ, which is the primary analyte in biosolids, the twofold hydroxylated and thus, more polar metabolite 10,11-dihydro-10,11-dihydroxy-carbamazepine (CBZ-DiOH), is the primary analyte in the aqueous phase and exhibited a significantly reduced affinity for the sediment, reflecting higher hydrophilicity of this compound as compared to CBZ (Miao et al., 2005; Stein et al., 2008). It seems to be very persistent with DT_{90} values exceeding 365 d. CBZ-DiOH was used as a substrate in biotic conversions (Loffler et al., 2005). As like as CBZ, CBZ-DiOH was not removed in WWTPs (Miao et al., 2005). It was detected in UK effluent from three WWTPs at average concentration of approximately 1.3 µg L^{-1} (Miao & Metcalfe, 2003).

5.8.1.2 Occurrence of selected pharmaceuticals in wastewater resource recovery facilities influents

Concentration ranges of selected pharmaceuticals in untreated WRRF influents available in the literature are summarized in Table 5.4. ACE was the most abundant compound measured in CSSs, with the highest concentrations in the range 1529 and 9279 µg L^{-1} in Settled Sediment and SS, respectively. ACE concentration in the aqueous phase of Settled Sediment ($C_{w,\ sed}$) was comparable with its concentration in WRRF influents from the literature, whereas in the aqueous phase of SS (($C_{w,\ SS}$), it was higher by one order of magnitude (see Table 5.4). Others have also noted the higher concentrations of ACE compared to other pharmaceuticals in WRRF influent concentrations in both in summer and winter (157 and 127 µg L^{-1}, respectively) (Ibrahim, 2013) or in estuarine sediments impacted by CSOs (Stewart et al., 2014). The particularly high levels of ACE in CSSs could be explained by the high consumption and easy accessibility (no medical prescription needed) of the compound (Beausse, 2004; Jones et al., 2002). Concentrations of biodegradable compounds such as ACE (k_{biol}>10 L g_{SS}^{-1} d^{-1}) (Joss et al., 2006) in the sewers are expected to be higher than in WRRF influents because of less dilution from infiltration and less degradation will have occurred because of the shorter travel time from the source to the sampling location.

CAF was also detected in high total concentrations (147 and 278 µg L^{-1} in Settled Sediment and SS matrices, respectively). Its concentrations in aqueous phases of Settled Sediment and SS matrices were comparable to those found in the influents of WRRFs in Norway (54.7 µg L^{-1}) (Weigel, Berger, et al., 2004); China (50 µg L^{-1}) (Zhou et al., 2010) and Canada (63.2 µg L^{-1})

(Miao et al., 2005). The concentrations were higher than those in WRRFs in Sweden (3.69 μg L^{-1}) (Bendz et al., 2005), Korea (8.810 μg L^{-1}) (Ryu et al., 2014), Milwaukee, WI area (5.86 μg L^{-1}) (Blair et al., 2013), Spain (2.17 to 3.84 μg L^{-1}) (Santos et al., 2007) and Canada (3.500 μg L^{-1}) (Madoux-Humery et al., 2013). The presence of caffeine was attributed to excretion of coffee from consumers because the amount of caffeine used in clinical medicine is negligible (Ternes et al., 2001). The abundant presence of CAF is likely associated with the high consumption of coffee, tea and soft drinks as well as the disposal of these items (Luo et al., 2014).

ACE and CAF have much higher use than CBZ, but have chemical properties that make them less persistent. CBZ is among the most frequently detected pharmaceutical compounds in wastewater (1.85 μg L^{-1}) (Clara et al., 2005). The greater detection frequency of CBZ compared to other compounds with much higher use (e.g., ACE and CAF) reflects the recalcitrant nature of the molecule (Clara et al., 2004). In the current study, CBZ was present in high total concentrations of 48.6 and 18.7 μg L^{-1} in Settled Sediment and SS matrices, respectively. CBZ-DiOH was detected in lower total concentrations (0.572 and 1.383 μg L^{-1} in Settled Sediment and SS matrices, respectively) as compared to its parent compound (CBZ) and compounds from other groups. CBZ concentrations in the aqueous phases were comparable but higher than those in WRRFs. CBZ-DiOH concentrations in the aqueous phases were comparable to those found in WRRF influents (see Table 5.4). Theophylline THEO (as confounded with paraxanthine, see Section 5.8.1.1) was also present in high total concentrations of 310 and 548 μg L^{-1} in SS and Settled Sediment matrices, respectively. Their concentrations in the aqueous phases were higher than those observed in WRRFs. For most of the pharmaceuticals studied in the WRRF influents (including CAF and CBZ), no significant differences by season were observed (Sui et al., 2011).

Table 5.4. Occurrence of selected pharmaceuticals in water resource recovery facilities (WRRFs) influents.

Compound	Influent concentration (μg L^{-1})	Reference
Acetaminophen	0.13–26.09; 10.194[a]	(Gros et al., 2006)
	29–246; 134[a]	(Gomez et al., 2007)
	74.552[a]	(Kim et al., 2012)
	18[b]; 150[c]	(Blair et al., 2013)
	157.43[a]; 88.20[b]; 1290.63[c]	(Ibrahim, 2013)
	1.817[a]; 1.051[b]; 9.142[c]	(Madoux-Humery et al., 2013)
Caffeine	25.06[a]	(Kim et al., 2012)
	8.810[a] (dry weather); 2.758[a] (wet weather)	(Ryu et al., 2014)
	9.2[b]; 130[c]	(Blair et al., 2013)
	5.86[a]; 5.65[b]; 11.4[c]	(Sui et al., 2011)
	48.8[a]; 89.5[c]	(Zhou et al., 2010)
	88.71[a]; 60.60[b]; 524.41[c]	(Ibrahim, 2013)
	147 ±76[a]	(Ternes et al., 2001)
	54.7[a]	(Weigel, Berger, et al., 2004)
	63.2[a]	(Miao et al., 2005)
	3.500[a]; 3.611[b]; 6.766[c]	(Madoux-Humery et al., 2013)
Theophylline	4.195[a]	(Kim et al., 2012)
	4.613[a]; 2.916[b]; 22.532[c]	(Madoux-Humery et al., 2013)
Carbamazepine	0.015–0.27; 0.054[b]	(Nakada et al., 2006)
	1.85[a]; 1.2[a]; 0.704[a]; 0.67[a]; 0.325[a]	(Clara et al., 2005)
	0.12–0.31; 0.15[a]	(Gomez et al., 2007)
	0.188[a] (dry weather); 0.082[a] (wet weather)	(Ryu et al., 2014)
	0.072[b]; 0.310[c]	(Blair et al., 2013)
	0.3561[a]	(Miao et al., 2005)
	0.0763[a]; 0.0707[b]; 0.233[c]	(Sui et al., 2011)
	0.279[a]; 0.261[b]; 0.493[c]	(Ibrahim, 2013)
	1.68[a]	(Bendz et al., 2005)
	0.28-0.36[a]	(Santos et al., 2007)
	0.215[a]; 0.231[b]; 0.363[c]	(Madoux-Humery et al., 2013)
10,11-dihydro-10,11-dihydroxycarbamazepine	1.0012[a]	(Miao et al., 2005)

[a]Mean, [b]Median, [c]Maximum concentrations.

5.8.2 Experimental methods

5.8.2.1 Materials

Table 5.5. Experimental K_d and K_{OC} values for sorption onto different matrices from the literature.

Compound	K_d (L Kg^{-1})	K_{OC}	Matrix	Reference
ACE	0.41	62	Sewage sludge	(Jones et al., 2002)
CAF	25	690	Soil	(Barron et al., 2009)
	14	46	Sludge	(Barron et al., 2009)
THEO				
CBZ	0.1		Sewage sludge	(Ternes et al., 2004)
	1.3	3.5	Creek sediment	(Loffler et al., 2005)
	25	83	Sewage sludge	(Jones et al., 2002)
	1.4 (low OC)/4.4 (high OC)	3900	Soil	(Drillia et al., 2005)
	13	360	Soil	(Barron et al., 2009)
	43	140	Sludge	(Barron et al., 2009)
CBZ-DiOH	0.3	29	Creek sediment	(Loffler et al., 2005)

5.8.2.2 Sampling and sample pre-treatment

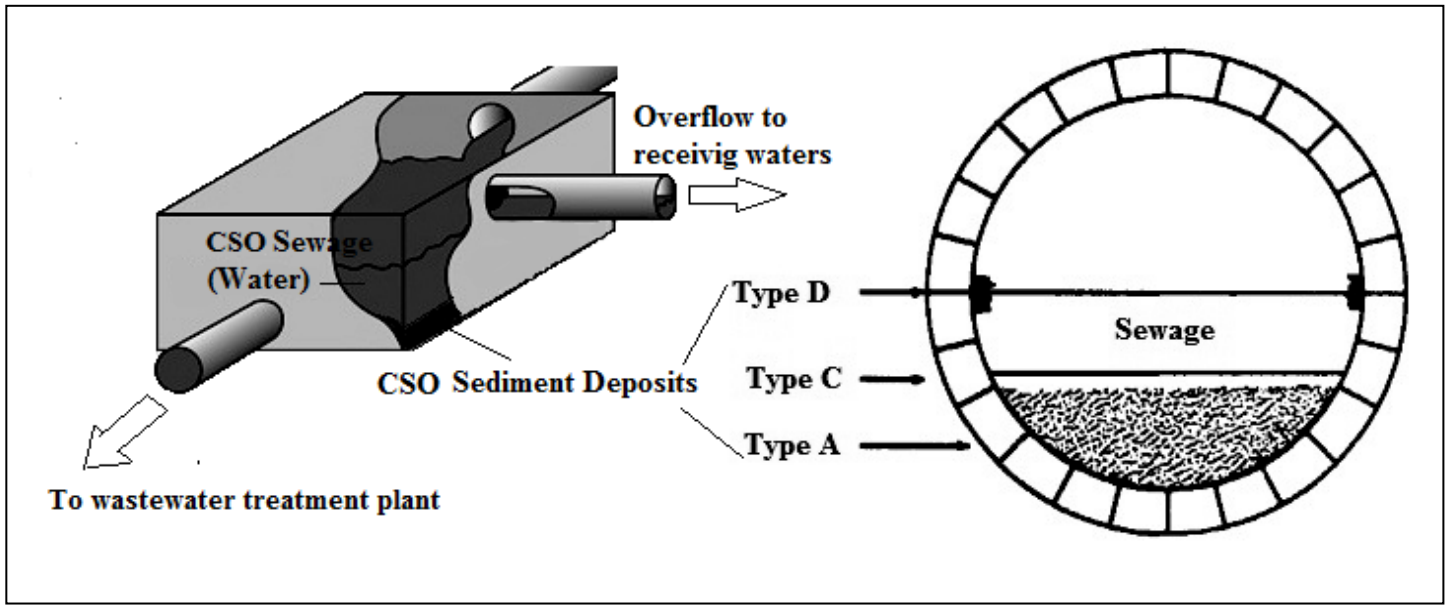


Figure 5.5. Combined sewer system (CSS) with three types of sediment deposits (modified from (Crabtree, 1989)).

The samples in this study included Type C and Type A deposits, as presented in Figure 5.5 of a CSS collection system.

Figure 5.6. Sampling and pre-treatment steps.

The settled sediment (StS) was a few cm deep while the supernatant containing SS was turbid.

Table 5.6. Matrix properties.

	SS	**settled sediment**
Moisture content (%)	97.5 ± 0.07	87.9 ± 0.15
Total solid (mg L^{-1})	26500	156060
Organic content of particles (f_{OC}) (%)	51.7	6.3
Organic carbon relative to total solid (mg L^{-1})	13700	9900
pH	7.05 – 7.29	7.28 – 7.35

5.8.2.3 Analysis of suspended and settled sediments

Freeze-dried solids (0.1 g) were extracted with 5 and 3 ml of methanol-acetone (3:1, v/v) in a 15 mL conical polypropylene centrifuge tube. In each extraction cycle, the sample was vigorously shaken for 30s, kept in an ultrasonic bath (frequency 40 KHz, Bransonic 5510, Connecticut, USA) for 20 min at 30 °C, mixed for 30 minutes on a vertical tube rotator (Scientific Equipment Products Co., Baltimore, US) and then centrifuged for 5 min at 6000 rpm. After each extraction step, the supernatants were collected, combined and evaporated to total dryness under a nitrogen stream at 30 °C. Samples were subsequently diluted in 4 mL of methanol-0.1% formic acid (pH 2.65) (1:20, v/v), ultrasonicated for 5 min in an ultrasonic bath at 30 °C, filtered using a 0.3-µm glass fiber membrane filter and analytes were pre-concentrated by online solid phase extraction (SPE), followed LC tandem MS detection for separation and quantification.

5.8.2.4 Analytical method

A schematic diagram of the system set-up (parallel SPE and elution) is shown in Viglino, Aboulfadl, Prévost, et al. (2008). The pre-concentration of samples was performed using the EQuan MAX system (Thermo Fisher Scientific, Waltham, MA). It consists of a sample delivery system, a dual switching-column array and an LC–MS/MS system. The delivery system comprised a HTC Thermo-pal autosampler (CTC analytics AG, Zwingen, Switzeraland) fitted with a cooled sample holder at 4 °C, a 2.5 mL syringe, used for in-loop sample injection and a quaternary pump Accela 600 (Thermo Finnigan, San Jose, CA) used to load the SPE column with the contents of the sample loop (1 mL). The column switching system was composed of two-position six-port and ten-port valves (VICI® Valco Instruments Co. Inc., Houston, TX) and a quaternary pump Accela 1250 (Thermo Finnigan, San Jose, CA) used for sample elution from the SPE column and separation on the analytical column. The on-line SPE was done with a Hypersil GOLD aQ column (20 mm×2.1 mm, 12 µm particle size) and chromatographic separation was achieved using a reversed phase Hypersil GOLD C18 UPLC column (50 x 2.1 mm, 1.9 µm) kept at 55 °C. All columns were manufactured by Thermo Fisher Scientific (Thermo Finnigan, San Jose, CA). Mobile phase A was 0.1 % formic acid in ultrapure water, and mobile phase B was methanol with 0.1 % formic acid. The mobile phase gradient used in this method is shown in Figure 5.7. The flow rate of the mobile phase was 525 µL min^{-1} and the injection volume was 1 mL. This configuration allowed for a total analysis time of 7 min per sample. To generate ions,

the Ion Max API Source was configured on a Quantum Ultra AM triple quadrupole mass spectrometer by Thermo Fisher Scientific (Waltham, MA) to operate in atmospheric pressure chemical ionization (APCI) mode. Selected reaction monitoring (SRM) mode has been applied for quantification and detection. The MS analyses were performed in positive ionization mode. The optimal collision voltage for each of the precursor to product ion transitions of each compound is listed in Table 5.7. The collision gas pressure and the offset energy for the collision quadrupole Q2 were two important factors for determining the major product ion intensity for each compound; they were optimized at 1.5 mTorr and between 17 and 36 eV, respectively (Table 5.7 and Table 5.8). Retention times are shown in LC–MS/MS chromatograph of pharmaceuticals (Figure 5.8).

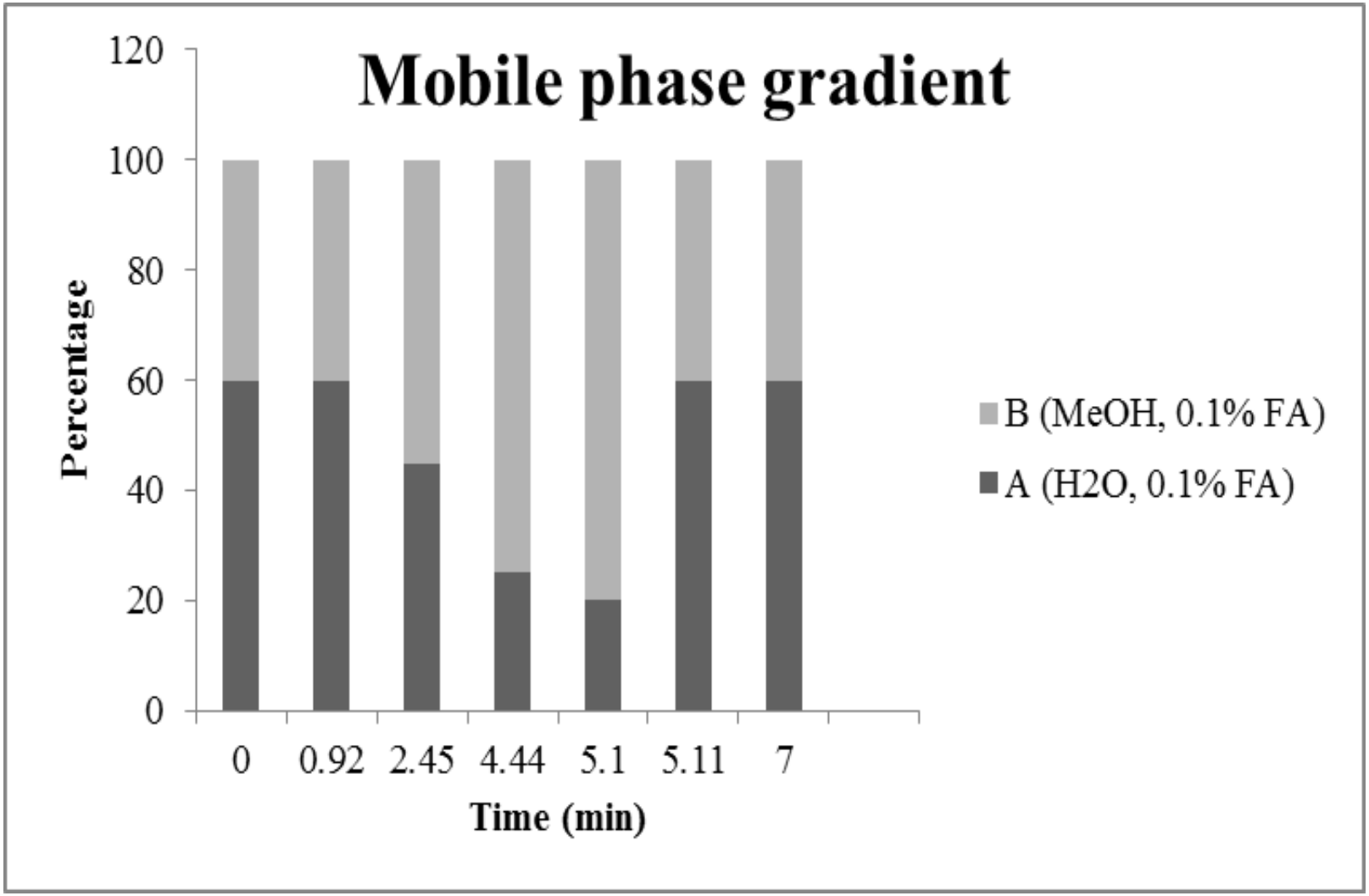


Figure 5.7. Binary gradient of mobile phase A (water/0.1% Formic acid) and mobile phase B (methanol/0.1% Formic acid) by using LC Pump Accela 1250.

Table 5.7. Precursor ion, product ions, and optimal collision voltage for targeted analytes.

Compound	**Precursor Ion (m/z)**	**Major Product Ion (m/z)**	**CE (eV)**	**Tub Lens**	**Minor Product Ion (m/z)**	**CE (eV)**	**Tub Lens**
Acetaminophen	152.100-152.101	110.100	17	74	65.200	30	74
$^{13}C_2$-acetaminophen	155.100	111.100	19	74	-	-	-
Caffeine	195.100-195.101	138.080	19	80	110.120	23	80
$^{13}C_3$-Caffeine	198.094	140.100	18	81	-	-	-
Theophylline	181.100-181.101	124.090	21	72	96.180	26	72
Carbamazepine	237.100-237.101	194.090	18	77	192.090	23	77
Carbamazepine-d_{10}	247.150	204.160	20	82	-	-	-
10,11-dihydro-10,11-dihydroxycarbamazepine	271.200-271.202	180.000	36	80	235.000	11	80

Table 5.8. MS parameters.

Ionization mode	Positive
Discharge current	5 µA
Vaporizer temperature	490 °C
Capillary temperature	350 °C
Sheath gas pressure	50 arb units
Aux. gas pressure	15 arb units
Collision gaz pressure	1.5 mTorr
Scan time	0.01 s

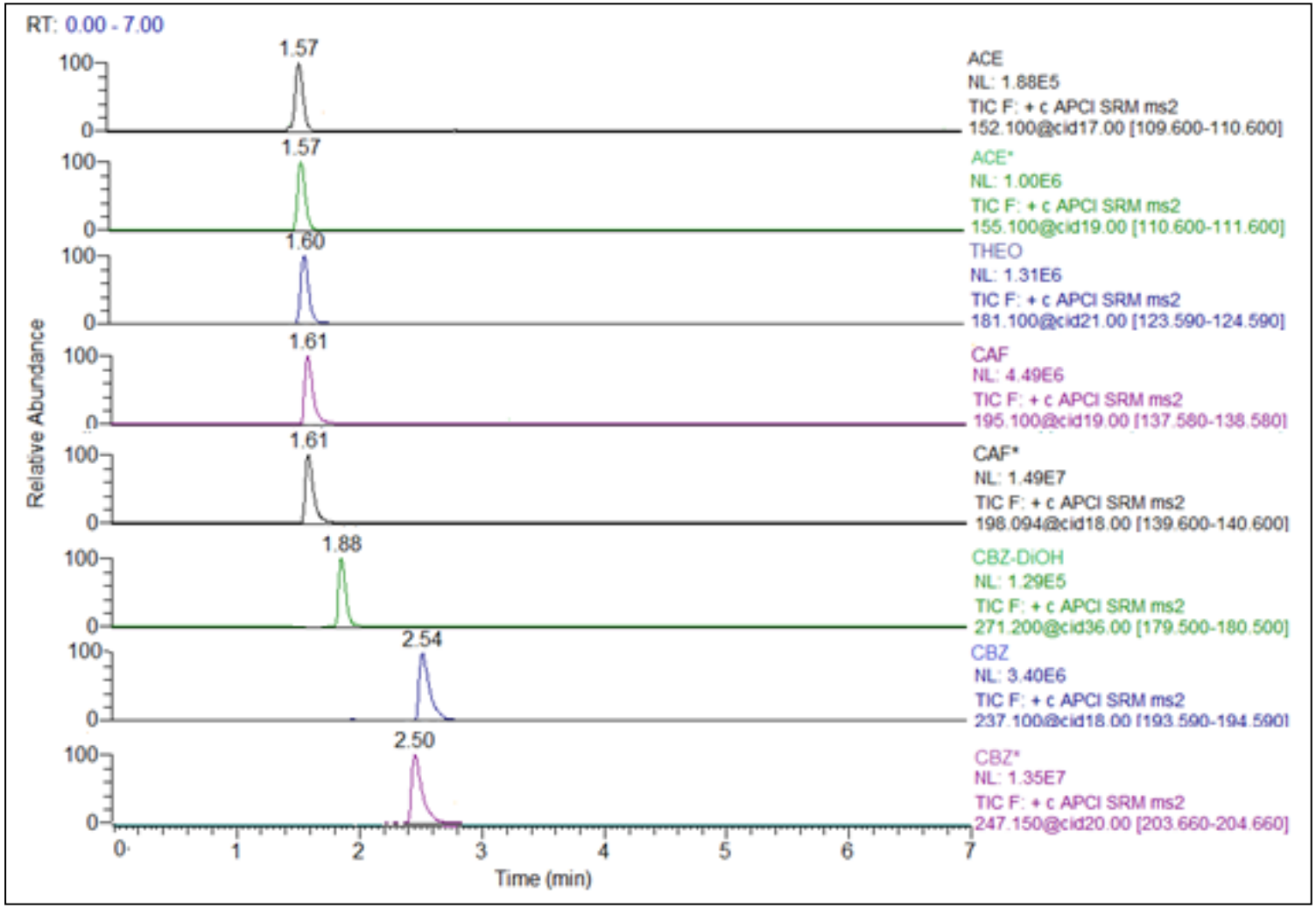


Figure 5.8. LC–MS/MS chromatograph of a standard mix solution of pharmaceuticals (1 µg L^{-1}) in the acidified water. * refers to labeled internal standard.

5.8.2.5 Method Validation

Solid-phase extraction recoveries and detection limits of analytical method

0.1 g of freeze-dried combined sewer sediments and sieved through 1.25-mm screen (n=3) were placed in different 15-mL conical polypropylene centrifuge tubes, covered with acetone (500 µL) and spiked with a mixture of standards (0.2 and 0.05 µg g^{-1}). The samples were shaken at 100 rpm in a refrigerated incubator shaker Innova 4230 (New Brunswick Scientific Edison, NJ. USA) and allowed to equilibrate overnight at 20 °C in the dark before ultrasonic-assisted extraction (USE), in order to obtain a dry and homogenous material. Blank samples (without analytes) and control samples (without sediment, but spiked with analytes) were included in each samples series. Compound-specific recoveries were determined by comparing the peak area of a sediment extract (spiked prior to USE) to the area obtained from detecting a standard solution spiked after SPE in the sediment sample extract. Before analysis, 20 µL of instrument internal standard containing 3 isotope-labeled compounds (0.1 mg L^{-1}) was added to correct for variations in sample recovery and instrumental performance. The analytical method detection limit (LOD) is referred to 3.3 times the standard deviation of the intercept divided by slope of the calibration curve (Glaser et al., 1981; Viglino, Aboulfadl, Daneshvar, et al., 2008).

5.8.2.6 Sorption Experimental design

Table 5.9. Experimental conditions and batch reactor design for sorption of selected pharmaceuticals CSO suspended and settled sediment.

Reactor (n=2)	Study	Concentrations of Selected pharmaceuticals	Incubation	Volume of Tap Water /Volume of removed supernatant Ratio	Time of Replacing Supernatant with Tap Water	Time Profile	Analysed Samples
A	Adsorption	Spiked at 250 µg L^{-1} for ACE and THEO, 50 µg L^{-1} for CAF and CBZ and 2.5 µg L^{-1} for CBZ-DiOH	With shaking at 120 rpm	-	-	0, 12 and 22 h	All supernatants All sediments
B	Continual desorption			1	0 h	0, 1, 2, 8, 12, 25, 48, 72, 120, 216, 288 and 383 h	All supernatants Sediments (0, 8, 72 and 120 h) Filters (120 h)
C		Natural		1	0 h	0, 0.5, 1, 2, 12, 24 48 and 71 h	All supernatants Sediments (0, 2, 24, 71 h) Filters (2 h)
D			Without shaking	1	0 h		
E				2	0 h		
F	Sequential desorption		With shaking at 120 rpm	2	0, 2, 8 h	0, 0.25, 0.5, 1, 2, 4, 8, 21, 31, 51 and 72 h	All supernatants All sediments
G			Without shaking	1	0, 12, 24 and 48 h	0, 2, 4, 6, 12, 24, 48 and 72 h (for SS) 0, 2, 4, 8, 13, 24, 32, 54 and 72 h (for Settled Sediment)	

5.8.3 Results and discussion

5.8.3.1 Extraction and analytical Methods

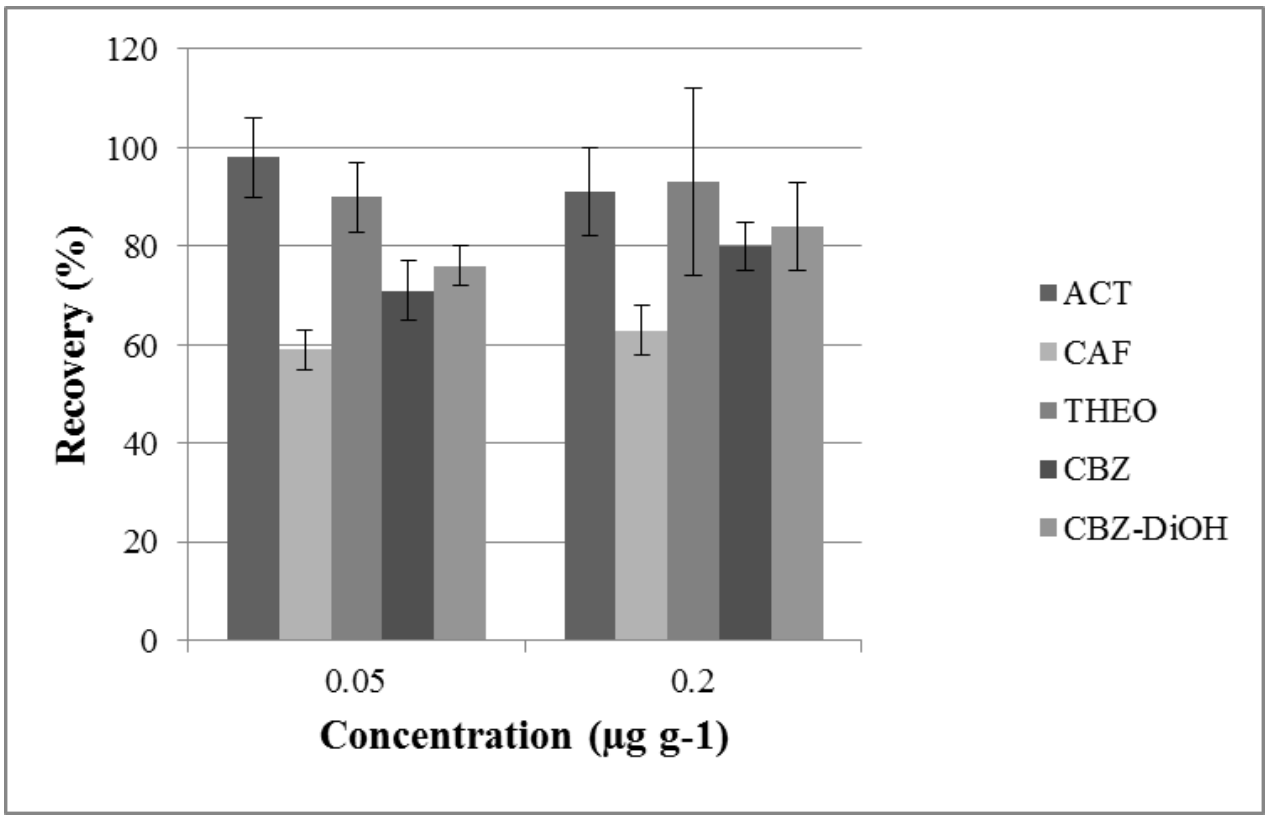


Figure 5.9. Solid-phase extraction recoveries comparing two different concentrations of pharmaceuticals in spiked standard solution, the error bars indicate standard deviations.

All calibration curves were linear ($R^2 > 0.99$). LOD values of each compound in the CSS water and CSS sediment extract samples are given in Table 5.10.

Table 5.10. LOD values of selected compounds in the liquid phase and CSS sediment extract samples.

	Liquid Phase		CSS Sediment Extract	
Compound	**LOD µg L^{-1}**	**R^2**	**LOD µg Kg^{-1}**	**R^2**
Acetaminophen	1.21	0.998	2	0.997
Caffeine	0.53	0.999	2	0.995
Theophylline	0.33	0.999	2	0.996
Carbamazepine	0.49	0.998	2	0.997
10,11-dihydro-10,11-dihydroxycarbamazepine	0.38	0.999	1	0.999

5.8.3.2 Sorption behaviour of pharmaceuticals to SS and Settled Sediment

Mass balances

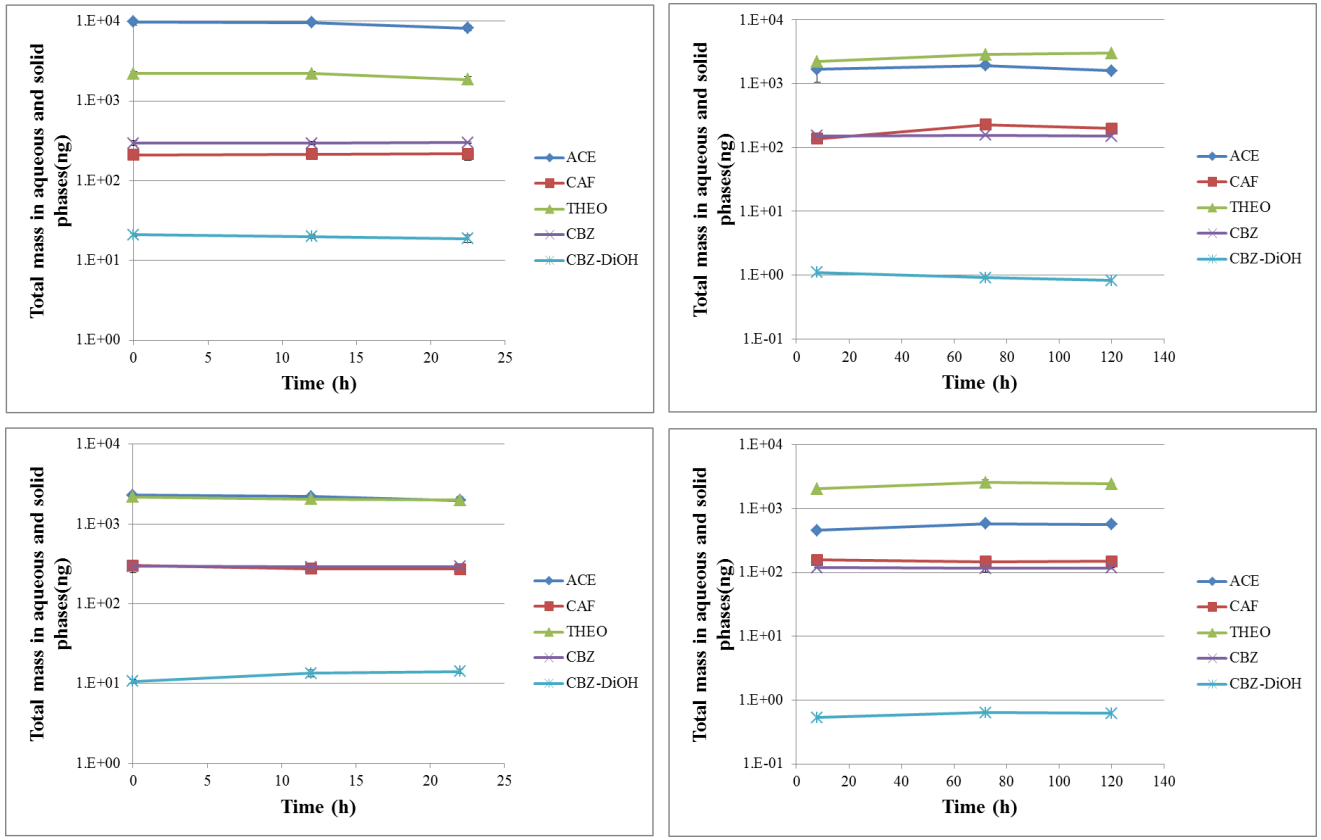


Figure 5.10. Total analyte mass (ng) in the aqueous and the solid phases at both adsorption in Reactors A (on the left) and desorption points in Reactors B (on the right) in SS (top panels) and Settled Sediment (bottom panels), error bars indicating standard deviations (n=2).

Solid-water distribution coefficients in non-spiked batch reactors under different conditions (with shaking, without shaking and with dilution)

Table 5.11. Solid water distribution coefficients log $K_{d,\ app}$ (L Kg^{-1}) of selected pharmaceuticals for the desorption of selected pharmaceuticals on native solid phases SS and Settled Sediment (Reactors C, D, and E).

Desorption condition	**With shaking in SS (Reactor C)**				**Without shaking in SS (Reactor D)**				**Without shaking and with dilution in SS (Reactor E)**			
Incubation Time (h)	**des 0**	**des 2**	**des 24**	**des 72**	**des 0**	**des 2**	**des 24**	**des 72**	**des 0**	**des 2**	**des 24**	**des 72**
Compound												
ACE	**-1.83**	**-2.07**	**-2.15**	**-2.19**	**-2.06**	**-1.94**	**-2.19**	**-2.18**	**-1.99**	**-2.06**	**-2.16**	**-2.25**
CAF	**0.79**	**0.52**	**0.30**	**0.10**	**0.81**	**0.58**	**0.33**	**0.08**	**1.11**	**0.76**	**0.73**	**0.54**
THEO	**-0.55**	**-0.70**	**-0.80**	**-1.00**	**-0.51**	**-0.64**	**-0.84**	**-1.00**	**-0.30**	**-0.44**	**-0.50**	**-0.66**
CBZ	**0.16**	**0.09**	**0.09**	**-0.02**	**0.19**	**0.17**	**0.08**	**0.00**	**0.44**	**0.32**	**0.33**	**0.23**
CBZ-DiOH	**-1.72**	**-2.68**	**-2.76**	**-3.22**	**-1.51**	**-1.82**	**-1.72**	**-2.33**	**-0.72**	**-2.32**	**-2.92**	**-2.56**
Desorption condition	**With shaking in StS (Reactor C)**				**Without shaking in StS (Reactor D)**				**Without shaking and with dilution in StS (Reactor E)**			
Incubation Time (h)	**des 0**	**des 2**	**des 24**	**des 72**	**des 0**	**des 2**	**des 24**	**des 72**	**des 0**	**des 2**	**des 24**	**des 72**
Compound												
ACE	**-2.27**	**-2.25**	**-2.56**	**-2.48**	**-2.33**	**-2.42**	**-2.46**	**-2.46**	**-2.03**	**-2.27**	**-2.32**	**-2.39**
CAF	**0.46**	**0.43**	**0.05**	**-0.08**	**0.56**	**0.33**	**-0.16**	**-0.03**	**0.74**	**0.45**	**0.23**	**0.18**
THEO	**-0.76**	**-0.79**	**-0.96**	**-1.12**	**-0.80**	**-0.89**	**-1.15**	**-1.07**	**-0.46**	**-0.69**	**-0.79**	**-0.92**
CBZ	**0.01**	**0.03**	**-0.17**	**-0.21**	**0.03**	**-0.02**	**-0.23**	**-0.12**	**0.24**	**0.13**	**0.05**	**-0.09**
CBZ-DiOH	**-2.22**	**-1.98**	**-2.53**	**-2.23**	**-2.10**	**-2.67**	**-2.12**	**-2.34**	**-1.80**	**-2.30**	**-2.58**	**-2.16**

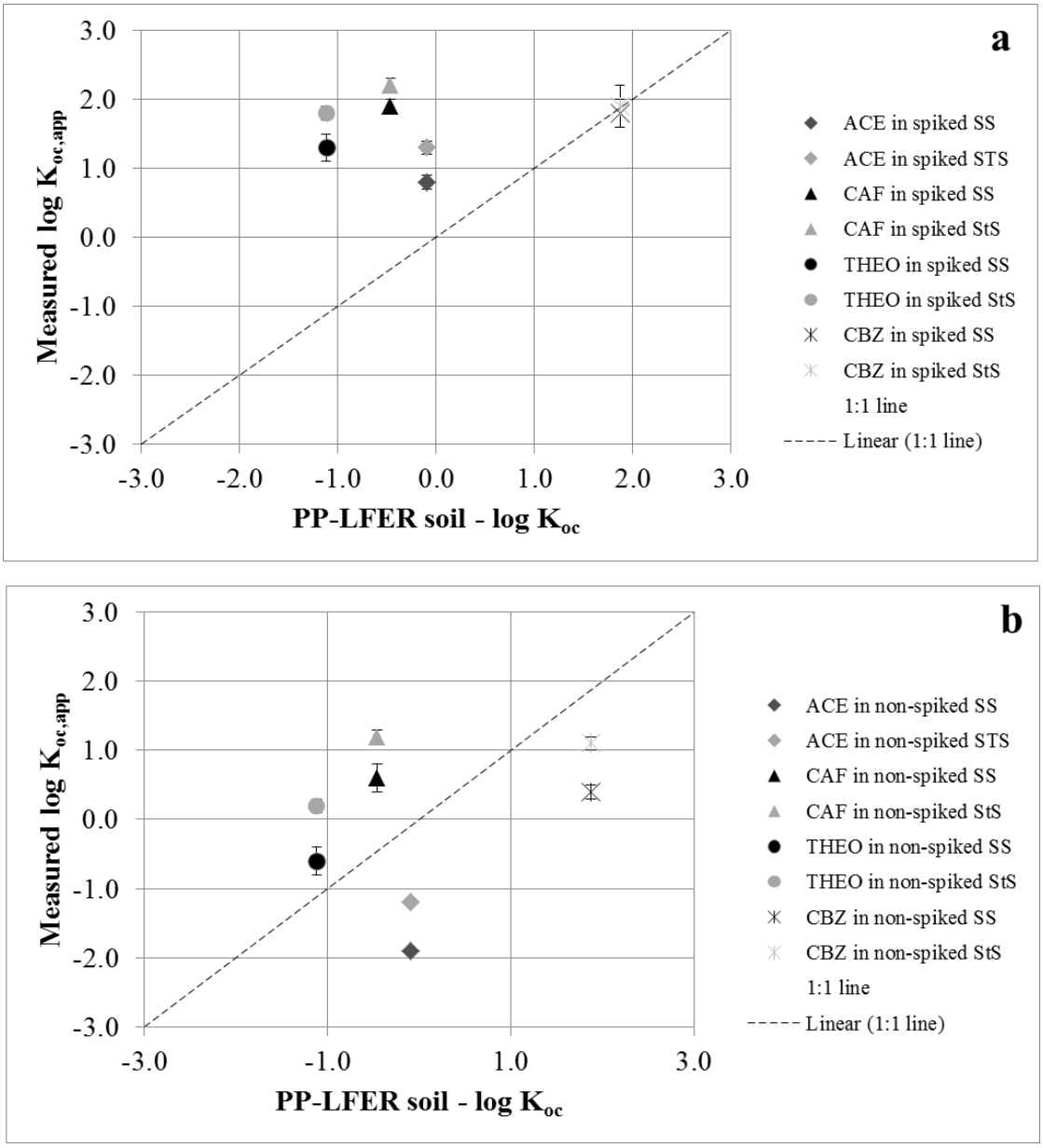


Figure 5.11. Average log $K_{OC,\,app}$ values (L Kg^{-1}) of pharmaceuticals and caffeine in both spiked (a) and non-spiked (b) SS and StS matrices as a function of PP-LFER soil-log K_{OC}, error bars indicate standard deviations (n=2), PP-LFER soil–log K_{OC} values were derived from the literature (Abraham & Acree Jr, 2004; Abraham et al., 2009; Abraham et al., 2008; Bronner & Goss, 2011; Tülp et al., 2008).

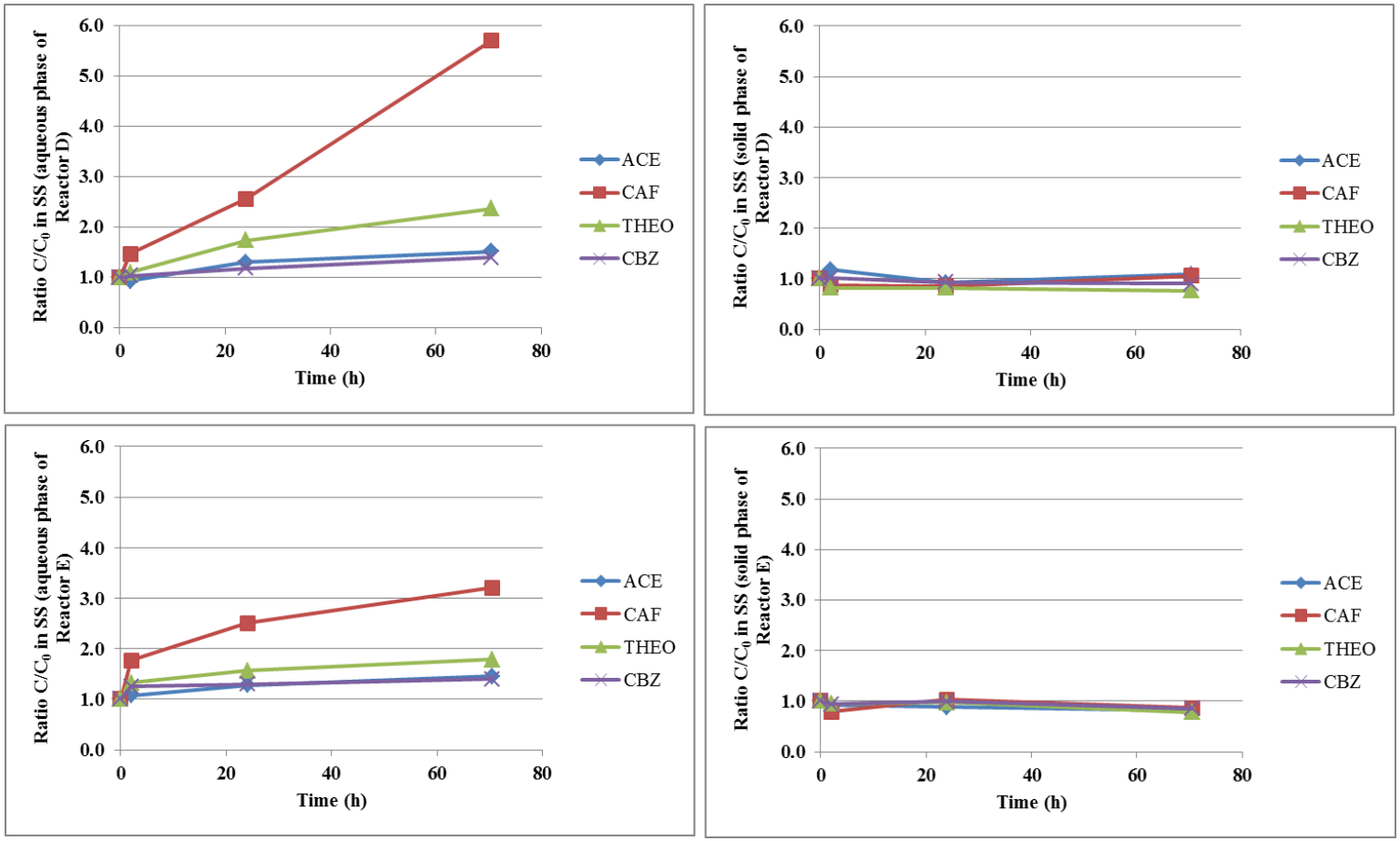


Figure 5.12. Desorption kinetics without spiking expressed in C/C_0 ratio of the selected pharmaceuticals and caffeine to the suspended solids SS (top pannels) and the settled sediment (bottom pannels) under 2 conditions (without shaking (Reactors D) and with dilution (Reactors E)) except for CBZ-DiOH, under 3 conditions (with shaking (Reactors C), without shaking (Reactors D), and with dilution (Reactors E)). C and C_0 correspond to the concentrations in aqueous phase (ng L^{-1}) or in solid phase (ng g^{-1}) at defined time and 0h of desorption respectively.

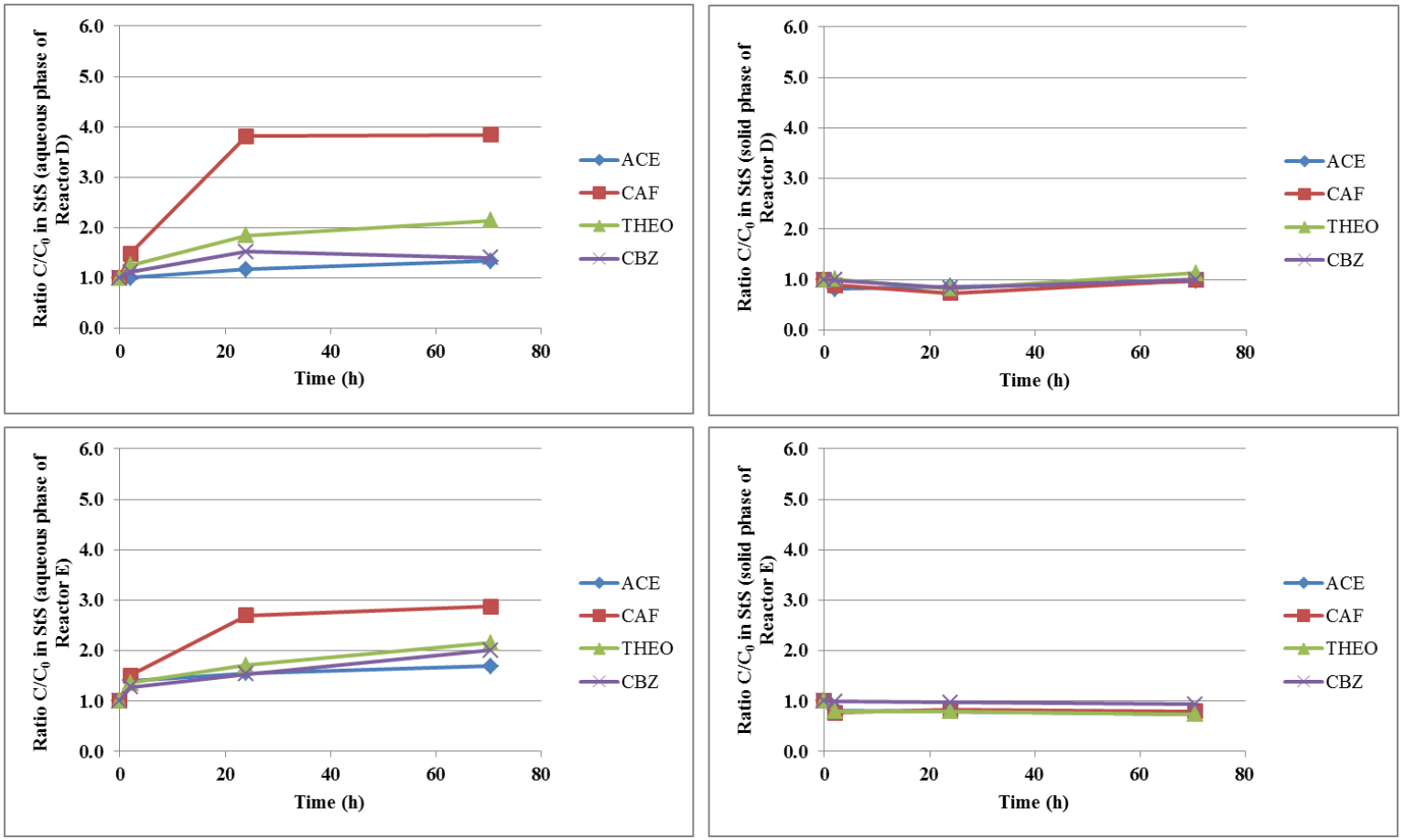


Figure 5.12. Desorption kinetics without spiking expressed in C/C_0 ratio of the selected pharmaceuticals and caffeine to the suspended solids SS (top pannels) and the settled sediment (bottom pannels) under 2 conditions (without shaking (Reactors D) and with dilution (Reactors E)) except for CBZ-DiOH, under 3 conditions (with shaking (Reactors C), without shaking (Reactors D), and with dilution (Reactors E)). C and C_0 correspond to the concentrations in aqueous phase (ng L^{-1}) or in solid phase (ng g^{-1}) at defined time and 0h of desorption respectively (cont'd).

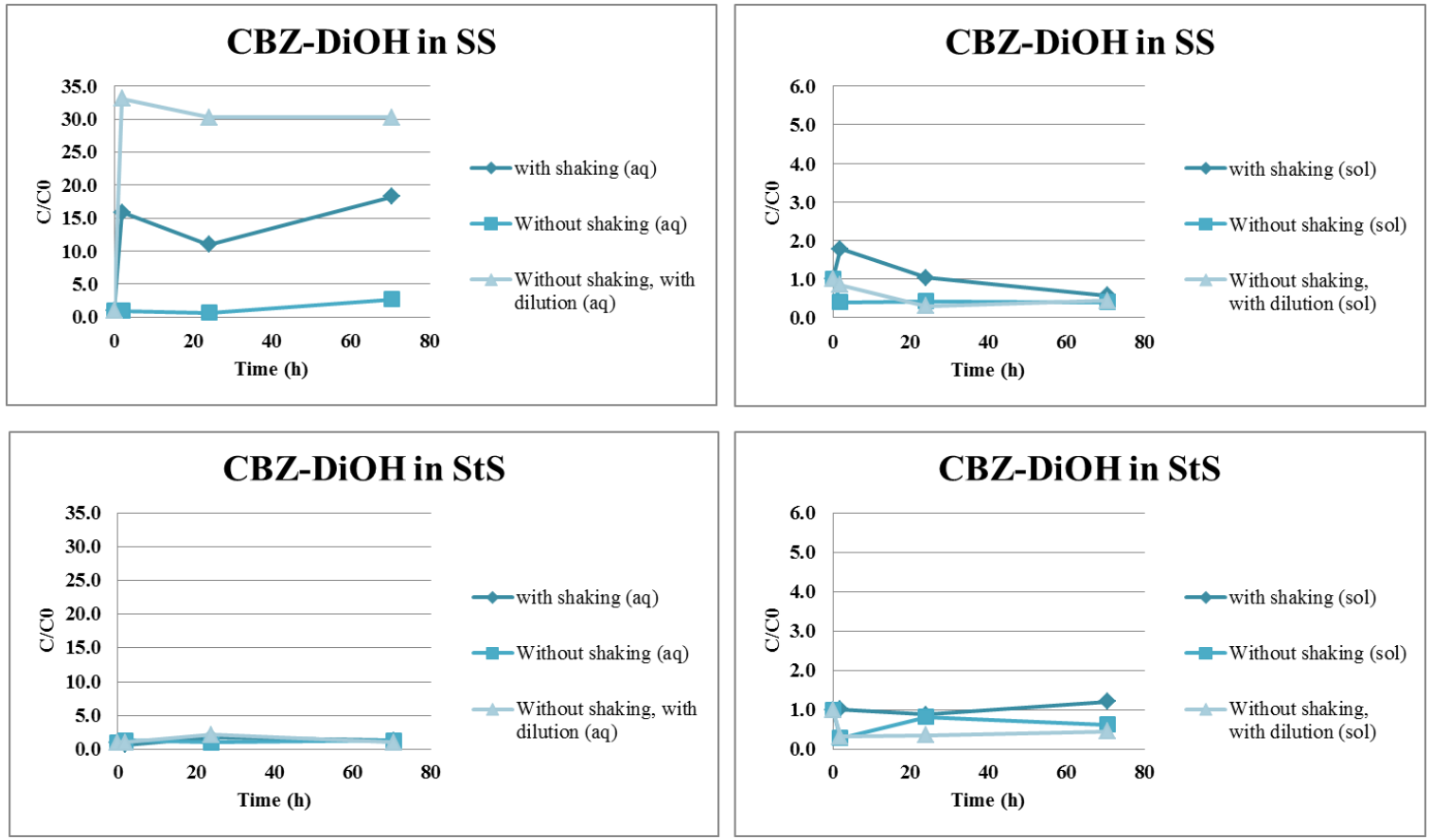


Figure 5.12. Desorption kinetics without spiking expressed in C/C_0 ratio of the selected pharmaceuticals and caffeine to the suspended solids SS (top pannels) and the settled sediment (bottom pannels) under 2 conditions (without shaking (Reactors D) and with dilution (Reactors E)) except for CBZ-DiOH, under 3 conditions (with shaking (Reactors C), without shaking (Reactors D), and with dilution (Reactors E)). C and C_0 correspond to the concentrations in aqueous phase (ng L^{-1}) or in solid phase (ng g^{-1}) at defined time and 0h of desorption respectively (cont'd).

Table 5.12. *P* values of Kruskal wallis test comparing $K_{d(des,\ T),app}/K_{d(des,\ T0),app}$ ratios of selected compounds in non-spiked reactors (C, D, and E).

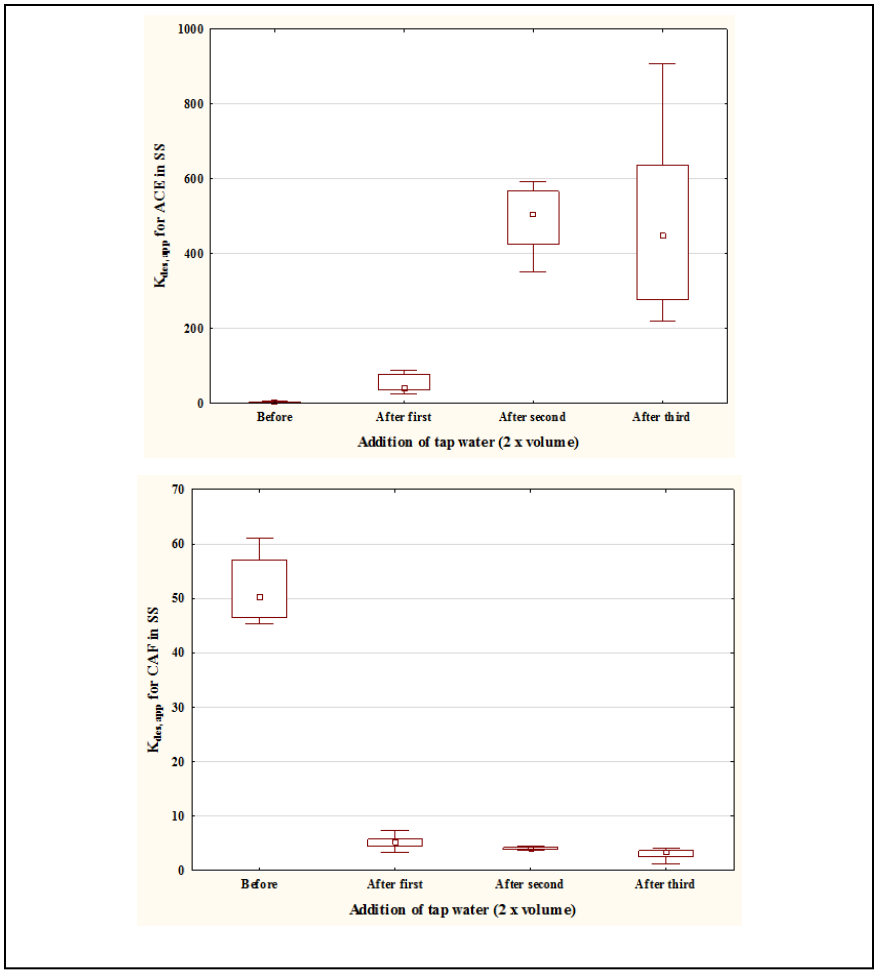
***P* (Kruskal wallis test), Desorption without spiking**		
Compound	**SS**	**Settled Sediment**
	Between Reactors C (with shaking), Reactors D (without shaking), and Reactors E (with dilution)	
ACE	0.0434	0.0120
CAF	0.9132	0.2238
THEO	0.8712	0.2811
CBZ	0.2831	0.4594
CBZ-DiOH	0.0018	0.0254
	Between Reactors C (with shaking) and Reactors D (without shaking)	
ACE	0.0446	0.3367
CAF	0.7150	0.1093
THEO	0.5839	0.6310
CBZ	0.5839	0.7488
CBZ-DiOH	0.0285	0.0782
	Between Reactors D (without shaking) and Reactors E (with dilution)	
ACE	0.3367	0.0039
CAF	0.7488	0.3367
THEO	0.7488	0.3367
CBZ	0.2623	0.3367
CBZ-DiOH	0.0039	0.1495

Table 5.13. *P* values of ANOVA-test comparing $K_{d(des)}$ of selected compounds in non-spiked reactors (C, D, and E) at different time of desorption under three conditions (without shaking, with shaking, and with dilution).

***P* (ANOVA test), Desorption without spiking**						
Compound	**SS (0, 2, 24, and 71h)**			**Settled Sediment (0, 2, 24, and 71h)**		
	Without shaking	**With shaking**	**With dilution**	**Without shaking**	**With shaking**	**With dilution**
ACE	0.1489	0.0478	0.0456	0.0258	0.0002	0.00003
CAF	0.00000	0.0085	0.00006	0.00000	0.00000	0.00000
THEO	0.0057	0.0002	0.0009	0.00000	0.0001	0.00000
CBZ	0.0003	0.4121	0.0029	0.00000	0.00002	0.0002
CBZ-DiOH	0.3093	0.0099	0.0050	0.3758	0.0127	0.0113
	SS (2, 24, and 71h)			**Settled Sediment (2, 24, and 71h)**		
	Without shaking	**With shaking**	**With dilution**	**Without shaking**	**With shaking**	**With dilution**
ACE	0.00001	0.5851	0.1872	0.3201	0.0001	0.0162
CAF	0.00003	0.1590	0.0010	0.00000	0.00000	0.0003
THEO	0.0002	0.0046	0.0002	0.00001	0.00004	0.00009
CBZ	0.0006	0.6556	0.0761	0.0001	0.0003	0.0024
CBZ-DiOH	0.3295	0.3125	0.3684	0.0921	0.0201	0.1991

Sequential desorption in non- spiked batch reactors

Effects of successive stormwater dilution on the K_{des}



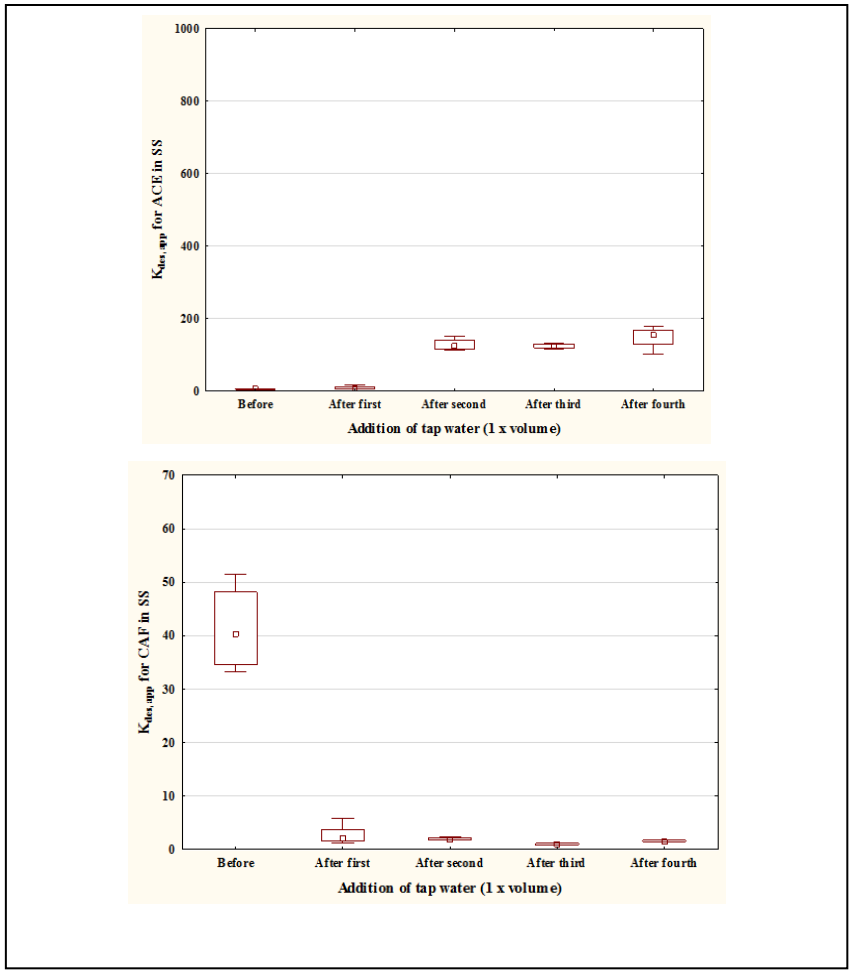
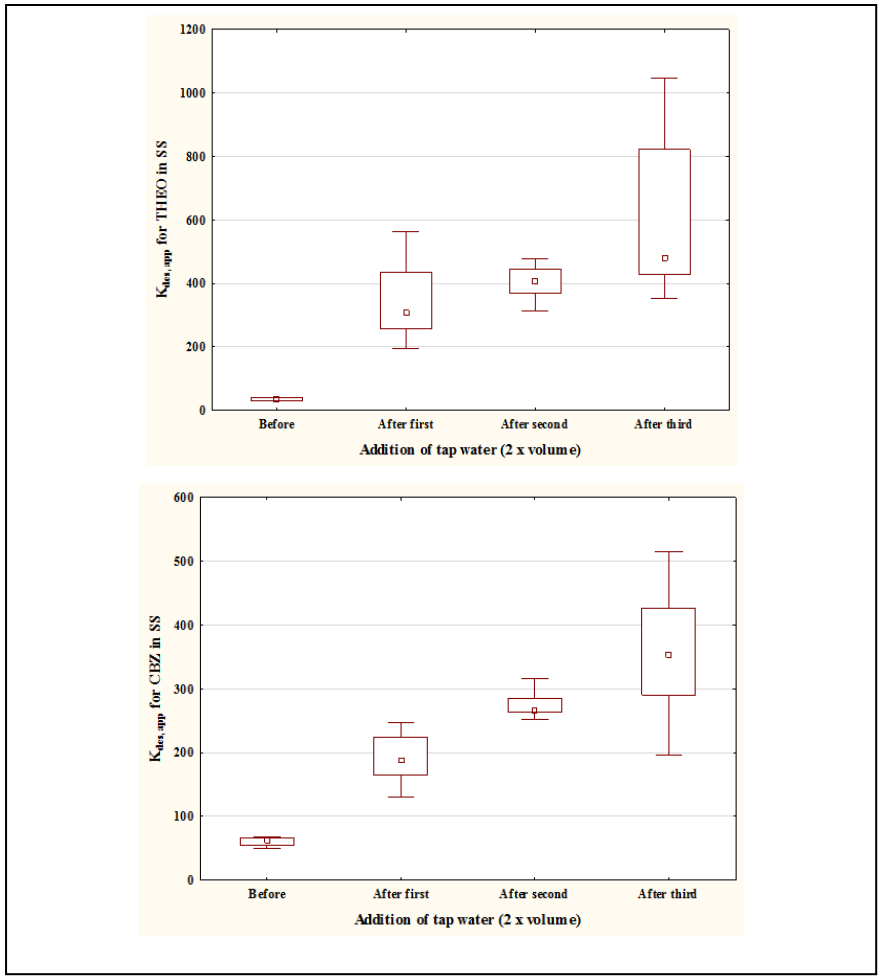


Figure 5.13. Box plots of the solid water distribution coefficients ($K_{des,\ app}$, L kg^{-1}) during sequential desorption in two different matrices (SS and Settled Sediment) with shaking (on the left) and without shaking (on the right), bars indicating 25/75 percentiles, whiskers indicating minimum and maximum values, circles indicating outliers, stars indicating extremes and middle points indicating median values.



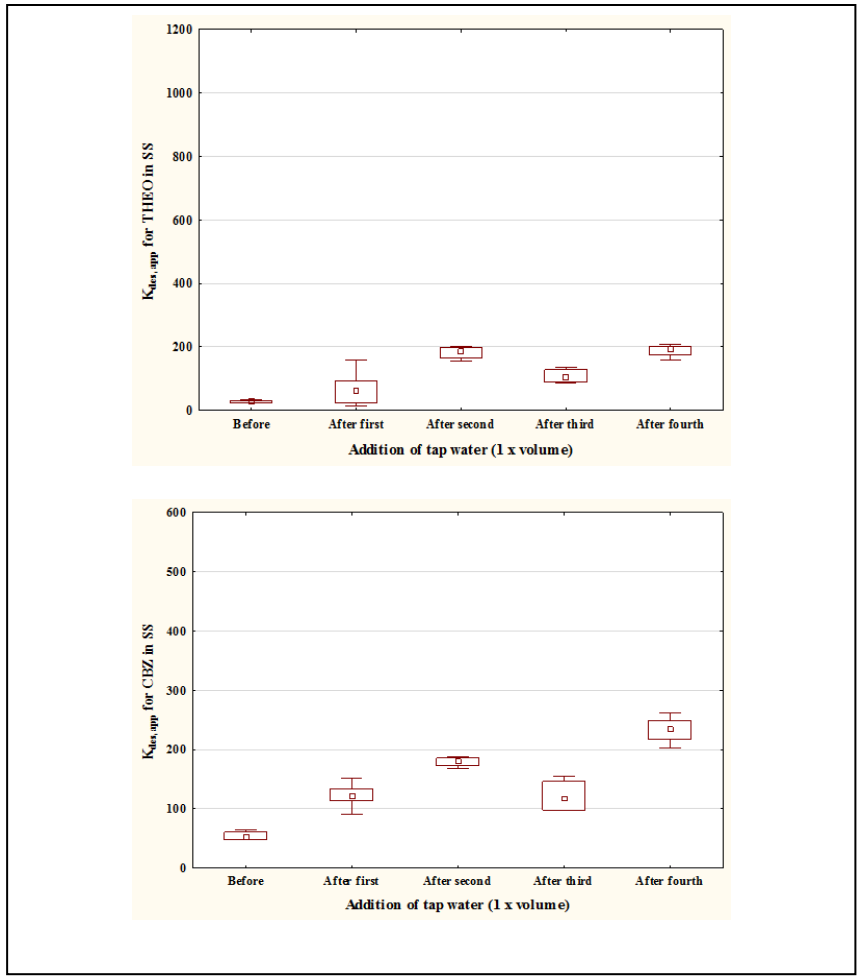
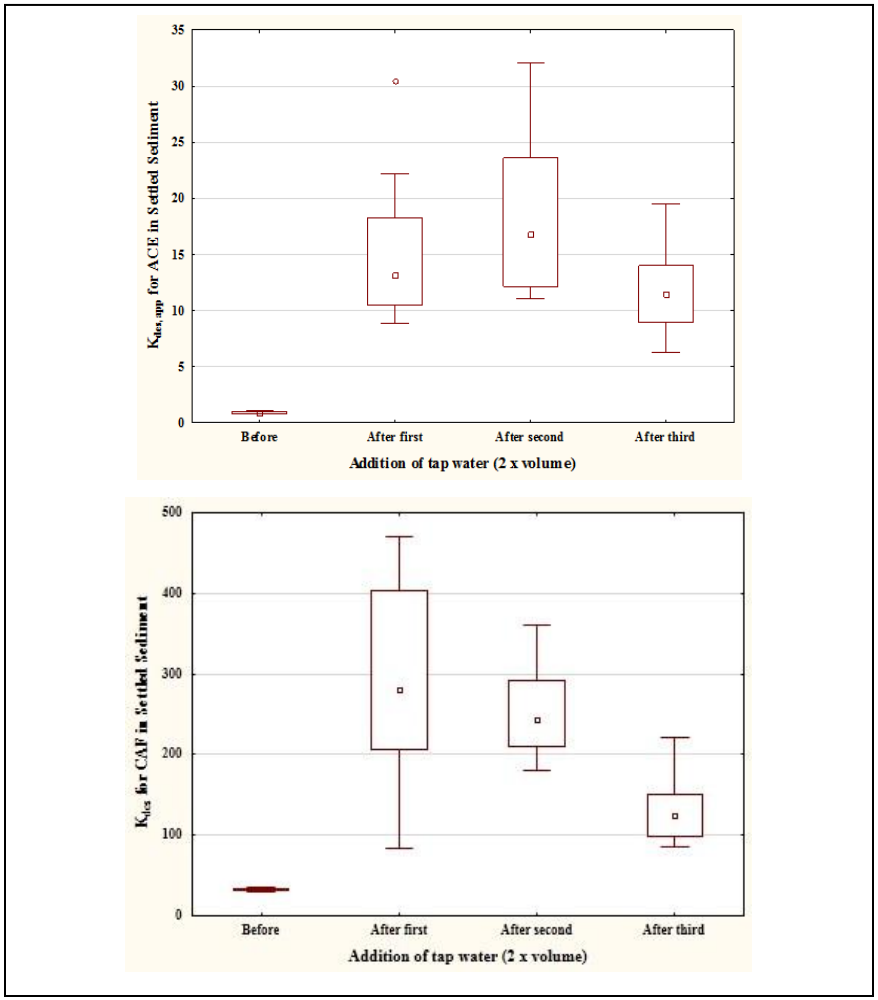


Figure 5.13. Box plots of the solid water distribution coefficients ($K_{des,\ app}$, L kg^{-1}) during sequential desorption in two different matrices (SS and Settled Sediment) with shaking (on the left) and without shaking (on the right), bars indicating 25/75 percentiles, whiskers indicating minimum and maximum values, circles indicating outliers, stars indicating extremes and middle points indicating median values (cont'd).



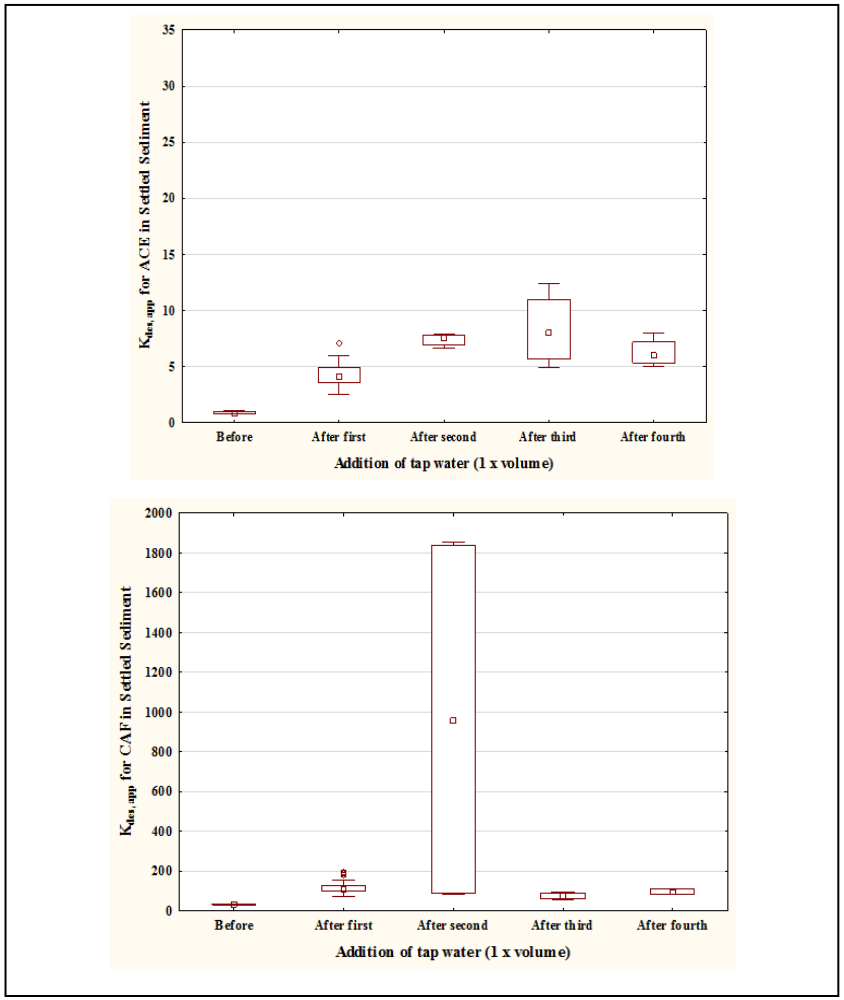
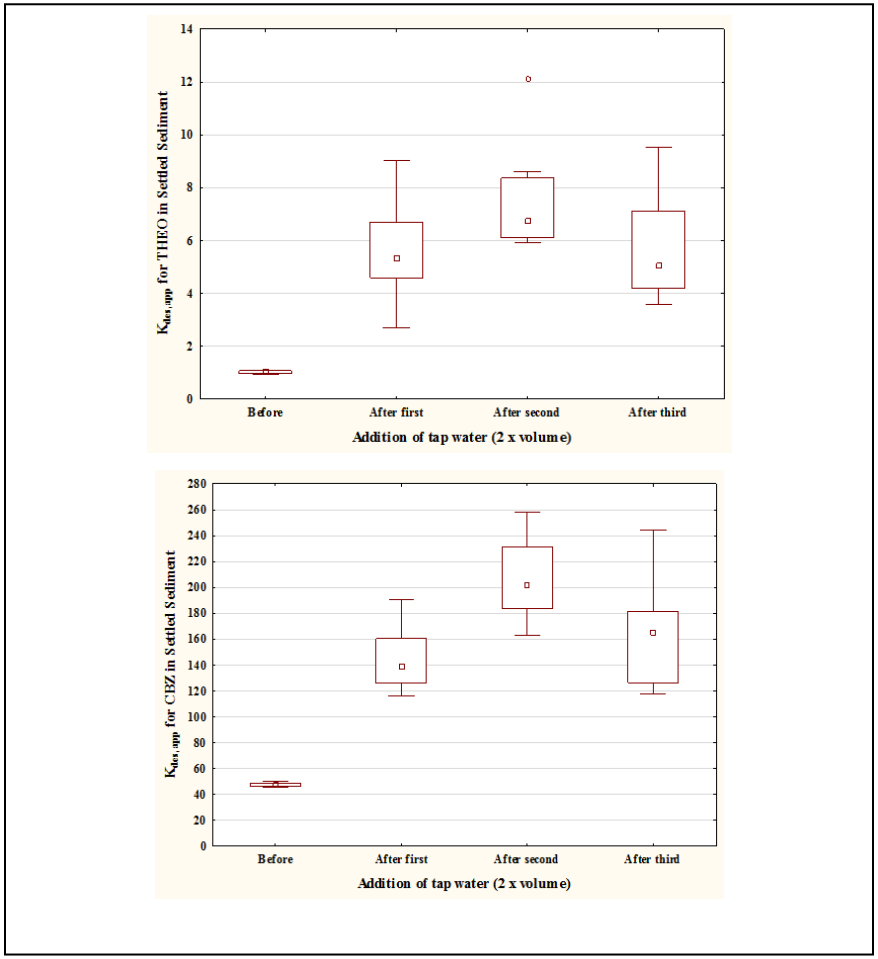


Figure 5.13. Box plots of the solid water distribution coefficients ($K_{des, app}$, L kg^{-1}) during sequential desorption in two different matrices (SS and Settled Sediment) with shaking (on the left) and without shaking (on the right), bars indicating 25/75 percentiles, whiskers indicating minimum and maximum values, circles indicating outliers, stars indicating extremes and middle points indicating median values (cont'd).



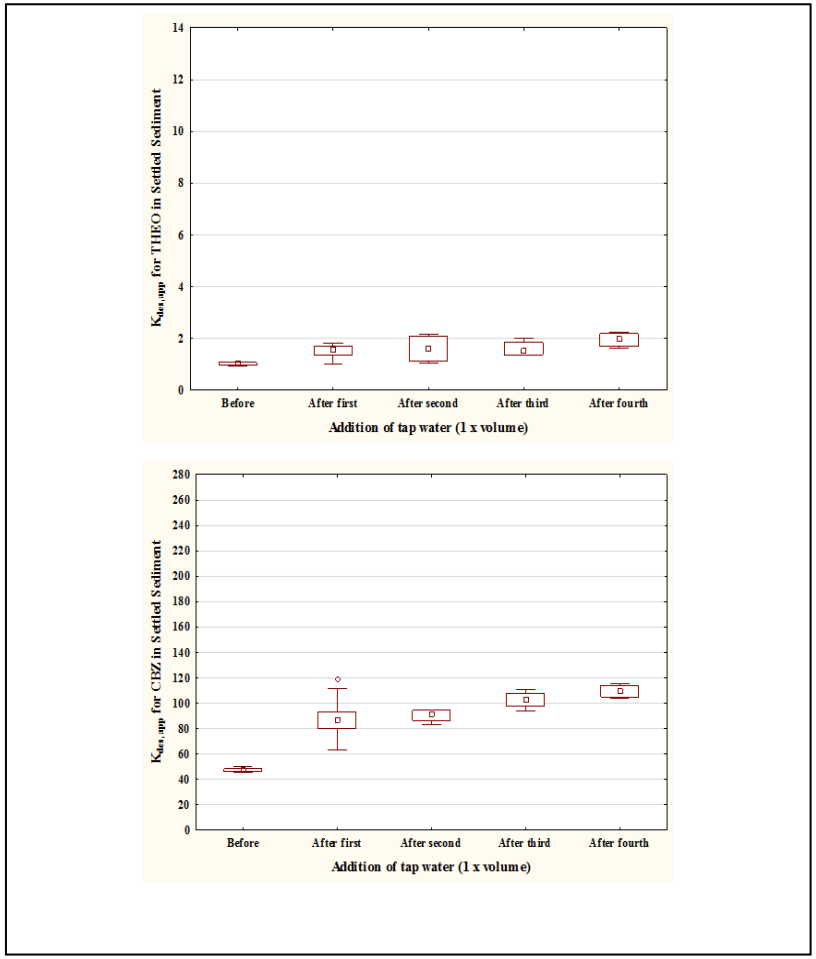


Figure 5.13. Box plots of the solid water distribution coefficients ($K_{des, app}$, L kg^{-1}) during sequential desorption in two different matrices (SS and Settled Sediment) with shaking (on the left) and without shaking (on the right), bars indicating 25/75 percentiles, whiskers indicating minimum and maximum values, circles indicating outliers, stars indicating extremes and middle points indicating median values (cont'd).

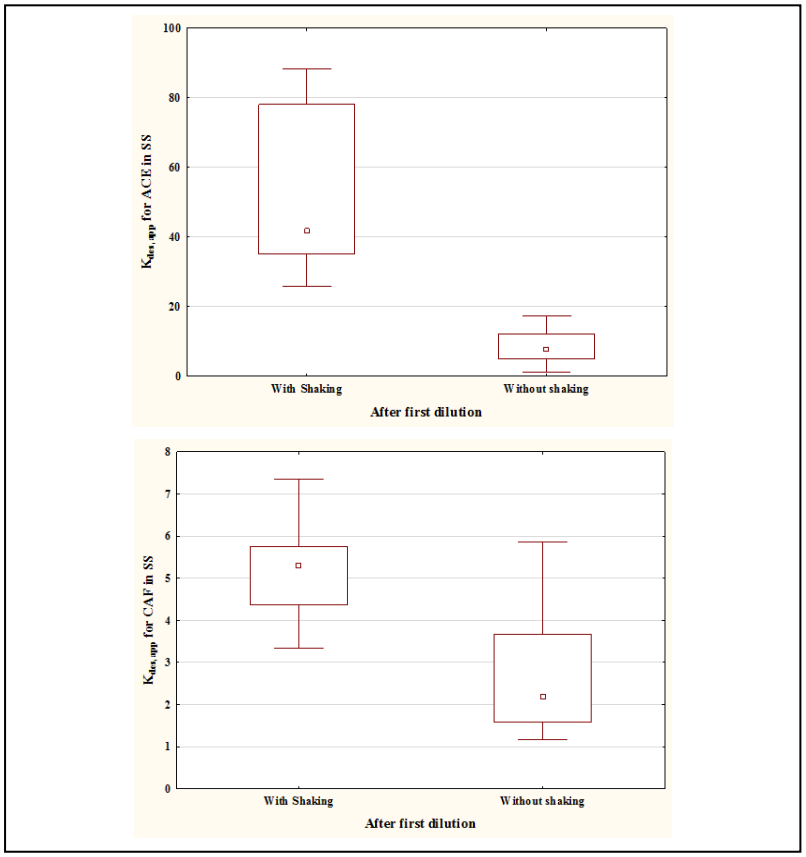
Table 5.14. *P* values of Kruskal wallis test comparing K_d of selected compounds in non-spiked reactors (F and G) before and after flushing under two conditions (with and without shaking).

***P* (Kruskal wallis test), Desorption without spiking**				
Compound	**SS**		**Settled Sediment**	
	Before, after first, second, third, and fourth dilution			
	Without shaking	**With shaking**	**Without shaking**	**With shaking**
ACE	0.00007	0.00000	0.00009	0.0008
CAF	0.0004	0.00000	0.0022	0.00000
THEO	0.0004	0.00002	0.0113	0.0013
CBZ	0.00006	0.00000	0.0006	0.00004
	Before and after first dilution			
	Without shaking	**With shaking**	**Without shaking**	**With shaking**
ACE	0.2152	0.0019	0.0019	0.0019
CAF	0.0019	0.0019	0.0019	0.0019
THEO	0.1632	0.0019	0.0042	0.0019
CBZ	0.0019	0.0019	0.0019	0.0019
	After first and second dilution			
	Without shaking	**With shaking**	**Without shaking**	**With shaking**
ACE	0.0019	0.00005	0.0025	0.1545
CAF	0.5355	0.0027	0.6985	0.4158
THEO	0.0032	0.0838	0.8162	0.0221
CBZ	0.0019	0.00005	0.4386	0.0002

Table 5.14. *P* values of Kruskal wallis test comparing K_d of selected compounds in non-spiked reactors (F and G) before and after flushing under two conditions (with and without shaking) (cont'd).

***P* (Kruskal wallis test), Desorption without spiking**				
Compound	**SS**		**Settled Sediment**	
	After second and third dilution			
	Without shaking	**With shaking**	**Without shaking**	**With shaking**
ACE	0.7728	0.5403	-	0.0221
CAF	0.0209	0.0012	0.0833	0.0004
THEO	0.0209	0.0071	0.7728	0.0371
CBZ	0.0209	0.0120	0.0833	0.0168
	After third and fourth dilution			
	Without shaking		**Without shaking**	
ACE	0.2482		0.3865	
CAF	0.0209		0.2482	
THEO	0.0209		0.1489	
CBZ	0.0209		0.1489	

Comparison of desorption coefficients among selected pharmaceuticals



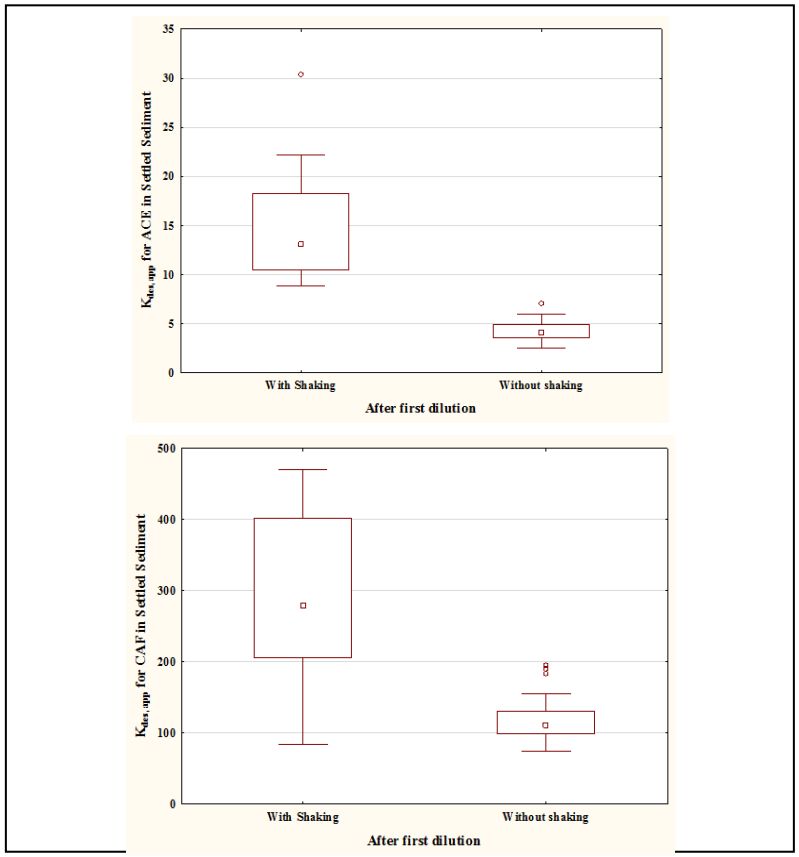
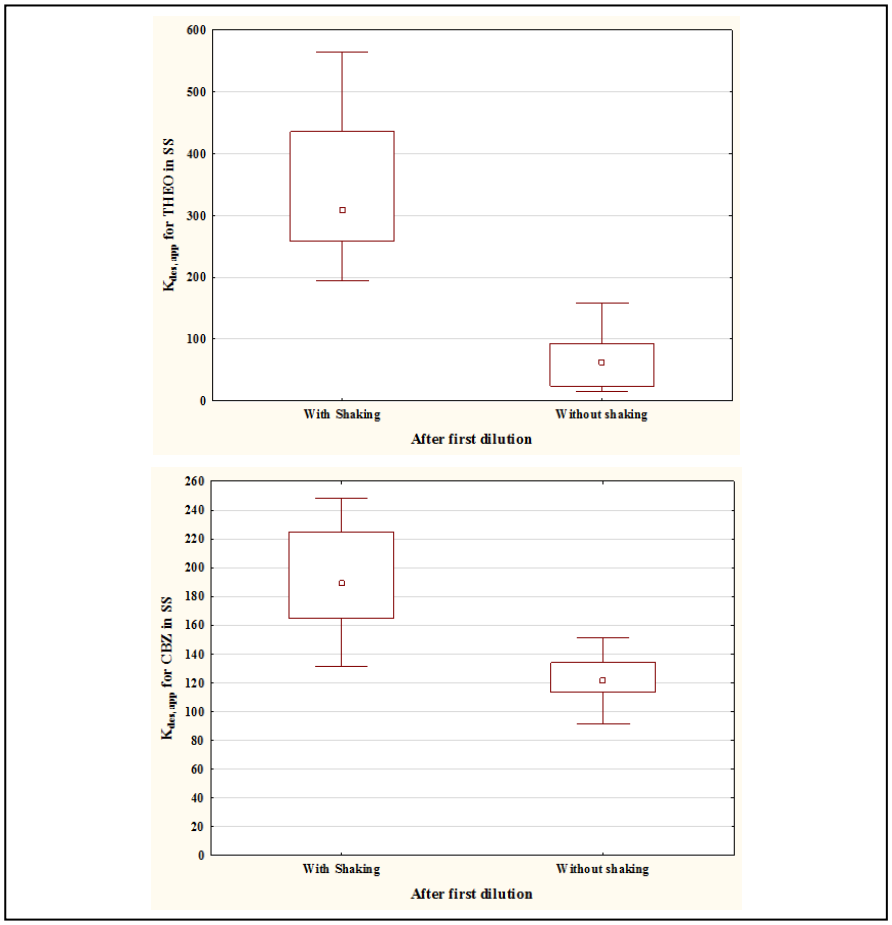


Figure 5.14. Box plots to compare distributions of pharmaceuticals after the first dilution with shaking (2x dilution) and without shaking (1x dilution) in SS (on the left) and Settled Sediment (on the right).



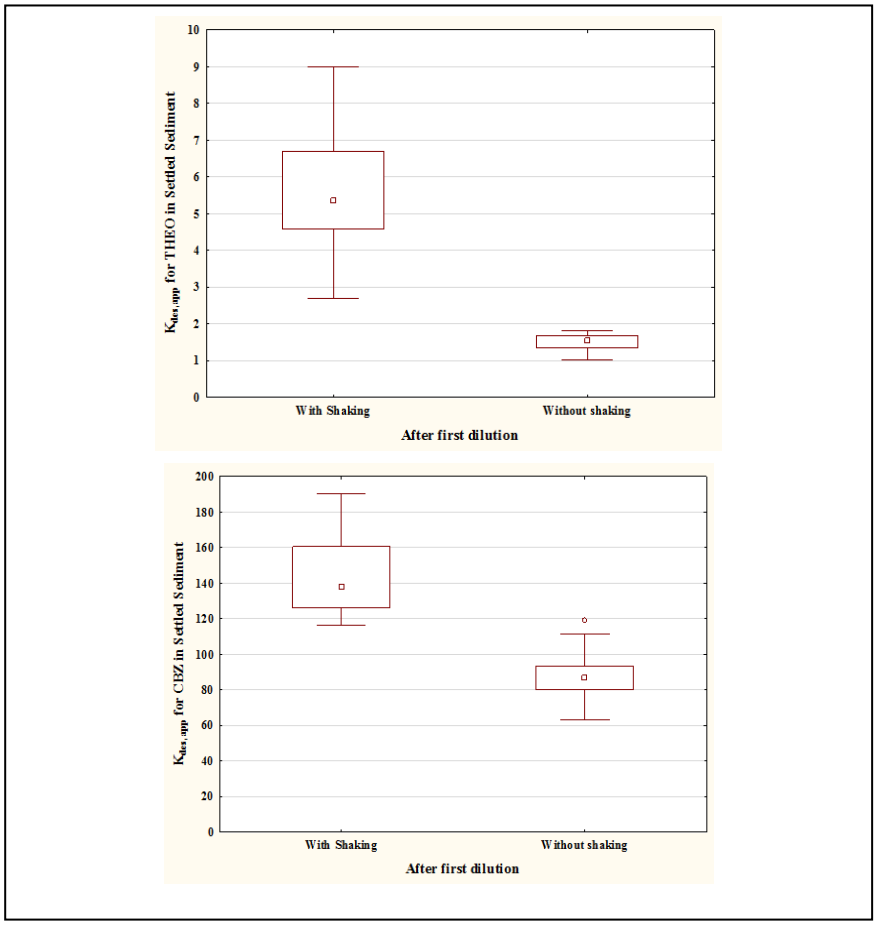
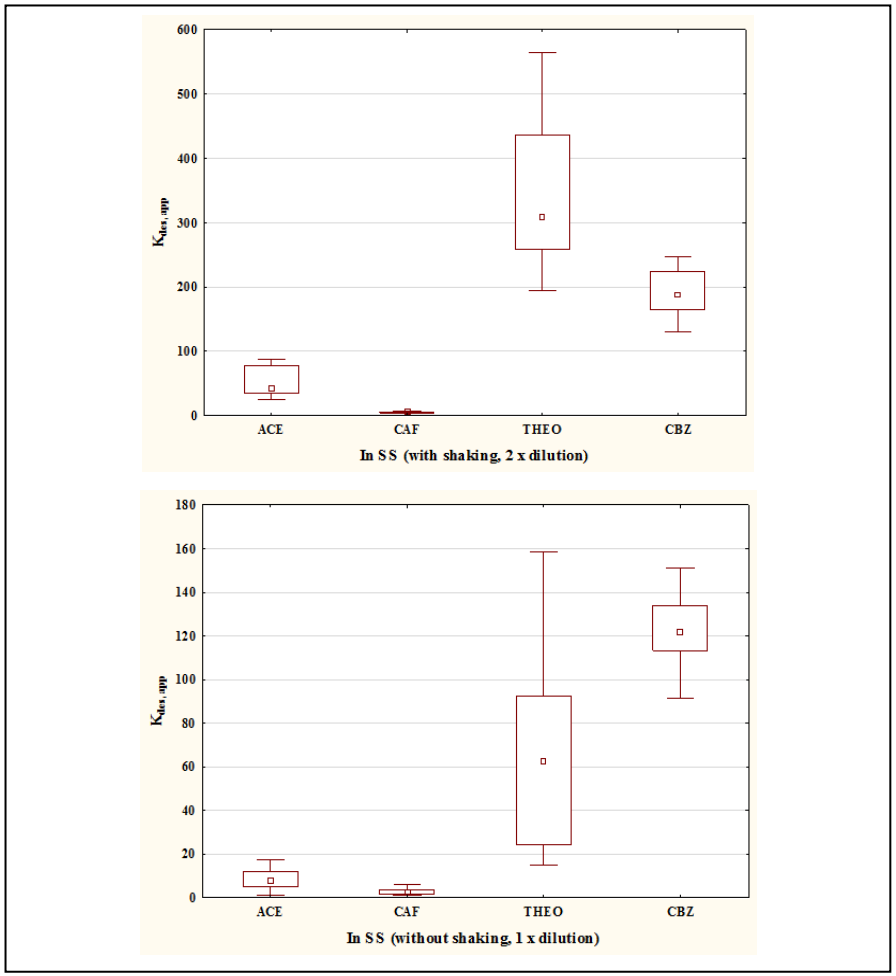


Figure 5.14. Box plots to compare distributions of pharmaceuticals after the first dilution with shaking (2x dilution) and without shaking (1x dilution) in SS (on the left) and Settled Sediment (on the right) (cont'd).



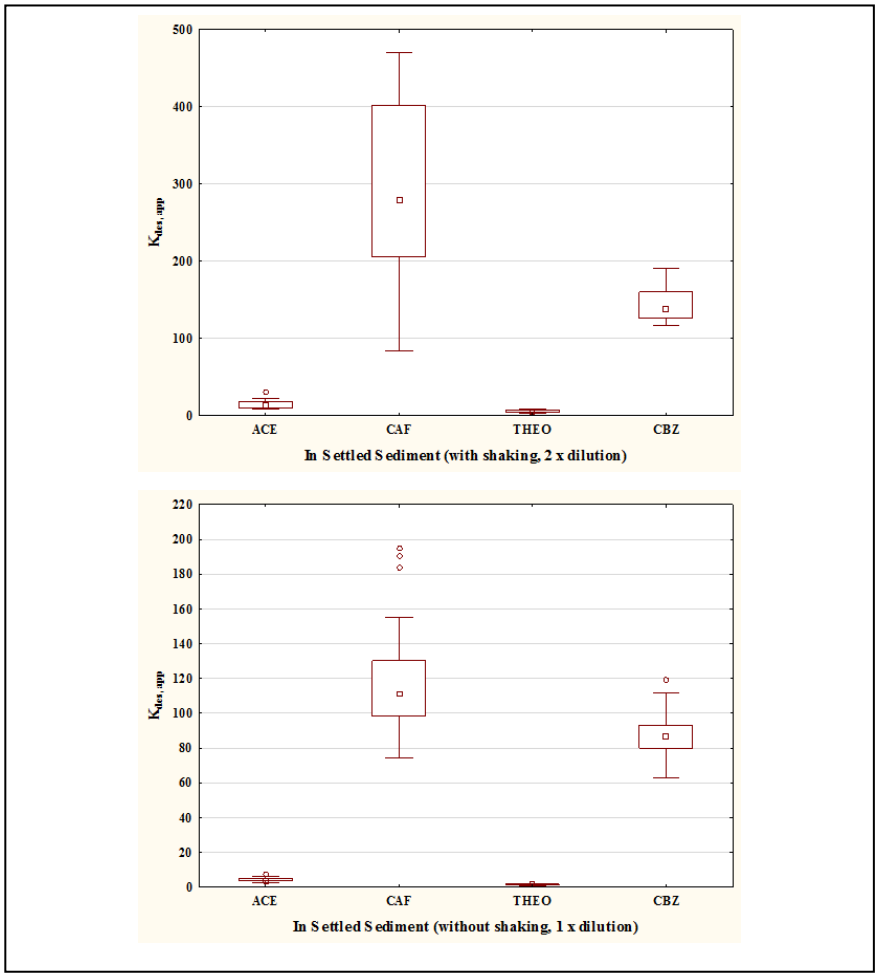


Figure 5.15. Box plots to compare distributions between pharmaceuticals after the first dilution with shaking (2x dilution) and without shaking (1x dilution) in SS (on the left) and Settled Sediment (on the right).

Sorption kinetics in spiked batch reactors (Reactors A and B)

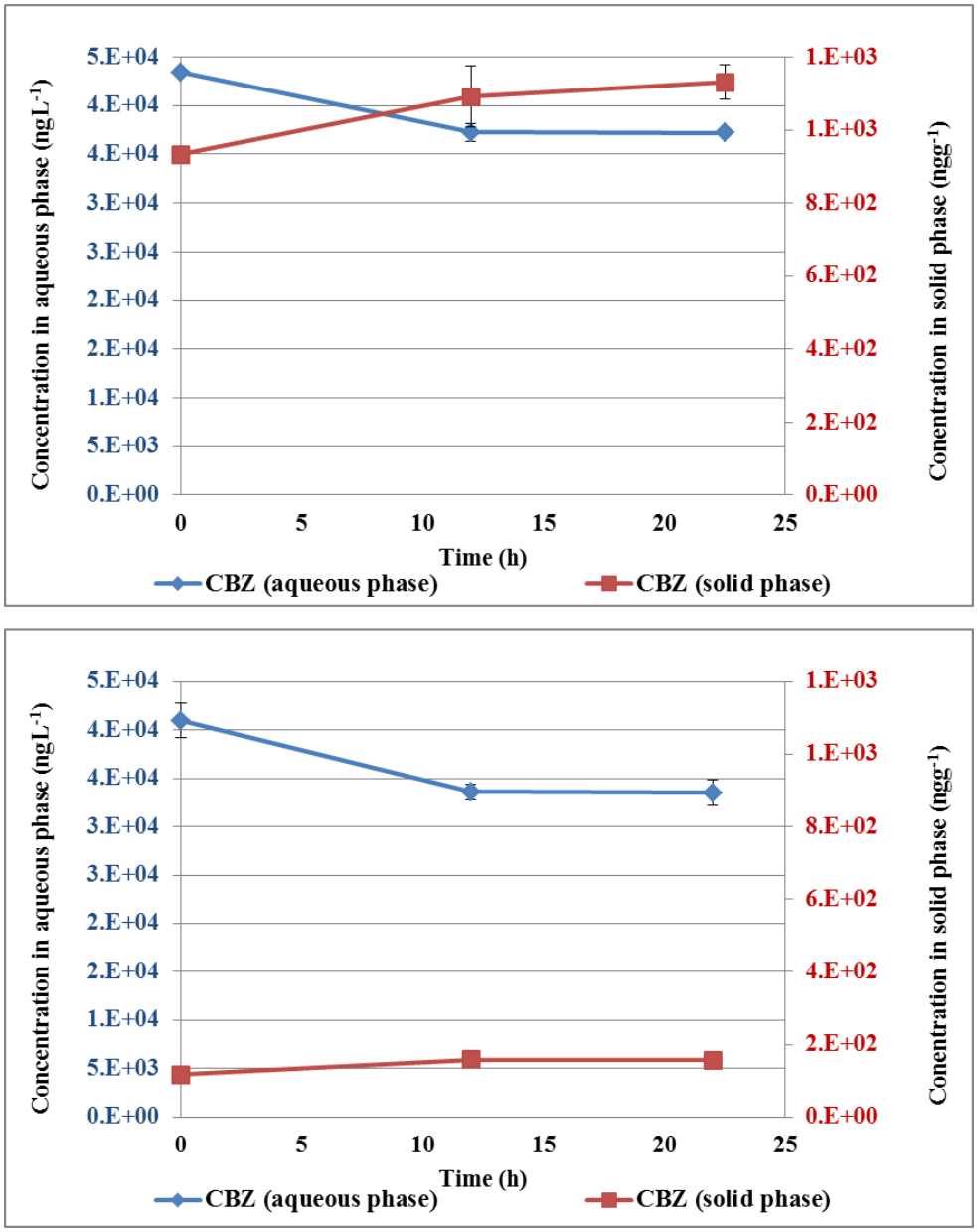


Figure 5.16. Adsorption kinetics of CBZ (Reactors A) on SS (top) and Settled Sediment (bottom), error bars indicating standard deviations (n=2). X-axis indicates the time (h); Y-axis indicates the concentration of compound in aqueous phase (ng L^{-1}) and solid phase (ng g^{-1}).

At adsorption equilibrium, concentrations of selected compounds should be higher in the solid phase and lower in the aqueous phase as compared to their initial concentrations at the time of spiking.

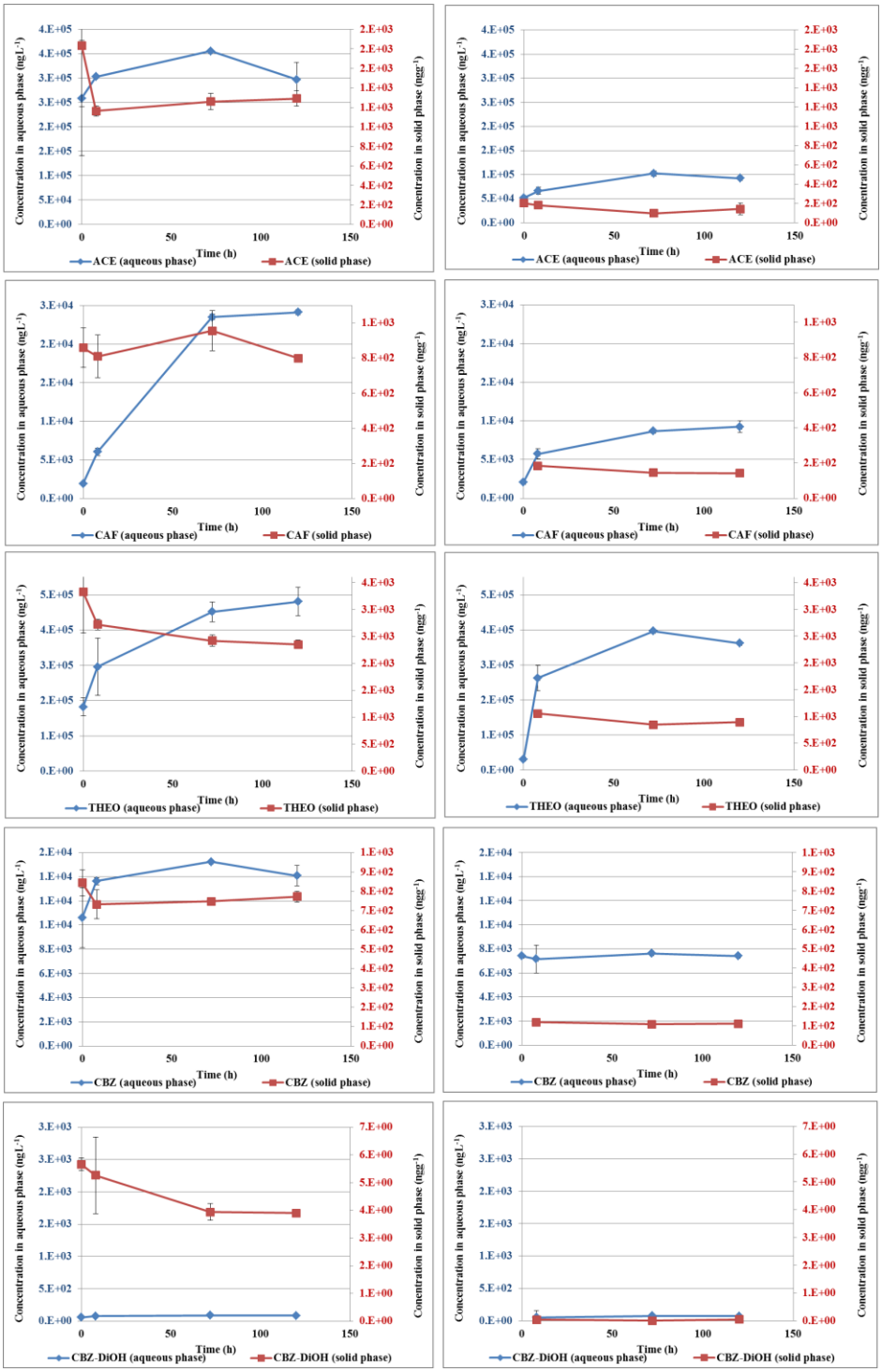


Figure 5.17. Desorption of selected pharmaceuticals as a function of time from spiked SS (column on the left) and StS (column on the right) in Reactor B, error bars indicating standard deviations (n=2). X-axis indicates the time (h); Y-axis indicates the concentration of compound in aqueous phase (ng L^{-1}) and solid phase (ng g^{-1}).

Desorption rate constants

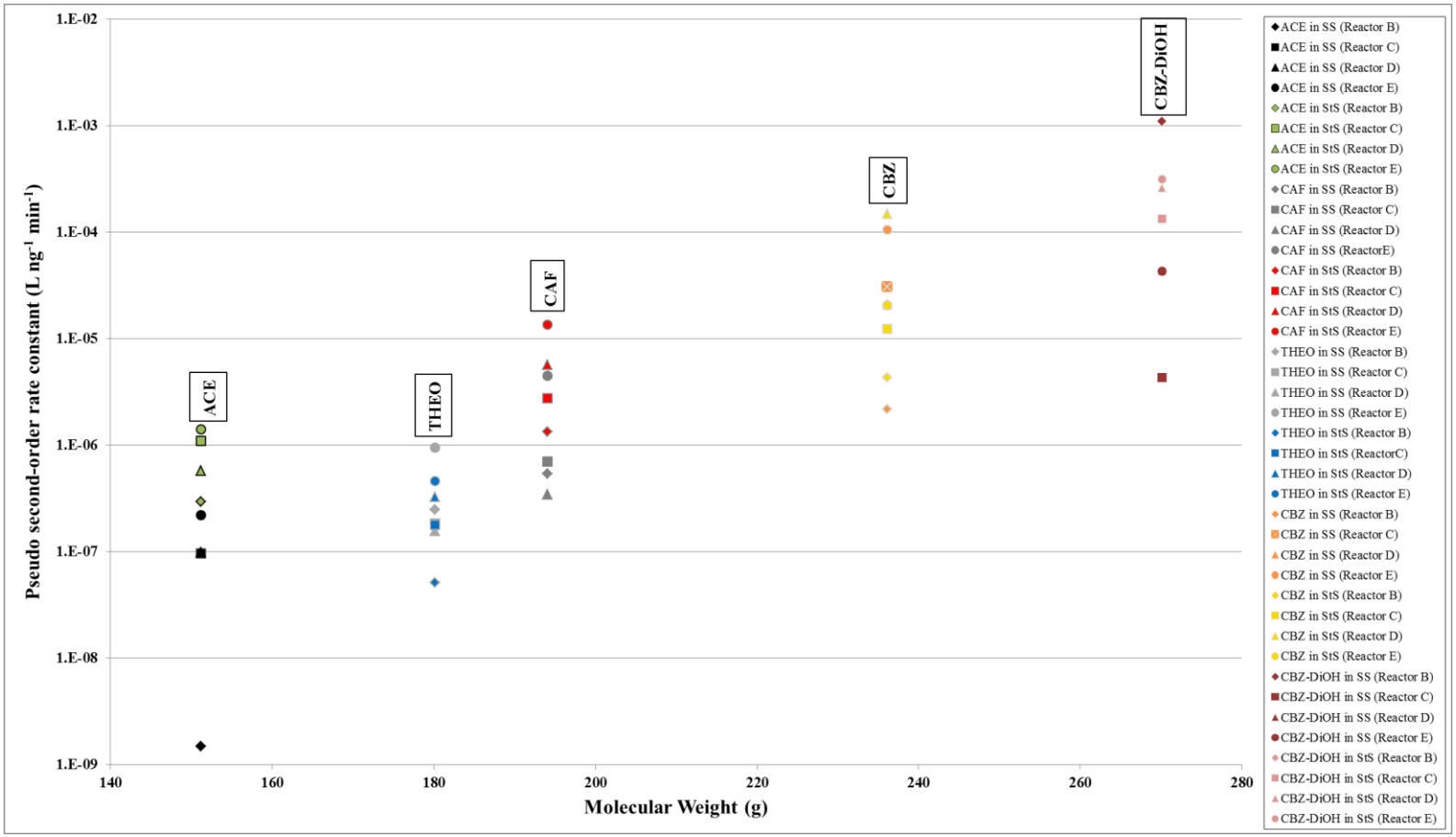


Figure 5.18. Desorption rate constants of pharmaceuticals as a function of their molecular weights.

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CHAPITRE 6 ARTICLE 3 : FECAL CONTAMINATION OF STORM SEWERS: EVALUATING WASTEWATER MICROPOLLUTANTS, HUMAN-SPECIFIC *BACTEROIDES* 16S rRNA, AND MITOCHONDRIAL DNA GENETIC MARKERS AS ALTERNATIVE INDICATORS OF SEWER CROSS CONNECTIONS

Ce chapitre présente le dépistage des sources de contamination fécale humaine dans une section de l'infrastructure hydrique dans la région métropolitaine de Montréal. Cette section est caractérisée par un système d'égouts séparés et affectés par le problème des raccordements inversés qui se produit lorsque les égouts sanitaires sont branchés ou lorsqu'ils s'écoulent dans les égouts pluviaux, entraînant alors une contamination fécale des cours d'eau urbains. En regard des travaux présentés dans ce chapitre, deux objectifs ont été ciblés. Le premier était d'effectuer une analyse de corrélation entre le niveau de marqueurs traditionnels (*Escherichia coli* et coliformes fécaux) et alternatifs spécifiques aux humains (génétiques (l'ARN ribosomal 16s de *Bacteroides* (HF183) et l'ADN mitochondrial (Hmt)) et chimiques (l'acétaminophène (ACE), la caféine (CAF), la théophylline (THEO) et la carbamazépine (CBZ)) de contamination fécale. Ceci permettrait de fournir des éléments qui pourront démontrer que les marqueurs alternatifs sont aussi efficaces, voire complémentaires lors de la détection des sources de contamination fécale humaine. Le deuxième objectif était de développer un indice de contamination sanitaire basé sur des marqueurs alternatifs qui pourrait permettre de faire des recommandations aux municipalités pour définir leurs priorités en matière d'infrastructures.

HF183, Hmt, ACE, CAF et THEO ont été proposés comme candidats traceur appropriés pour l'identification de raccordements inversés. Leur fiabilité a surtout été démontrée dans les secteurs résidentiels. Les résultats obtenus au cours de ces travaux de recherche ont été soumis à *Water Research* en février 2016.

FECAL CONTAMINATION OF STORM SEWERS: EVALUATING WASTEWATER MICROPOLLUTANTS, HUMAN-SPECIFIC BACTEROIDES 16S rRNA, AND MITOCHONDRIAL DNA GENETIC MARKERS AS ALTERNATIVE INDICATORS OF SEWER CROSS CONNECTIONS

M. Hajj-Mohamad[a,b*], M. Hachad[a], G. Deschamps[c], S. Sauvé[d], R. Villemur[e], M-A. Blais[e], M. Prévost[b], S. Dorner[a]

[a] Canada Research Chair in Source Water Protection, Department of Civil, Geological and Mining Engineering, Polytechnique Montréal, C.P. 6079, Succursale Centre-ville, Montréal, Qc, Canada H3C 3A7; [b] NSERC Industrial Chair in Drinking Water Treatment, Department of Civil, Geological and Mining Engineering, Polytechnique Montréal, C.P. 6079, Succursale Centre-ville Montréal, Qc, Canada H3C 3A7; [c] Service du Développement et des Opérations, Direction de L'environnement et du Développement Durable, Division de la Planification et du Suivi Environnemental, Réseau de Suivi du Milieu Aquatique, Ville de Montréal, Montreal, QC, Canada H2M 2T8; [d] Department of Chemistry, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, QC, Canada H3C 3J7; [e] INRS-Institut Armand-Frappier, Institut National de la Recherche Scientifique, Laval, QC, Canada H7V 1B7; *Corresponding author. Phone: +1 514 340-4711 ext. 2983, e-mail: mariam.hajj-mohamad@polymtl.ca

6.1 Abstract

A set of fecal indicator bacteria (thermotolerant (fecal) coliforms (FC) and *Escherichia coli* (*E. coli*)), and alternative markers (human-specific *Bacteroidales* (HF183) marker and human mitochondrial DNA (Hmt) marker, carbamazepine (CBZ), caffeine (CAF), theophylline (THEO) and acetaminophen (ACE)) were tested for their use as tracers to find illicit household sewage connections into storm drainage systems. A high incidence of human fecal contamination was observed, illustrating the need for a method to appropriately prioritize sectors for the rehabilitation of sewer cross-connections. Concentrations of alternative markers were not significantly different between the residential and industrial/commercial/institutional (ICI) sectors. However, median *E. coli* concentrations were higher in the residential as compared to ICI sectors ($p < 0.05$). Hmt marker, CAF, and THEO were well correlated to *E. coli* in the ICI sector, whereas all alternative markers were not in the residential sector, possibly as a result of higher *E. coli* inputs from other sources such as domestic animals or fauna. All alternative markers were correlated to FC only in the ICI sector, except CBZ. Thresholds were determined to relate alternative markers to *E. coli* for sewer cross-connection decision-making. An arbitrary threshold of 3 Log_{10} copies HF183 100 mL^{-1}, 2 Log_{10} copies Hmt 100 mL^{-1}, 2 Log_{10} ng CAF L^{-1}, 2 Log_{10} ng THEO L^{-1}, and 1 Log_{10} ng ACE L^{-1} allow us to identify sites with elevated fecal

contamination, as defined by more than 235 MPN 100 mL^{-1} of *E. coli*. HF183, Hmt, CAF, THEO, and ACE were identified as suitable markers for identifying sewer cross-connections and are more reliable than *E. coli* alone, most importantly in residential sectors.

6.2 Introduction

In urban areas, high loads of fecal indicator bacteria (FIB) and pathogens are often discharged into streams and rivers through urban infrastructure, even in dry weather (Sercu et al., 2009; Wu, Rees, et al., 2011). Urban streams with high concentrations of FIB may require municipalities to investigate corrective actions for reducing fecal loading when environmental concentrations do not meet regulatory requirements. Many cities have programs to investigate and correct cross-connections between sanitary sewers and storm drains. Control programs have typically been based on visual inspection and grab sampling followed by laboratory analysis, including the enumeration of culturable fecal coliforms (FC) and/or *Escherichia coli* (*E. coli*) (Figueras et al., 2000; MAMROT, 2000). A threshold of 12000 *E. coli* (CFU) 100 mL^{-1}, was proposed as indicative of wastewater contamination in storm sewers (Pitt, 2004). However, there is generally no clear indication of sources of FIB in storm drains that could be the result of resident animal populations, sewer cross-connections or sanitary sewer exfiltration (Sercu et al., 2009) that flows through unsaturated soil into storm drains (Schirmer et al., 2013). The ability to reliably identify the origin of fecal pollution is critical for the timely and cost-effective management of remediation efforts (Seurinck et al., 2005). Therefore, it is important to investigate other water quality indicators and use a reliable method to determine the source of fecal pollution (Okabe et al., 2007). Various studies have reported on chemical and biological indicators to distinguish between human and animal contamination in water from storm sewer outfalls (Daneshvar et al., 2012; Haack et al., 2009; Sauvé et al., 2012).

Although relationships between land-use classes and fecal contamination may suggest their potential sources (Young & Thackston, 1999), there are limitations using traditional FIB for identifying sewer cross-connections. FC and *E. coli* can survive and grow after being released into the receiving water and can become temporally inactive in some cases (Byappanahalli et al., 2003; Desmarais et al., 2002; Luo et al., 2011; Stevens et al., 2004; Tallon et al., 2005). Moreover, they are often poorly correlated with the presence of pathogenic microorganisms such as *Cryptosporidium*, *Salmonella* or *Campylobacter spp.* (Harwood et al., 2005; Wu, Long, et al.,

2011). Non-fecal sources can be associated with the coliform group (Gavini et al., 1985; Whitman et al., 2003) that remain present in the natural water environment (Desmarais et al., 2002; Ishii et al., 2006; Solo-Gabriele et al., 2000; Walk et al., 2007) with widely varying survival rates (Anderson et al., 2005), making it difficult to distinguish between fecal bacteria associated with recent contamination events and those found in the natural environment (Badgley et al., 2010; Whitman et al., 2003).

Combining genetic and chemical indicators having differential environmental persistence, fate and transport, enhance fecal pollution detection through a multiple lines of evidence approach and inform remediation decisions (Haack et al., 2009; Sidhu et al., 2013). There have been limited studies concerning the coexistence of host-specific mitochondrial DNA with other fecal indicators in aquatic environments (Kapoor et al., 2013; Villemur et al., 2015). Furthermore, the relationships between the occurrence of mitochondrial DNA and chemical fecal indicators (caffeine (CAF), acetaminophen (ACE), carbamazepine (CBZ), and theophylline (THEO)) remain uncharted. Before these newer approaches can be widely used for targeting storm sewers for rehabilitation of cross-connections, more information is needed regarding the relations between chemical, microbial, and biomolecular indicators of fecal pollution sources.

Given the variety of methods that are available for detecting sewer cross-connections, there is a need for method validation and guidance on their application (Panasiuk et al., 2015). Our main objective was to determine the most appropriate human fecal indicators among a suite of genetic and chemical markers to identify cross connections between sewer lines and storm drains considering measured concentrations, detection limits, and land-use. The secondary objective was 1) to investigate the relationships among the concentrations of thermotolerant coliforms, *E. coli*, wastewater microplollutants (WWMPs) such as ACE, CAF, THEO, and CBZ, and human-specific genetic markers (HF183 *Bacteroides* 16S ribosomal RNA (rRNA) gene sequence and human mitochondrial DNA (Hmt)), 2) to confirm a standard reference value of CAF (400 ng L^{-1}) proposed by Sauvé et al. (2012) as a threshold for the prioritization of sectors with cross-connections for remedial action, and 3) to propose threshold reference values of alternative markers for an index for the identification of cross-connections and the prioritization of drainage basin sectors for remedial work.

6.3 Materials and methods

6.3.1 Watersheds Description

MEA and DEN Creek watersheds are located in the Greater region of Montréal and have separate storm water systems. The MEA and DEN watersheds cover approximately 11.8 and 4.7 km^2, respectively. Both creeks drain to a fluvial lake used as a source of drinking water in the region. The MEA Creek is almost entirely channelized and covered. However, it is open along a busy public park at which dogs and their feces were observed. Most of the MEA Watershed has been developed, and over 90 percent of the development is residential. Commercial/Institutional and industrial activities account for an additional 8 percent. Other urban spaces, including parks and schools, comprise another 2 percent; the remainder is open space (Figure 6.4). The DEN Creek is an urban creek passing through industrial, residential and airport areas before flowing into the fluvial lake.

During dry weather, MEA and DEN Creeks have little or no flow. Provincial water quality standards for bacteria during dry or wet weather have generally not been met. Fecal coliforms and *E. coli* analyses in this area conducted by the municipality have suggested that the high concentrations of indicator bacteria measured in dry weather were primarily the result of cross-connections to storm drains.

6.3.2 Sampling sites

A total of 30 sampling points were selected, 11 within the MEA Creek Watershed and 19 within the DEN Creek watershed. Sites were chosen based on potential impact from human fecal pollution by cross connections (see Table 6.5 in Supporting Information†[2]). The majority of sampling locations (25/30) represented independent branches within the sewer network.

6.3.3 Sample collection

Sampling was conducted during dry weather conditions (less than 0.1 inches within the previous 72 hours) to prevent runoff from interfering with results. Sampling was conducted downstream to

[2] † Electronic Supporting Information (ESI) is available in Section 5 of this chapter.

upstream. From the outfall to the most upstream storm water manhole, samples were collected between 8:00 a.m. and 15:00 p.m. in sterile polypropylene bottles and transported on ice the samples to the laboratories and processed within 2 h of collection. In total, 30 water samples from 2 watersheds were obtained in summer 2012 and tested for the presence of FC colonies and *E. coli* by culture, general *Bacteroidales* (GenBac) by PCR, human-specific *Bacteroides* 16S rRNA gene sequence (HF183) and human-specific mitochondrial DNA (Hmt) by qPCR, and WWMPs by SPE-LC-ESI-MS/MS. Water samples were separated in three and sent in different laboratories (Villemur's laboratory for Hmt and HF183 markers, Sauvé's laboratory for WWMPs, and Dorner's laboratory for remaining analyses).

6.3.4 Analyses of conventional and alternative fecal indicators

FCs were analysed using the standardized membrane filtration method (APHA et al., 1998; Centre d'expertise en analyse environnementale du Québec, 2011). The detection limit (DL) for this method is 1 CFU (colony forming units) per volume or dilution filtered.

The enumeration of *E. coli* was performed by using the Colilert-18/Quanti-Tray system/2000 (IDEXX, Portland ME, USA). Results were reported as MPN per 100 mL. The most probable number (MPN) could be estimated from a chart provided by the manufacturer as per the Environmental Analysis Center of Québec (Centre d'expertise en analyse environnementale du Québec, 2009). 1 MPN 100 mL^{-1} for water was used as minimal detectable threshold for statistical validation.

Methodology for DNA extraction, and PCR detection of the *Bacteroidales* general marker (BAC32F/BAC708R) was previously described in detail (Besner et al., 2010). Results were expressed as presence/absence data. Detection (absence/presence) and quantification of the human-specific HF183 marker and human-specific mitochondrial marker (Hmt) using previously validated methods were carried out in the Villemur laboratory as described in (Villemur et al., 2015). The DLs were 100 copies 100 mL^{-1}.

The four WWMPs selected for the study (CAF, CBZ (an anti-seizure drug primarily excreted in the faeces (about 70%)), THEO, used for the treatment of bronchial asthma, present in soft drinks and also a metabolite of CAF, and ACE, an analgesic) were analysed by an on-line solid-phase extraction combined with liquid chromatography electrospray tandem mass spectrometry with

positive electrospray ionisation (SPE-LC-ESI-MS/MS) first developed by Viglino, Aboulfadl, Daneshvar, et al. (2008). Detailed information on the analytical method is available in Sauvé et al. (2012). DLs were estimated as three times the standard deviation of 5 replicate measurements of a field sample and were 9 ng L^{-1} for CAF, 0.2 ng L^{-1} for CBZ, 6 ng L^{-1} for THEO and 10 ng L^{-1} for ACE. All samples were analyzed in duplicate. For each event, laboratory blanks and field blanks were analyzed. All blank values were below DLs.

6.3.5 Data processing and statistical analysis

Goodness-of-fit tests were performed by ProUCL version 5.0.00 to determine whether or not data were normally distributed and to address values below the detection limit (estimated values by ProUCL Software). Following log transformation, data followed a normal distribution. Therefore, a parametric test (Pearson's correlation) was used to assess relationships among indicators. A *p* value of < 0.05 was considered significant. Pearson's correlation coefficients (*r*) were determined for the data sets. To assess the impact of surrounding land use on concentrations of fecal markers in watersheds, the Kruskal-Wallis ANOVA test was used. All statistical tests were performed with statistical software (STATISTICA 12 - Ultimate Academic Bundle, StatSoft Inc., Tulsa OK, USA).

6.3.6 Index for the prioritization of drainage basin sectors with cross-connections

A simple index was calculated based upon alternative specific marker thresholds to classify each marker concentration as being low, average, or high. Thresholds values were determined from measured concentrations. Values of the calculated index for each site ranged from 0-1 with 1 representing sites with strong evidence of human fecal contamination. The index was calculated as follows:

$$Index = \sum_{i=1}^{n} \frac{marker_i}{n}$$

Where n is the number of alternative human specific markers used (6 in this study), and the values of $marker_i$ were 0 if its measured concentration was classified as low, 0.5 if medium, and 1

if high. All markers were given equal weight, although this index could be modified according to the specific marker measurements available and the thresholds modified to reflect local conditions that could be affected by dry weather inflows from groundwater infiltration and cooling waters.

The index enabled a classification of sites into low (index < 0.4) medium (0.4-0.6), and high (> 0.6) priority. We considered that high priority sites were sites contaminated by cross-connections, whereas medium priority sites demonstrated mixed sources of contamination. Low priority sites were either dominated by wild or domestic animal sources of fecal contamination or had low levels of mixed fecal contamination.

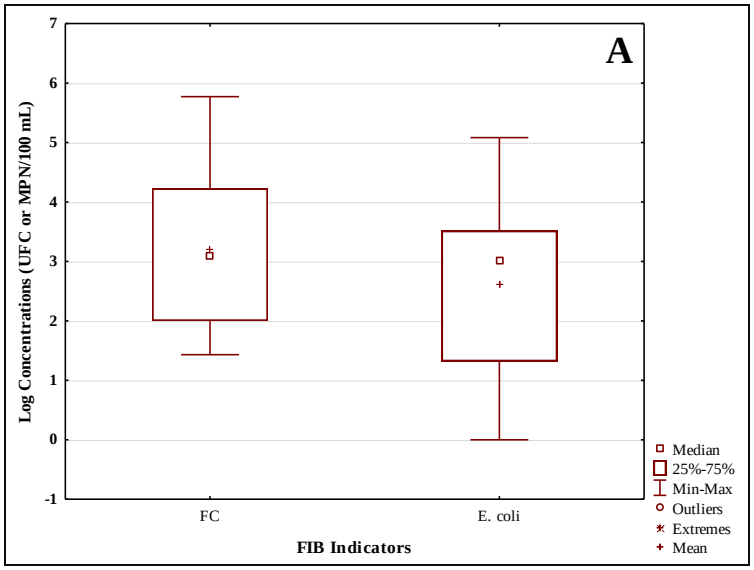
6.4 Results and discussion

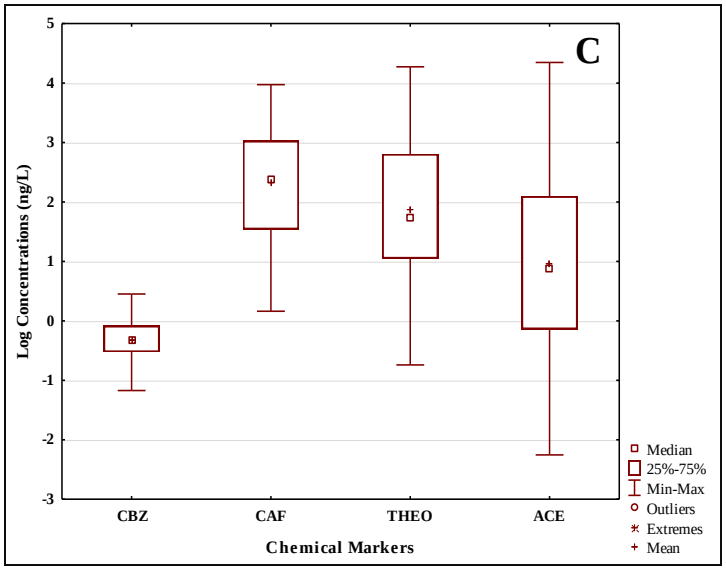
6.4.1 Occurrence and concentrations of markers in water samples

E. coli and FC were detected in 100% of samples from both watersheds (n=11+19, MEA and DEN, respectively). GenBac, HF183, and Hmt were detected in 11/11 (100%), 10/11 (91%) and 10/11 (91%) of MEA watershed samples, and 17/19 (89%), 8/19 (42%) and 11/19 (58%), respectively, of DEN watershed samples (Table 6.6). WWMP markers were present in all samples, demonstrating that sewage commonly enters stormwater systems in urban sectors and is a major source of fecal contamination in storm sewers as was also observed by Sauer et al. (2011). Although all samples were positive for WWMPs, 7/30, 1/30, 4/30 and 19/30 of samples had concentrations below the detection limit for CBZ, CAF, THEO and ACE, respectively. GenBac, HF183, and Hmt detection was found in 100%, 79% and 79% of the water samples from residential sectors (n=14), respectively. It was found in 88%, 44% and 63% of the water samples from industrial/commercial/institutional (ICI) sectors (n=16), respectively. For the 5 sites representing downstream to upstream samples, no increasing or decreasing trend was observed, as concentrations varied as a result of fecal contamination inputs from sewer branches and dilution processes.

Box plots of marker concentrations or relative abundance are shown in Figure 6.1 (see also Table 6.7 in ESI†). Concentrations of FIB and alternative markers per site (in order of most contaminated to least contaminated) are provided in Figure 6.2. The variability of FIB and marker values reflects the range of pollution loads from fecal inputs and is predominantly related

to the number of cross-connections in a given sector, but is also influenced by groundwater infiltration and the addition of cooling waters. No significant differences in FIB and marker concentrations were observed between the two watersheds. Table 6.8 shows the concentrations of selected alternative specific markers from different potential wastewater sources in previously published studies. Concentrations of CBZ, and CAF were comparable to those of (Sauvé et al., 2012) in storm sewers. Mean WWMP concentrations were between 1 and 2.75 orders of magnitude lower than mean concentrations from wastewater treatment plant influents in the Greater Montréal Area (Madoux-Humery et al., 2013). Mean concentrations of WWMPs are generally lower in storm sewers because dry weather flow consists primarily of groundwater infiltration and cooling waters that do not contain high concentrations of WWMPs. Maximum measured concentrations of WWMPs in the MEA and DEN watersheds were approximately the same as mean wastewater treatment plant influent concentrations (Madoux-Humery et al., 2013) and are strong indicators of cross-connections.





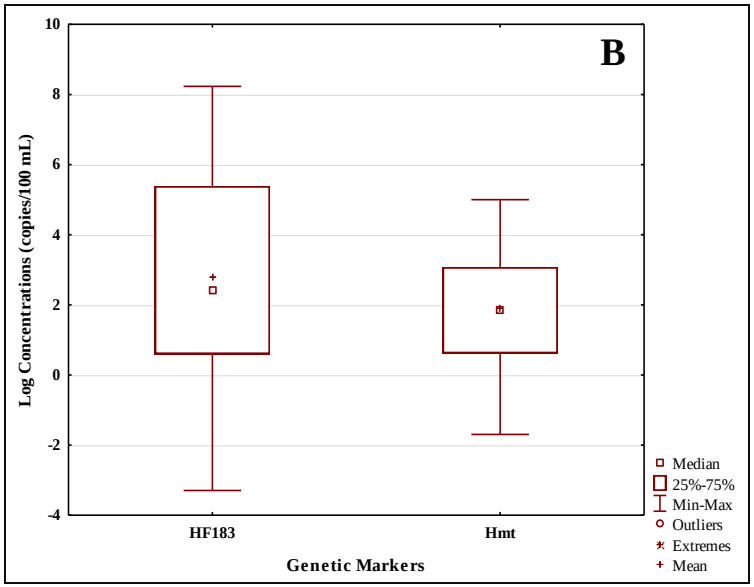


Figure 6.1. Box plots of marker concentrations (FIB indicators (conventional fecal indicator bacteria) (A), genetic markers (B), and chemical markers (C)) in urban watersheds, bars indicating 25/75 percentiles, whiskers indicating minimum and maximum values, circles indicating outliers, stars indicating extremes, middle points indicating median values, and cross indicating mean values.

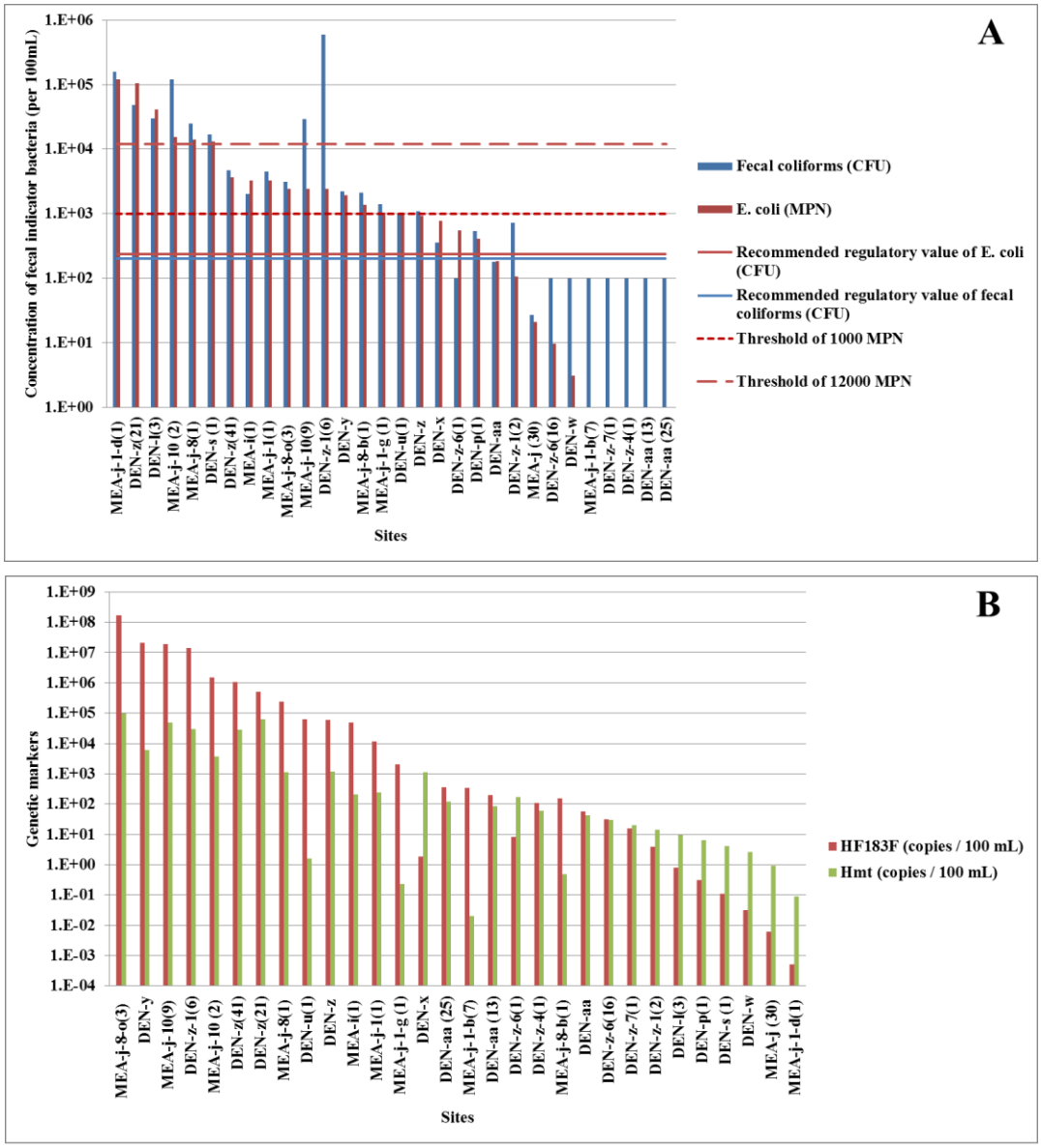


Figure 6.2. Concentrations of FC and *E. coli* (A), HF183 and Hmt (B), and CBZ, CAF, THEO, and ACE (C) per sampling site. The blue and red lines in (A) represent threshold limits for FC at 200 CFU 100 mL^{-1} and *E. coli* at 235, 1000, and 12000 MPN 100 mL^{-1}, respectively.

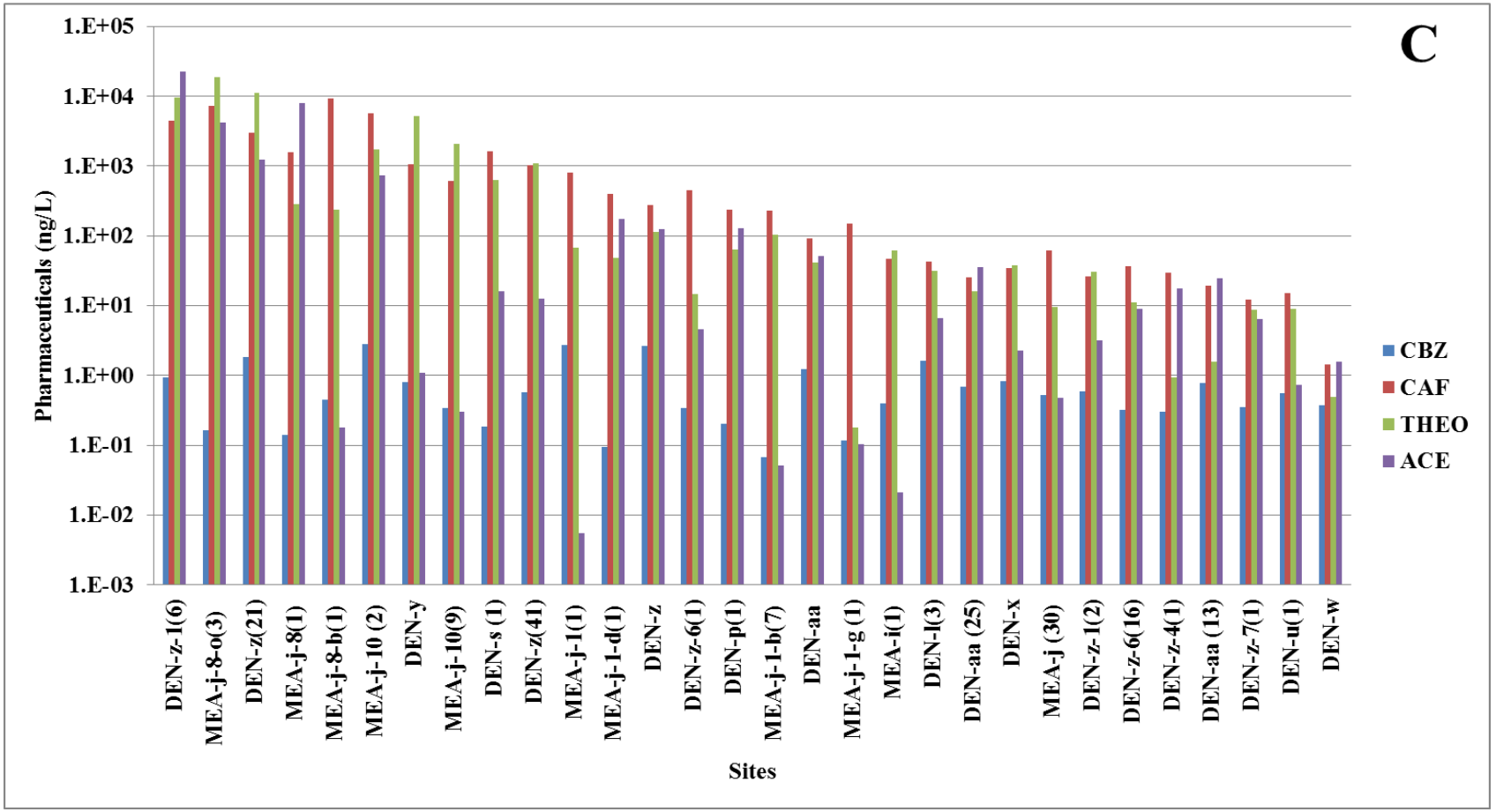


Figure 6.2. Concentrations of FC and *E. coli* (A), HF183 and Hmt (B), and CBZ, CAF, THEO, and ACE (C) per sampling site. The blue and red lines in (A) represent threshold limits for FC at 200 CFU 100 mL^{-1} and *E. coli* at 235, 1000, and 12000 MPN 100 mL^{-1}, respectively (cont'd).

Observed alternative marker concentrations in relation to their detection limits provide a useful indication of their potential use as wastewater tracers (Benotti & Brownawell, 2007; Hajj-Mohamad et al., 2014). Thus, among the WWMP tracers, CAF, THEO and ACE are the more suitable tracers of cross-connections. CBZ is an excellent wastewater tracer in combined sewers to determine the fraction of total flow coming from sewage, but its use as a tracer of sewer cross-connections is more limited given its lower use within the population as compared to ACE or CAF. Although THEO is a metabolite more closely representing fecal contamination as compared to CAF (coffee grinds are not directly related to fecal contamination), both fecal and greywater sources of contamination are present in sewer cross-connections. Among the genetic markers, the frequency of Hmt above detection limits was approximately the same as HF183.

Given that the goal of cross-connected sewer identification is typically to meet regulatory standards for FIB, samples with an elevated fecal contamination were identified as being those with more than 200 colony-forming units per 100 mL (CFU 100 mL^{-1}) of FC which is approximately equivalent to 235 CFU 100 mL^{-1} of *E. coli*, a regulatory value recommended by U.S. EPA for the protection of primary contact recreation in both coastal and non-coastal waters (USEPA, 1986). The combined data for both watersheds showed that 67 % of samples had FC and *E. coli* concentrations greater than 200 or 235 (CFU) 100 mL^{-1}, respectively (Figure 6.2.A), demonstrating widespread fecal contamination of the storm sewer network, common in urban areas (Ellis & Butler, 2015).

Even with very low *E. coli* concentrations (including those with 1 MPN 100 mL^{-1} *E. coli*), sites were positive for the alternative markers. Given that *E. coli* in raw sewage sources are not as quickly diluted to below detection limits as compared to most WWMPs (Guérineau et al., 2014; Madoux-Humery et al., 2013) and the short travel times in these sewer networks, the different excretion patterns of the contaminants could explain this trend. *E. coli* discharges to the sewer are highly dependent on human defecation patterns, with peaks occurring in the very early morning (Heaton et al., 1992), whereas WWMPs excreted with urine will be more widely present and thus more likely to be measured throughout the day (Madoux-Humery et al., 2013).

Passive sampling techniques (Panasiuk et al., 2015) would be recommended in cases where *E. coli* concentrations demonstrate that a fecal contamination source exists yet WWMPs are close to detection limits. Another possibility is to sample settled particulate matter in storm sewers (if

sediment accumulation occurs between wet weather events) and urban stream networks (Hajj-Mohamad et al., 2014). Passive sampling offers several advantages as compared to grab sampling (Stuer-Lauridsen, 2005; Zhang, Hibberd, et al., 2008), particularly for intermittent sources of contamination such as cross-connected sewers that depend on human activities. However, microbial contaminants and genetic markers cannot be passively sampled. As FIB can grow in the environment, any techniques to culture them *in situ* for passive sampling would result in false positives. Furthermore, where large numbers of cross-connections are located, passive sampling techniques are not necessary because there is a strong likelihood that concentrations will be higher and well above detection limits as they were in the present study. Timing sample collection according to human activities in the sector (during the morning (around 7-8 am) and the evening (around 6-7 pm) in residential sectors and during working hours in ICI sectors) will increase the probability of collecting samples when sewage cross connections lead to wastewater discharges into storm drains. Highly targeted identification of a small number of wastewater inflows would have a higher number of false negatives without passive sampling but false negatives are less likely to occur in problematic sectors that should be the focus of targeted corrective action.

6.4.2 Relationships among markers

All indicator data sets were lognormal. The complete data set was subjected to correlation analysis using the Pearson test. Several significant correlations were found among markers. A summary of all statistical correlations and significance among markers is available in Table 6.1. Of note, significant positive correlations were observed between the presence of FC and human-specific *Bacteroidales* (HF183), human mitochondrial (Hmt) markers, CAF, THEO, and ACE ($r \geq 0.39$) and between *E. coli* and CAF and THEO ($r \geq 0.59$). To the best of our knowledge, no studies have examined as wide a range of genetic and chemical markers for sewer cross-connection identification as in this study. Table 6.9 summarizes correlations among fecal contamination markers from the literature and demonstrates that some studies have found correlations (*e.g.* (Layton et al., 2006; Sauvé et al., 2012)), whereas others have not (*e.g.* (Sidhu et al., 2013; Villemur et al., 2015)).

Table 6.1. Summary of marker correlations (Pearson's correlation, *r*) in storm drain samples.

Target	***E. coli***	**FC**	**HF183 marker**	**Hmt marker**	**CBZ**	**CAF**	**THEO**
FC	0.83*	-	-	-	-	-	-
HF183 marker	0.27	0.40*	-	-	-	-	-
Hmt marker	0.32	0.39*	0. 74*	-	-	-	-
CBZ	0.18	0.16	0.26	0.46*	-	-	-
CAF	0.65*	0.66*	0.53*	0.43*	0.04	-	-
THEO	0.59*	0.66*	0.62*	0.66*	0.20	0.81*	-
ACE	0.24	0.42*	0.21	0.46*	0.07	0.36	0.43*

E. coli (*Escherichia coli*), FC (fecal coliforms), HF183 marker (human-specific *Bacteroidales* marker), Hmt marker (human mitochondrial DNA marker), CBZ (carbamazepine), CAF (caffeine), THEO (theophylline), ACE (acetaminophen), (*) denotes significance for correlations at $p < 0.05$.

The correlation between HF183 and Hmt markers in the samples (not considering live/dead cells) suggests their similar persistence in environmental waters. The HF183 concentrations consistently exceeded those of Hmt that could be related to differences in amplification methods. Interestingly, HF183 and Hmt showed the highest correlation ($r = 0.74$) in comparison with other alternative indicators. All WWMPs assayed showed a significant correlation to the Hmt marker ($r \geq 0.43$). No significant correlation was shown between CBZ and other markers and between ACE and HF183 marker and CAF. (Sauvé et al., 2012) also found no correlation between CAF and CBZ in storm drains, whereas CBZ and *E. coli* were significantly correlated in combined sewer overflows (Madoux-Humery et al., 2013). The poor correlation between CBZ and other markers can be explained by sources and travel times. CBZ use is not as widespread in the population as compared to CAF and ACE, however it is highly stable in the environment. CBZ is indicative of distant fecal sources in the absence of FIB and other markers.

The GenBac marker, an indicator of general fecal contamination was present in all samples except for two (DEN-aa (13) and DEN-aa (25)). This indicated that there is a presence of fecal contamination at 93% of sampling sites (data not shown). Given that *E. coli* and FC were present in all samples above detection limits, they are more sensitive as fecal indicators than the GenBac marker. The use of a general *Bacteroidales* marker (presence/absence test) with the specific human marker helped to validate negative results for the specific marker. Furthermore, the use of both markers helped identify the presence of non-human fecal contamination. Thus, for the majority of storm sewers, a mixed contamination (human and animal) was demonstrated in both the MEA and DEN watersheds.

For some storm sewers, there were moderate or high concentrations of *E. coli* or FC whereas there was a low concentration of the HF183 or Hmt marker. Low concentration of the specific molecular markers compared to *E. coli* could be the result of an animal fecal source or human fecal source that was not detected by them. In contrast, some sampling points had a high concentration of the HF183 or Hmt marker and a low concentration of *E. coli* and FC. Once discharged, *E. coli* and FC will be subjected to a number of processes including predation, settling, dispersion and dilution (Dorner et al., 2006). Furthermore, once these bacteria are subjected to these conditions of environmental stress, they demonstrate changes in their shape and size, their hydrophobicity, and they can lose their ability to replicate while maintaining their viability (Anderson et al., 2005; Bernhard et al., 2003; Craig et al., 2004; Garcia-Armisen &

Servais, 2009; Ishii et al., 2006; Maiga et al., 2009; McLellan, 2004; Muela et al., 2000; Pote et al., 2009). Such bacteria are considered to be viable but non-culturable (VNBC) and cannot be enumerated using traditional culture methods (Balleste & Blanch, 2010). However, real-time quantitative PCR is a sensitive technique that enables the quantification of viable and non-viable *Bacteroidales* spp. DNA and may explain the higher concentrations of the HF183 marker (Nielsen et al., 2007) as compared to *E. coli* in some instances. The higher presence of HF183 relative to *E. coli* could be indicative of fecal sources in the upper reaches of the sewer network. It is also not beyond the realm of possibility that *Bacteroides* could grow in the environment under certain conditions as has been demonstrated by others (Weidhaas et al., 2015).

The human mitochondrial DNA marker (Hmt) was detected and quantified in 70 % (21/30) and 43 % (13/30) of samples, respectively (Figure 6.2.B). It confirms the majority of partitioning of sources based on the HF183 marker (detected and quantified in 60 % (18/30) and 50 % (15/30) of samples, respectively). Sites contaminated by human-specific *Bacteroidales* are not necessarily the same sites contaminated by Hmt. A marked discrepancy was observed between HF183 and Hmt in 5/30 of samples because of differential persistence of the markers in the environment. *Bacteroidales* are likely to grow under ambient surroundings where alternate habitats such as sediments with anaerobic conditions do exist. In the case of storm sewers, the travel time is on the order of hours, and thus PCR detection would target a mixture of nucleic acids from live and dead cells.

The slow environmental degradation of WWMPs (particularly for CBZ) can lead to the disappearance of genetic markers and FIB before the elimination of WWMPs. Many of the sites with low *E. coli* concentrations had average CBZ concentrations (*e.g.* DEN-aa (13)), suggesting a distant fecal source (Figure 6.2.C).

Various factors affect correlations among WWMPs. Metabolism rate, excretion rate and time, environmental occurrence and persistence, sample pre-treatment and analysis methods, beverages and food products/coming from human activities are related to observed differences. For example, 2 to 4% of the ingested CAF (Arnaud, 1993; Tang-Liu et al., 1983), CBZ (Clara et al., 2004), and ACE (Prescott, 1983) are excreted unchanged in the urine. THEO is among the primary monodemethylated metabolites of caffeine in humans (Lelo et al., 1986) and accounts for 19 ± 5% of total excretion after its ingestion (Rodopoulos & Norman, 1997). Along with the use

of these WWMPs in pharmaceuticals, THEO and a large amount of CAF can be found in common food and beverage products. In addition, ACE is widely used as a non-prescription medication. As a result, CAF, THEO, and ACE were the most abundant indicators in aquatic environment in comparison with CBZ despite its higher environmental persistence and lower detection limit (Hajj-Mohamad et al., 2014). The disposal of unconsumed CAF may also be a greater contributor than its actual consumption but would nevertheless highlight a sewer connection – more to the kitchen sink than to bathroom (Sauvé et al., 2012; Seiler et al., 1999), which leads to an overestimation of its fecal sources. However, as an indicator of sewer cross-connections, it is not necessary that it be directly related to fecal sources.

6.4.3 Land use and fecal contamination

Relationships between marker concentrations and *E. coli* concentrations are displayed in Figure 6.3 include all sampling sites in the MEA and DEN storm sewer networks. A more detailed explanation of the linear regression models are provided in Figure 6.5 in ESI†. Land-use is an important determinant of drain water quality (Ekklesia et al., 2015). The non-parametric Kruskal-Wallis test was used to evaluate the differences among the distributions of markers in relation to the general dominant land-use classes (R and ICI). Only the median concentration of *E. coli* was significantly different ($p = 0.0372$) and higher in residential areas of both storm sewersheds. This demonstrates the importance of non-human fecal sources of contamination in residential catchments where *E. coli* and FC concentrations are elevated. To determine if the dominant land-use in proximity to the sample location influences the relationship of the concentration of markers, data from MEA and DEN watersheds were grouped under two categories (residential (R) and industrial/commercial/institutional (ICI)), and we separately compared alternative marker concentrations to *E. coli* concentrations in these different areas (Figure 6.3 and Table 6.10). As expected, residential areas showed a strong significant correlation between FC and *E. coli* concentrations ($r = 0.89$, $p < 0.05$). However, no significant correlations were observed between *E. coli* and alternative marker concentrations in residential areas, even when considering samples from MEA alone. In contrast, ICI areas showed significant correlations between FC, Hmt, CAF, and THEO concentrations with *E. coli* concentrations ($p < 0.05$), but no significant correlation between HF183, CBZ, or ACE and *E. coli* concentrations ($p > 0.05$).

6.4.4 Alternative marker thresholds for the classification of *E. coli* concentrations

Decisions for undertaking remedial action in urban streams and sewer networks are made based upon FIB and not alternative marker concentrations. Thus, we compared alternative marker thresholds in relation to *E. coli* concentrations above a regulatory threshold. Arbitrary thresholds of 470 copies of HF183 100 mL^{-1}, 67 copies Hmt 100 mL^{-1}, 0.5 ng CBZ L^{-1}, 167 ng CAF L^{-1}, 56 ng THEO L^{-1}, and 8 ng ACE L^{-1} were used to identify samples with elevated fecal contamination, as defined by more than 235 MPN 100 mL^{-1} of *E. coli* (Table 6.11). A quadrant analysis of the data in Figure 6.3 is summarized in Table 6.2 for the markers that were significantly correlated with *E. coli*. The defined thresholds for the HF183 marker, Hmt marker, CAF, THEO and ACE correctly predicted *E. coli* concentrations that were either above or below the 235 CFU 100 mL^{-1} threshold for over 50% of samples. The markers more accurately predicted concentrations of *E. coli* below the threshold than above it. CAF was the most accurate marker for predicting *E. coli* above the 235 CFU 100 mL^{-1} threshold, a success rate of 75% with ACE being the lowest with a success rate of 50%. A similar analysis was conducted for FC (Figure 6.6, Table 6.12). The threshold concentrations of alternative indicators HF183, Hmt, CAF, THEO, and ACE allow the correct classification of FC concentrations greater than 200 CFU 100 mL^{-1} with 70%, 60%, 75%, 90%, and 60% accuracy, respectively.

Alternative markers were further classified into low, medium and high concentrations. The class was then used to predict the *E. coli* class as being above or below the threshold of 235 CFU 100 mL^{-1} (Table 6.3). Table 6.3 shows that samples with the highest concentrations of the alternative markers were all associated with *E. coli* concentrations above the regulatory threshold and these values are suggested as thresholds for the identification of priority cross-connections. However, the converse did not hold true and even with the lowest concentrations of alternative markers, *E. coli* were frequently above the regulatory threshold. Apart from the possible presence of non-fecal contaminants due to tracers dumped along streets and into storm drains or to the disposal of unconsumed tracers down the sink (Seiler et al., 1999), the presence of high concentrations of alternative tracers of contamination can be explained by a widespread or severe contamination of storm sewers by wastewater via cross-connections (Panasiuk et al., 2015). The concentration of chemical tracers was controlled among other factors by the number of users of pharmaceutical-

containing drugs and by their fate and transport in receiving waters (Daneshvar et al., 2010; Monteiro & Boxall, 2010). A lower concentration of alternative indicators is not necessarily indicative of the absence of FC and *E. coli*, due to background contamination from non-human faecal sources. In aggregate, HF183 (100%) and CAF (75%) were the best indicators to track fecal contamination from human sources regardless of the region that surrounds the sampling site when *E. coli* concentrations were below and above 235 CFU 100 mL^{-1}, respectively Table 6.2. In addition, ACE correctly predicted 50% of samples at any area when *E. coli* concentrations were below or above 235 CFU 100 mL^{-1}. In another study, ACE and CAF were recommended as chemical tracers of surface waters in high-density and low-density residential areas, respectively (Ekklesia et al., 2015). In this study, ACE concentrations were more variable than CAF concentrations. The more constant and widespread use of CAF in the population means that it is more likely to be present when a cross-connection is present. However, a high concentration of ACE is a stronger indication of the presence of a cross-connection upstream as CAF could be present in storm drains as a result of the improper disposal of caffeinated drinks and containers in the street.

Table 6.13 showed the prediction frequency for wastewater cross connection decision-making based on land use. In residential areas and the MEA watershed, the HF183 and Hmt markers, and ACE correctly classified (100%) of non-exceedances of the *E. coli* standard (235 CFU 100 mL^{-1}). In ICI areas and DEN watershed, HF183 marker, CAF, and THEO correctly predicted (100%) of non-exceedances of the 235 colonies of *E. coli* per 100 mL. In general, the prediction frequencies of non-exceedances of the threshold (235 colonies of *E. coli* per 100 mL) were higher than the prediction frequencies of exceedances of this threshold. In residential areas and the MEA watershed, the prediction frequencies of exceedances of the threshold (235 colonies of *E. coli* per 100 mL) were the highest with HF183 marker, CAF and THEO (67% and 78%, respectively). In ICI areas, the prediction frequencies of exceedances of the threshold were the highest with Hmt marker and CAF (88%) whereas in DEN watershed, CAF correctly and highly predicted (73%) of exceedances of the 235 colonies of *E. coli* per 100 mL.

Table 6.2. Prediction frequency for wastewater cross connection decision-making based on the quadrant analysis of the linear regression of all samples in Figure 6.3.

Prediction description (quadrant in Figure 6.3)	**Number of samples/total number possible samples (%)**				
	HF183	**Hmt**	**CAF**	**THEO**	**ACE**
Incorrect *E. coli* non-exceedance (I)	0/10 (0.0)	2/10 (20.0)	1/10 (10.0)	1/10 (10.0)	5/10 (50.0)
Correct *E. coli* non-exceedance (III)	10/10 (100.0)	8/10 (80.0)	9/10 (90.0)	9/10 (90.0)	5/10 (50.0)
Correct *E. coli* exceedance (II)	13/20 (65.0)	13/20 (65.0)	15/20 (75.0)	14/20 (70.0)	10/20 (50.0)
Incorrect *E. coli* exceedance (IV)	7/20 (35.0)	7/20 (35.0)	5/20 (25.0)	6/20 (30.0)	10/20 (50.0)

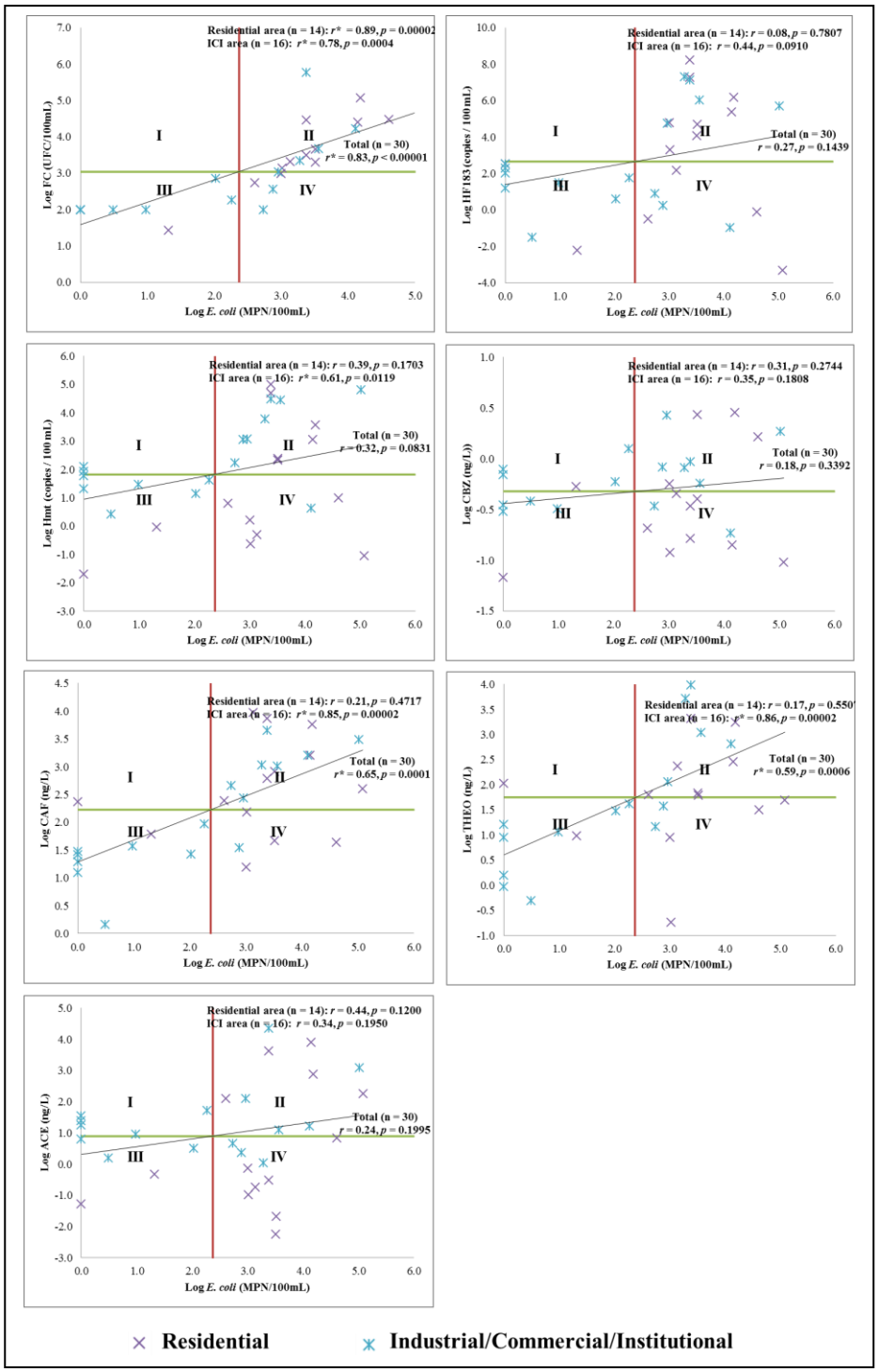


Figure 6.3. Quadrant analysis (threshold of 235 CFU 100 mL^{-1}, n = 30) and Pearson correlation coefficients (*r*-values) of *E. coli* with marker concentrations considering land use (Residential area (n =14) and ICI area (n = 16)). Data "$\log_{10}$ (x)" transformed, with x for value of given variable. Significant correlations (*p*-values < 0.05) are indicated by asterisks.

Table 6.3. *E. coli* exceedance prediction using alternative markers as determined from quadrant analysis of Figure 6.3.

Log marker concentration*	**(I) Incorrect *E. coli* non-exceedance (%)**	**(III) Correct *E. coli* non-exceedance (%)**	**(IV) Incorrect *E. coli* exceedance (%)**	**(II) Correct *E. coli* exceedance (%)**
HF183				
< 1	0/9 (0.0)	3/9 (33)	6/9 (67)	0/9 (0.0)
1–3	0/8 (0.0)	7/8 (88)	1/8 (13)	0/8 (0.0)
> 3	0/13 (0.0)	0/13 (0.0)	0/13 (0.0)	13/13 (100.0)
Hmt				
< 1	0/10 (0.0)	3/10 (30.0)	7/10 (70.0)	0/10 (0.0)
1–2	1/6 (17.0)	5/6 (83.0)	0/6 (0.0)	0/6 (0.0)
> 2	1/14 (7.0)	0/14 (0.0)	0/14 (0.0)	13/14 (93.0)
CAF				
< 1	0/1 (0.0)	1/1 (100.0)	0/1 (0.0)	0/1 (0.0)
1–2	0/12 (0.0)	8/12 (67.0)	4/12 (33.0)	0/12 (0.0)
> 2	1/17 (6.0)	0/17 (0.0)	1/17 (6.0)	15/17 (88.0)
THEO				
< 1	0/7 (0.0)	5/7 (71.0)	2/7 (29.0)	0/7 (0.0)
1–2	0/11 (0.0)	4/11 (36.0)	4/11 (36.0)	3/11 (27.0)
> 2	1/12 (8.0)	0/12 (0.0)	0/12 (0.0)	11/12 (92.0)
ACE				
< 0	0/8 (0.0)	2/8 (25.0)	6/8 (75.0)	0/8 (0.0)
0–1	1/8 (13.0)	3/8 (38.0)	4/8 (50.0)	0/8 (0.0)
> 1	4/14 (29.0)	0/14 (0.0)	0/14 (0.0)	10/14 (71.0)

(*) with threshold values for low, medium and high concentrations.

6.4.5 Prioritization of sites for additional investigation

The identification of sites for additional monitoring was based primarily on classes as determined through application of the index (Table 6.4). Threshold values used to classify each contaminant as "low ", "medium", and "high" are presented in Table 6.3 (Data not shown for CBZ (< (-1): low, (-1)-(-0.3): medium, and > (-0.3): high)). Direct contamination from cross connections was likely because there were visible sewage solids during sampling of several sites (MEA-j-10 (9), MEA-j-10 (2), MEA-j-8(1), MEA-j-O (3), DEN-aa). It can be seen that not all sites with high *E. coli* concentrations would be high priority for cross-connected sewers. This suggests that other source of *E. coli* such as domestic animals or wild fauna contribute to FIB concentrations. These observations reinforce the notion that studied alternative indicators in surface waters (especially in residential areas) is a more specific indicator of contamination by probable sanitary sewage discharge than FC and *E. coli.* 8/16 sites (5 sites in residential area and 3 sites in ICI area) and 8/16 (6 sites in residential area and 2 sites in ICI area) were shown with high and medium priority for cross-connected sewers, respectively. Otherwise, only 2 sites (in ICI area) with low *E. coli* concentrations (< 235 *E. coli* 100 mL^{-1}) had high priority for cross-connected sewers (Table 6.4). It is interesting to compare the median concentrations of sites classified as "high" or "low" priority with the threshold of 12000 CFU 100 mL^{-1}, proposed as indicative of wastewater contamination in storm sewers (Pitt, 2004). The median *E. coli* concentration for all sites classified as "high" using the alternative markers was 2419 MPN 100 mL^{-1} (mean of 12300 MPN 100 mL^{-1}) and is nearly an order of magnitude lower than the Pitt threshold. For sites classified as "low" priority, the median *E. coli* concentration was 284 MPN 100 mL^{-1}, a value of the same order of magnitude as the 235 CFU 100 mL^{-1} threshold. The median for low priority sites indicates that approximately a third of samples will respect the regulatory threshold of 235 CFU 100 mL^{-1} and two thirds will be above, but not likely as a result of human fecal contamination. Thus, the use of alternative specific indicators will permit the appropriate classification of sites and improve the prioritization of sectors for rehabilitation.

Table 6.4. Microbial, molecular and chemical marker concentrations (log_{10}), index values and potential sources of fecal contamination for sites with high and low/ average *E. coli* concentrations.

Site	Land use	*E. coli*[a]	HF183F	Hmt	CBZ	CAF	THEO	ACE	Index Value (Priority Class)	Dominant source of fecal contamination
MEA-j-1-d(1)	R	High*	Low	Low	Low	High	Medium	High	0.42 (medium)	Mixed
DEN-z(21)	ICI	High*	Medium	Medium	High	Medium	Medium	High	0.67 (high)	Cross-connections
DEN-l(3)	R	High*	Low	Low	High	Medium	Medium	Medium	0.42 (medium)	Mixed
MEA-j-10 (2)	R	High*	High	High	High	High	High	High	1.00 (high)	Cross-connections
MEA-j-8(1)	R	High*	High	High	Medium	High	High	High	0.92 (high)	Cross-connections
DEN-s (1)	ICI	High*	Low	Medium	High	Medium	Medium	Medium	0.50 (medium)	Mixed
DEN-z(41)	ICI	High	Medium	Medium	Medium	Medium	Medium	Medium	0.50 (medium)	Mixed
MEA-i(1)	R	High	High	High	Medium	Medium	Medium	Low	0.58 (medium)	Mixed
MEA-j-1(1)	R	High	High	High	High	High	Medium	Low	0.75 (high)	Cross-connections
MEA-j-8-o(3)	R	High	High	High	Medium	High	High	High	0.92 (high)	Cross-connections
MEA-j-10(9)	R	High	High	High	Medium	High	High	Low	0.75 (high)	Cross-connections
DEN-z-1(6)	ICI	High	High	High	High	High	High	High	1.00 (high)	Cross-connections
DEN-y	ICI	High	High	High	High	High	High	Medium	0.92 (high)	Cross-connections
MEA-j-8-b(1)	R	High	Medium	Low	Medium	High	High	Low	0.50 (medium)	Mixed
MEA-j-1-g (1)	R	High	High	Low	Medium	High	Low	Low	0.42 (medium)	Mixed
DEN-u(1)	R	High	High	Low	High	Medium	Low	Low	0.42 (medium)	Mixed

Table 6.4. Microbial, molecular and chemical marker concentrations (log_{10}), index values and potential sources of fecal contamination for sites with high and low/ average *E. coli* concentrations (cont'd).

Site	Land use	*E. coli*[a]	HF183F	Hmt	CBZ	CAF	THEO	ACE	Index Value (Priority Class)	Dominant source of fecal contamination
DEN-z	ICI	Average	Low	Low	Medium	High	High	High	0.58 (medium)	Mixed
DEN-x	ICI	Average	High	High	High	High	High	High	1.00 (high)	Cross-connections
DEN-z-6(1)	ICI	Average	Low	Low	Medium	Low	Low	Medium	0.17 (low)	Mixed
DEN-p(1)	R	Average	Low	Low	Medium	High	Medium	High	0.50 (medium)	Mixed
DEN-aa	ICI	Low	Medium	Medium	Medium	Medium	Low	Medium	0.42 (medium)	Mixed
DEN-z-1(2)	ICI	Low	High	High	High	High	High	High	1.00 (high)	Cross-connections
MEA-j (30)	R	Low	Low	Low	High	Medium	Low	Low	0.25 (low)	Low priority mixed
DEN-z-6(16)	ICI	Low	High	High	High	High	High	High	1.00 (high)	Cross-connections
DEN-w	ICI	Low	Low	High	High	Medium	Medium	Medium	0.58 (medium)	Mixed
MEA-j-1-b(7)	R	Low	Medium	Low	Low	High	High	Low	0.42 (medium)	Mixed
DEN-z-7(1)	ICI	Low	Low	High	Medium	High	Medium	Medium	0.58 (medium)	Mixed
DEN-z-4(1)	ICI	Low	Medium	Medium	Medium	Medium	Low	High	0.50 (medium)	Mixed
DEN-aa (13)	ICI	Low	Medium	Medium	High	Medium	Low	High	0.58 (medium)	Mixed
DEN-aa (25)	ICI	Low	Medium	High	High	Medium	Medium	High	0.75 (high)	Cross-connections

6.5 Conclusions

- WWMPs were detected in all samples demonstrating that human fecal contamination through sanitary sewer cross-connections is widespread in the tested urban storm water systems.
- Significant correlations were observed between the presence of FC and alternative markers (HF183, Hmt, CAF, THEO, and ACE) whereas *E. coli* showed significant correlations only with CAF and THEO. Given that the method used to measure FC was more precise than the method used for *E. coli*, it was possible to take into consideration all alternative indicators correlated with FC in order to find the best indicators for the identification of cross-connections.
- Human-specific markers were also detected in storm sewer systems when *E. coli* concentrations were low (below 235 MPN 100 mL^{-1}), illustrating the importance of using a suite of markers associated with cross-connections and not only those associated with direct fecal inputs heavily influenced by human defecation patterns.
- HF183 marker (100% of classification accuracy) and CAF (75% of classification accuracy) were the most sensitive indicators to predict *E. coli* concentrations below and above a threshold of 235 CFU or MPN 100 mL^{-1} in dry weather storm sewer samples, respectively.
- Concentrations above 3 Log for HF183, 2 Log for Hmt, CAF, and THEO, and 1 Log for ACE are the proposed thresholds for identifying cross-connected sewers with *E. coli* concentrations above 235 *E. coli* CFU 100 mL^{-1}.
- The use of an index to rank sites for cross-connection rehabilitation using alternative markers is more accurate than the *E. coli* threshold of 12000 CFU 100 mL^{-1} proposed by (Pitt, 2004). The 12000 CFU 100 mL^{-1} threshold is insufficiently conservative and would miss many sewer cross-connections, particularly if sampling is conducted at any time other than the early morning. In addition, sites with suspected non-human animal sources of fecal contamination had *E. coli* concentrations above 12000 CFU 100 mL^{-1} (see Table 6.4, 3/6 of sites had mixed sources of fecal contamination).

- Significant differences between residential versus ICI sectors suggests that different strategies should be used when monitoring these sectors for sewer cross-connections. The time of day of sampling and human defecation patterns might be more important with regards to *E. coli* concentrations in residential areas versus ICI areas. Thus cross-connections would be more apparent in ICI sectors throughout the day using WWMP tracers as compared to residential areas where inputs would be more transient. Passive sampling techniques would be useful in residential areas whereas they do not appear to be necessary in ICI sectors.
- The storm sewers analysed represent only a part of the whole storm sewer studied. 16/30 (53%) of studied sewers (8/16 (50%) of sites with medium and high degrees) had human fecal contamination (as defined by a high concentration of *E. coli*). The methods and thresholds proposed are for conducting a preliminary analysis of human fecal contamination in storm sewer networks. Once sectors are identified and prioritized, additional investigations involving property owners could be undertaken to identify the specific problematic connections.
- Animal-specific fecal indicators for urban sewer networks for which animals and wildlife could be useful in residential areas, but do not appear to be necessary in ICI sectors.

6.6 References

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6.7 Electronic supporting information

6.7.1 Supplementary Tables

Table 6.5. Description of the MEA and DEN Creeks watershed sampling sites, surrounding land-use and their primary fecal contamination source.

Sampling date	Site	Land use	Presumed primary fecal contamination source determined by the municipality
15/08/2012	MEA-j-1(1)	Residential	Cross-connections/animals
15/08/2012	MEA-i(1)	Residential	Cross-connections/animals
15/08/2012	MEA-j-1-b-(7)	Residential	Cross-connections
15/08/2012	MEA-j-1-d(1)	Residential	Animals
15/08/2012	MEA-j-1-g(1)	Residential	Animals
15/08/2012	MEA-j-8-b(1)	Residential	Animals
15/08/2012	MEA-j-8(1)	Residential	Cross-connections/animals
15/08/2012	MEA-j-8-o(3)	Residential	Cross-connections (corrections in August 2012)
15/08/2012	MEA-j-10(9)	Residential	Cross-connections
15/08/2012	MEA-j-10(2)	Residential	Cross-connections
15/08/2012	MEA-j(30)	Residential	Cross-connections (corrections in August 2012)
27/08/2012	DEN-u(1)	Residential	Cross-connections/animals
27/08/2012	DEN-y	ICI	Cross-connections
27/08/2012	DEN-w	ICI	Unknown
27/08/2012	DEN-s(1)	ICI	Unknown
27/08/2012	DEN-p(1)	Residential	Unknown
27/08/2012	DEN-l(3)	Residential	Unknown
27/08/2012	DEN-x	ICI	Cross-connections
24/09/2012	DEN-z-1(2)	ICI	Cross-connections
24/09/2012	DEN- z-6(1)	ICI	Unknown
27/08/2012	DEN-z	ICI	Cross-connections /Unknown
24/09/2012			
24/09/2012	DEN- z-7(1)	ICI	Unknown
24/09/2012	DEN- z-6(16)	ICI	Unknown
27/08/2012	DEN-aa	ICI	Cross-connections
24/09/2012			
24/09/2012	DEN-z(41)	ICI	Cross-connections
24/09/2012	DEN-z(21)	ICI	Cross-connections
24/09/2012	DEN-z-4(1)	ICI	Unknown
25/09/2012	DEN-z-1(6)	ICI	Unknown
25/09/2012	DEN-aa(13)	ICI	Cross-connections
25/09/2012	DEN-aa(25)	ICI	Cross-connections

Rainfall conditions of sampling days were 2.2, 1.0 and 0.0 mm and a trace on the 15th and 27th of August, 24th and 25th of September, respectively. (Unknown: probably animal origin, but no direct observations of feces).

Table 6.6. Alternative marker detection in stormwater.

Marker	***E. coli***	**Fecal coliform**	**GenBac marker[a]**	**HF183 marker**	**Hmt marker**	**CBZ**	**CAF**	**THEO**	**ACE**
MEA watershed	100%	100%	100%	91%	91%	100%	100%	100%	100%
DEN watershed	100%	100%	89%	42%	58%	100%	100%	100%	100%

Data is % detection of samples tested. n = 30 (11 for MEA watershed and 19 for DEN watershed), [a] presence/absence method.

Table 6.7. Concentrations of fecal coliforms, E. coli, human-specific Bacteroidales (HF183F), human mitochondrial DNA (Hmt) markers, carbamazepine (CBZ), caffeine (CAF), theophylline (THEO), and acetaminophen (ACE) per watershed.

Watersheds	Fecal coliforms[a]	*E. coli*[a]	HF183[b]	Hmt[b]	CBZ[c]	CAF[c]	THEO[c]	ACE[c]
MEA n=11								
Min	2.70E+01	1.00E+00	5.07E-04	2.02E-02	6.73E-02	4.65E+01	1.82E-01	5.56E-03
Max	1.57E+05	1.21E+05	1.70E+08	1.00E+05	2.83E+00	9.40E+03	1.87E+04	8.07E+03
Median	3.14E+03	2.42E+03	1.16E+04	2.07E+02	3.40E-01	6.12E+02	1.05E+02	3.02E-01
Mean	3.13E+04	1.50E+04	1.74E+07	1.40E+04	7.12E-01	2.39E+03	2.12E+03	1.20E+03
Standard deviation	5.46E+04	3.56E+04	5.09E+07	3.20E+04	1.03E+00	3.39E+03	5.56E+03	2.60E+03
DEN n=19								
Min	1.00E+02	1.00E+00	3.08E-02	1.60E+00	1.84E-01	1.45E+00	4.91E-01	7.28E-01
Max	5.90E+05	1.05E+05	2.09E+07	6.34E+04	2.67E+00	4.45E+03	1.10E+04	2.22E+04
Median	5.40E+02	5.48E+02	5.78E+01	6.01E+01	5.90E-01	4.30E+01	3.13E+01	1.25E+01
Mean	3.67E+04	8.98E+03	1.92E+06	6.85E+03	8.02E-01	6.57E+02	1.47E+03	1.26E+03
Standard deviation	1.35E+05	2.50E+04	5.58E+06	1.64E+04	6.44E-01	1.20E+03	3.34E+03	5.08E+03
Total n=30								
Min	2.70E+01	1.00E+00	5.07E-04	2.02E-02	6.73E-02	1.45E+00	1.82E-01	5.56E-03
Max	5.90E+05	1.21E+05	1.70E+08	1.00E+05	2.83E+00	9.40E+03	1.87E+04	2.22E+04
Median	1.25E+03	1.03E+03	2.66E+02	7.29E+01	4.90E-01	2.35E+02	5.50E+01	7.81E+00
Mean	3.47E+04	1.12E+04	7.59E+06	9.47E+03	7.69E-01	1.29E+03	1.71E+03	1.24E+03
Standard deviation	1.11E+05	2.89E+04	3.12E+07	2.31E+04	7.90E-01	2.36E+03	4.21E+03	4.29E+03

ROS estimation method was used to impute ND observations (Nondetects) using a lognormal model by ProUCL,. [a] Fecal coliform and *E. coli* are expressed in CFU and MPN 100 mL^{-1} respectively, [b] HF183 and Hmt are expressed in copies 100 mL^{-1}, [c] CBZ, CAF, THEO and ACE are expressed in ng L^{-1}.

Table 6.8. Occurrence of alternative human specific markers from different wastewater sources.

Compound	Wastewater source	Receiving water	Mean value of marker concentration	Reference
HF183[a]	Diverse sources (NS)	Watersheds, river, and stream	4.0	(Villemur et al., 2015)
Hmt[a]	Diverse sources (NS)	Watersheds, river, and stream	3.1	(Villemur et al., 2015)
CBZ[b]	Cross-connections Sewage exfiltration	Stream Canal	0.82 0.22	(Guérineau et al., 2014; Hajj-Mohamad et al., 2014)
	Cross-connections	Brooks Collectors Discharge outfalls	0.64 0.08 0.15 2.00, 2.49	(Sauvé et al., 2012)
		Combined sewer system WWTP influent	2.37	(Madoux-Humery et al., 2013)
CAF[b]	Cross-connections Sewage exfiltration	Stream Canal	1.96 1.74	(Guérineau et al., 2014; Hajj-Mohamad et al., 2014)
	Cross-connections	Brooks Collectors Discharge outfalls	2.56 3.07 3.03	(Sauvé et al., 2012)
		WWTP influent	3.83	(Madoux-Humery et al., 2013)
THEO[b]	Cross-connections Sewage exfiltration	Stream Canal	1.91 1.15	(Guérineau et al., 2014; Hajj-Mohamad et al., 2014)
		WWTP influent	4.36	(Madoux-Humery et al., 2013)
ACE[b]	Cross-connections Sewage exfiltration	Stream Canal	2.23 ND	(Guérineau et al., 2014; Hajj-Mohamad et al., 2014)
		Combined sewer system WWTP influent	3.51, 3.93 4.06	(Madoux-Humery et al., 2013)

The mean values are expressed in [a] $\log_{10}$ copy number per 100 mL of filter water, [b] in $\log_{10}$ ng per L, NS: not specified, ND: not detected, WWTP influent: Wastewater treatment plant influent.

Table 6.9. Summary of correlations (*r*-values) among fecal contamination markers from the literature.

Markers	Target sites	*r*-values	Reference
FC / CAF	Stormwater collection systems (large urban area)	0.75	(Sauvé et al., 2012)
FC / CBZ		0.27	
***E. coli* / *Bacteroides* species AllBac marker**	Creek waters	0.92	(Layton et al., 2006)
FC / *Bacteroides-Prevotella* group-specific 16S rRNA gene markers	River waters	0.70	(Okabe et al., 2007)
Total coliforms / Total *Bacteroidales* 16S rRNA gene marker	River waters	0.51	(Jeong et al., 2010)
FC / total *Bacteroidales*16S rRNA gene marker		0.49	
Total coliforms / Human-specific *Bacteroidales* 16S rRNA genetic markerFC / Human-specific *Bacteroidales* 16S rRNA genetic marker		0.57 0.61	
FC / CAF	Surface waters from watersheds in a rural-to-urban gradient	0.40-0.61	(Young et al., 2008b)
FC / Human-specific *Bacteroidales* HF183 marker	Surface waters from watersheds (representative of typical natural vs urban environments and of human activities (agricultural vs urban))	0.36	(Villemur et al., 2015)
FC / Human mitochondrial DNA qmitoHu marker		NS	
Human mitochondrial DNA qmitoHu marker / human-specific *Bacteroidales* HF183 marker		0.57	
Human-specific *Bacteroidales* / Human mitochondrial DNA	Surface water from watersheds (urban creek system impacted by combined sewer over flows)	0.62	(Kapoor et al., 2013)
E. coli* / Human-specific *Bacteroidales		0.33	
CAF / ACE	Urban stormwater runoff	NS	(Sidhu et al., 2013)

NS: Non significant correlation.

Table 6.10. Summary of Pearson's correlation coefficients (*r*) provided, of *E. coli* MPN concentration to alternative marker concentration based on surrounding land-use or watershed represented in Figure 6.3.

Land-use	Fecal coliforms	HF183 marker	Hmt marker	CBZ	CAF	THEO	ACE
Residential (n = 14)	0.89*	0.08	0.39	0.31	0.21	0.17	0.44
Industrial/Commercial/Institutional (n = 16)	0.78*	0.44	0.61*	0.35	0.85*	0.86*	0.34
Watershed MEA (n = 11)	0.89*	0.15	0.42	0.22	0.37	0.22	0.49
Watershed DEN (n = 19)	0.79*	0.30	0.38	0.37	0.71*	0.76*	0.23

(*) denotes significance correlations at $p < 0.05$. n correspond to the sample size for each sample surrounding. Data "$\log_{10}$ (x)" transformed, with x for value of given variable.

Table 6.11. Summary of arbitrary thresholds of alternative markers (corresponding to the thresholds of 235, 1000, and 12000 *E. coli* 100 mL^{-1}, consecutively).

Indicators	**Arbitrary thresholds (log unit)**		
E. coli	2.37	3.00	4.08
FC	3.05	3.44	4.10
HF183	2.67	3.01	3.58
Hmt	1.82	2.06	2.46
CBZ	-0.32	-0.29	-0.24
CAF	2.22	2.47	2.90
THEO	1.75	2.05	2.57
ACE	0.90	1.06	1.33

Table 6.12. Prediction frequency for making wastewater cross connection management decisions based on the quadrant analysis of the linear regression of all samples in Figure 6.6.

Prediction description (quadrant in Figure 6.6)	**Number of samples/total number possible samples (%)**				
	HF183	**Hmt**	**CAF**	**THEO**	**ACE**
Incorrect FC non-exceedance (I)	4/10 (40.0)	6/10 (60.0)	3/10 (30.0)	2/10 (20.0)	7/10 (70.0)
Correct FC non-exceedance (III)	6/10 (60.0)	4/10 (40.0)	7/10 (70.0)	8/10 (80.0)	3/10 (30.0)
Correct FC exceedance (II)	14/20 (70.0)	12/20 (60.0)	15/20 (75.0)	18/20 (90.0)	12/20 (60.0)
Incorrect FC exceedance (IV)	6/20 (30.0)	8/20 (40.0)	5/20 (25.0)	2/20 (10.0)	8/20 (40.0)

Table 6.13. Prediction frequency for wastewater cross connection decision-making based on the quadrant analysis of the linear regression in Figure 6.3 and surrounding land-use or watershed.

Prediction description (quadrant in Figure 6.3)	**Number of samples/total number possible samples (%)**				
Residential	**HF183**	**Hmt**	**CAF**	**THEO**	**ACE**
Incorrect *E. coli* non-exceedance (I)	0/2 (0.0)	0/2 (0.0)	1/3 (33.0)	1/3 (33.0)	0/2 (0.0)
Correct *E. coli* non-exceedance (III)	2/2 (100.0)	2/2 (100.0)	2/3 (67.0)	2/3 (67.0)	2/2 (100.0)
Correct *E. coli* exceedance (II)	8/12 (67.0)	6/12 (50.0)	8/12 (67.0)	8/12 (67.0)	5/12 (42.0)
Incorrect *E. coli* exceedance (IV)	4/12 (33.0)	6/12 (50.0)	4/12 (33.0)	4/12 (33.0)	7/12 (58.0)
ICI	**HF183**	**Hmt**	**CAF**	**THEO**	**ACE**
Incorrect *E. coli* non-exceedance (I)	0/8 (0.0)	2/8 (25.0)	0/7 (0.0)	0/7 (0.0)	5/8 (63.0)
Correct *E. coli* non-exceedance (III)	8/8 (100.0)	6/8 (75.0)	7/7 (100.0)	7/7 (100.0)	3/8 (38.0)
Correct *E. coli* exceedance (II)	5/8 (63.0)	7/8 (88.0)	7/8 (88.0)	6/8 (75.0)	5/8 (63.0)
Incorrect *E. coli* exceedance (IV)	3/8 (38.0)	1/8 (13.0)	1/8 (13.0)	2/8 (25.0)	3/8 (38.0)
Watershed MEA	**HF183**	**Hmt**	**CAF**	**THEO**	**ACE**
Incorrect *E. coli* non-exceedance (I)	0/2 (0.0)	0/2 (0.0)	1/2 (50.0)	1/2 (50.0)	0/2 (0.0)
Correct *E. coli* non-exceedance (III)	2/2 (100.0)	2/2 (100.0)	1/2 (50.0)	1/2 (50.0)	2/2 (100.0)
Correct *E. coli* exceedance (II)	7/9 (78.0)	6/9 (67.0)	7/9 (78.0)	7/9 (78.0)	4/9 (44.0)
Incorrect *E. coli* exceedance (IV)	2/9 (22.0)	3/9 (33.0)	2/9 (22.0)	2/9 (22.0)	5/9 (56.0)
Watershed DEN	**HF183**	**Hmt**	**CAF**	**THEO**	**ACE**
Incorrect *E. coli* non-exceedance (I)	0/8 (0.0)	2/8 (25.0)	0/8 (0.0)	0/8 (0.0)	5/8 (63.0)
Correct *E. coli* non-exceedance (III)	8/8 (100.0)	6/8 (75.0)	8/8 (100.0)	8/8 (100.0)	3/8 (38.0)
Correct *E. coli* exceedance (II)	6/11 (55.0)	7/11 (64.0)	8/11 (73.0)	7/11 (64.0)	6/11 (55.0)
Incorrect *E. coli* exceedance (IV)	5/11 (45.0)	4/11 (36.0)	3/11 (27.0)	4/11 (36.0)	5/11 (45.0)

6.7.2 Supplementary Figures

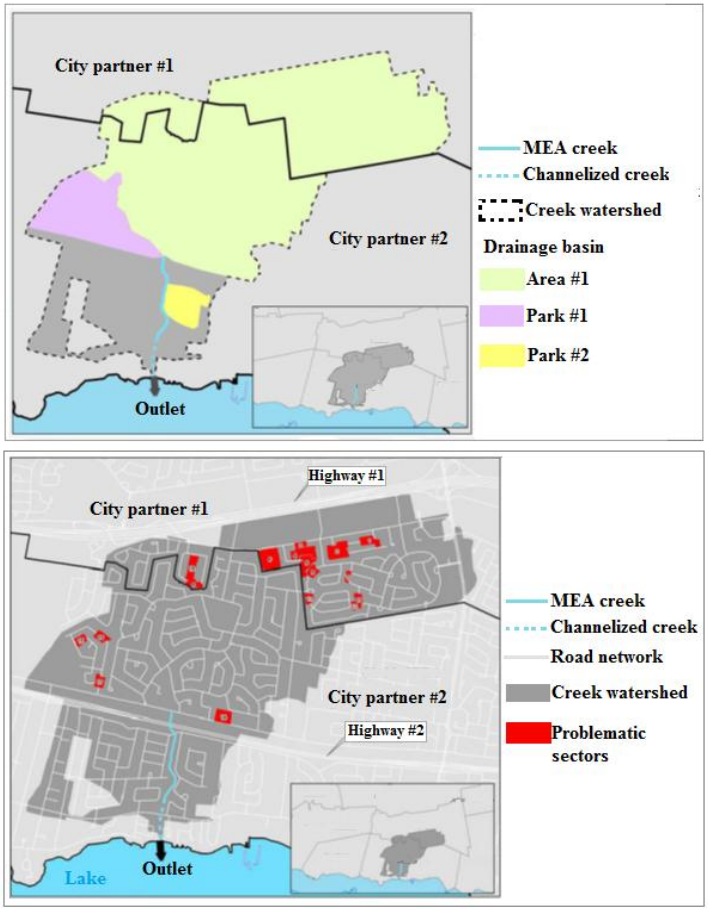

Figure 6.4. Map of the MEA Creek watershed: Watershed boundaries (top). Historical water quality sampling sites (highlighted in red) had shown high concentrations of fecal coliforms (bottom) and classified as problematic sectors.

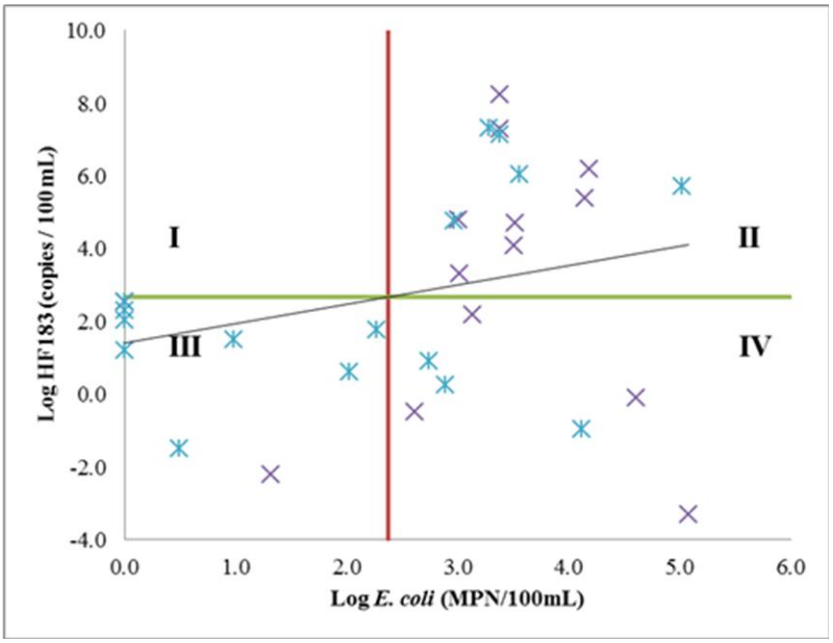

Figure 6.5. Linear regression model: linear regression (black line) of *E. coli* concentration to Human specific *Bacteroides* concentration. The red and green lines are the vertical and horizontal transects of the 235 *E. coli* MPN 100 mL^{-1} of water threshold. The quadrants arising from the red and green lines are labeled by roman numerals in the graph.

Figure 6.5 presents a linear regression model between *E. coli* and the HF183 marker. The figure was subdivided into 4 sections (I, II, III, and IV). The vertical line (red line in Figure 6.5) corresponds to the recommended *E. coli* limit in log concentration; the horizontal line corrresponds to the log concentration of the HF183 marker by qPCR (green line on Figure 6.5) that crosses the linear regression line (black line in Figure 6.5) at 235 MPN 100 mL^{-1}. With a good significant correlation, it was possible to determine through linear regression the concentrations of the HF183 marker that would enable an accurate classification of *E. coli* that are below (true negatives) or above (true positives) the 235 MPN 100 mL^{-1} threshold (Figure 6.5, quadrants II and III). Furthermore, it was possible to determine the concentrations of the HF183 marker that failed to accurately classify *E. coli* concentrations below (false negatives) or above (false positives) for the *E. coli* threshold (Figure 6.5, quadrants I and IV). For some sites, there were high *E. coli* concentrations and moderate or low alternative markers. In the case of HF183 marker, this may have been due to animal fecal pollution sources which are not detected using the HF183 marker.

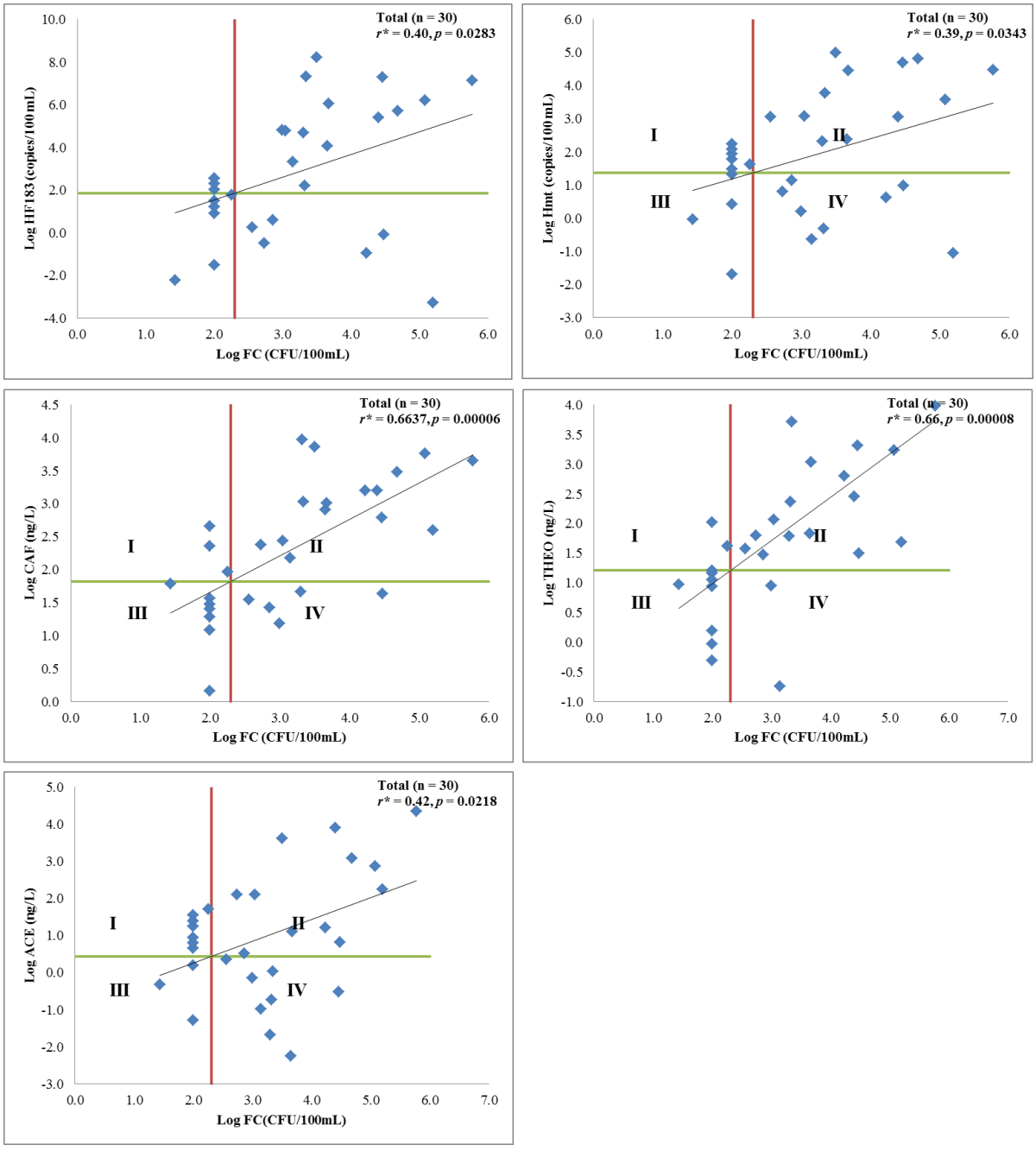

Figure 6.6. Linear regressions, with Pearson's Rank (*r*-values) and *p*-values provided, of fecal coliform CFU concentrations to other marker concentrations in samples. (*) denotes significance for correlations at $p < 0.05$.

6.7.3 References

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CHAPITRE 7 DISCUSSION GÉNÉRALE

L'objectif général de cette thèse était de développer une approche générale pour le dépistage des sources de contamination fécale dans les cours d'eau urbains qui aboutira à une meilleure priorisation de sources pour des mesures correctives. La première étape a été de développer des méthodes d'extraction et d'analyse des produits pharmaceutiques et de soins personnels (PPSPs) dans des matrices environnementales incluant les compartiments solides et aqueuses des trop-pleins et des cours d'eau urbains afin de répondre à deux des questions du projet : (1) quelles sont les concentrations réelles de PPSPs ciblés dans les sédiments des trop-pleins et des cours d'eau urbains et (2) quelle est l'importance de l'analyse de PPSPs dans les sédiments par rapport à leur analyse dans l'eau pour dépister les sources de contamination fécale d'origine humaine dans des sites à différents degrés de contamination. Par la suite, le développement de méthodes d'extraction et d'analyse de PPSPs particulaires et dissous nous a permis d'étudier un des processus (sorption) intervenant dans le devenir de cinq composés pharmaceutiques dans les trop-pleins en cas de forte pluie ou fonte des neiges. L'étude de la sorption s'est fait au travers de molécules modèles choisies en fonction de certains critères, citons entre autres, la variété de propriétés physico-chimiques, l'occurrence fréquente et l'atténuation faible ou raisonnable. Le but étant d'estimer si l'augmentation des concentrations en contaminants observée durant la surverse est associée à la remise en suspension de dépôts (sédiments) accumulés dans les égouts lors de l'augmentation du débit ou « first flush ». La dernière étape de ce projet a été de déterminer l'origine de la pollution fécale dans les eaux des réseaux d'égouts pluviaux des bassins versants urbains dotés des réseaux d'assainissements séparatifs en combinant des indicateurs classiques non discriminants et des marqueurs discriminants chimiques et moléculaires.

Cette discussion est présentée selon trois sous-thèmes. L'importance de la mise en place de mesures concrètes de PPSPs dans les sédiments parmi les outils utilisés visant à améliorer l'efficacité des programmes de surveillance de la qualité des eaux (Article 1). Le second sous-thème décrit, par la suite, les distributions et les cinétiques de cinq composés pharmaceutiques par l'étude en batch de leur sorption, avec une réflexion dans le contexte de l'origine de ces composés dans les surverses (Article 2). Le troisième sous-thème décrit la combinaison des indicateurs microbiologiques, chimiques et moléculaires pour dépister des raccordements

inversés dans deux territoires urbains à différentes activités afin d'établir des mesures de gestion des eaux usées non traitées provenant de raccordements inversés (Article 3). Les conclusions du projet de recherche ainsi que les recommandations concernant le dépistage des sources de contamination fécale dans les cours d'eau urbains sont présentées au dernier chapitre.

7.1 Développement de méthodes d'extraction et d'analyse des indicateurs chimiques utilisés pour le dépistage des sources de contamination fécale

En se basant sur les propriétés physico-chimiques des contaminants émergents d'origine humaine, les données de consommation, leur occurrence et leur ubiquité dans les compartiments environnementaux aquatiques et en prenant soin de représenter une certaine variété de ces contaminants indicateurs, dix molécules de huit classes différentes ont été sélectionnées pour le développement de méthodes d'extraction et d'analyse.

Différentes méthodes combinant les étapes d'extraction des matrices solides, de concentration et de purification des indicateurs émergents cibles sont disponibles dans la littérature. L'extraction en phase solide (SPE) assistée par ultrasons est l'une des approches les plus utilisées à cause de sa rapidité, sa simplicité et son économie. En parallèle, les méthodes analytiques ont connu des progrès remarquables au fil du temps et permettent de mesurer dans des matrices complexes de contaminants à l'état de traces, citons entre autres les méthodes chromatographiques en phase liquide couplées à la spectrométrie de masse en tandem (MS/MS) (Díaz-Cruz et al., 2009; Zuloaga et al., 2012).

Le principal défi de cette partie de mon projet réside dans le développement d'une méthode multi-résidus simple capable de faire face aux difficultés liées à la variété des propriétés physico-chimiques des composés ciblés, à l'extraction des matrices et aux effets d'interférences potentielles en spectrométrie de masse ainsi qu'à l'analyse de ces composés d'une façon précise et spécifique dans les compartiments solides et liquides par la même méthode analytique.

La méthode développée est validée dans des matrices de sédiments provenant des cours d'eau et des trop-pleins à travers l'évaluation des paramètres suivants : La limite de détection (LOD), le taux de recouvrement, les effets de matrices et la précision (dans une même journée ainsi que durant trois journées consécutives) exprimée en termes de %RSD. Par ailleurs, au meilleur de mes connaissances, la méthode développée dans cette étude a permis d'avoir une valeur de la

plus petite limite de détection pour les composés ciblés (Loffler & Ternes, 2003; Pérez-Carrera et al., 2010; Vazquez-Roig et al., 2010).

D'après les concentrations des composés ciblés mesurées dans les échantillons environnementaux (Table 4.3), on a trouvé que l'acétaminophène, l'aténolol, la caféine, la théophylline et la carbamazépine sont les plus importants composés à prendre en étude lors d'éventuels travaux ultérieurs.

La gamme dynamique, calculée avec le ratio de la concentration naturelle moyenne du composé sur la limite de détection et qui dépend du site et du compartiment étudiés ainsi que de la méthode d'analyse spécifique utilisée, a été estimée, pour la première fois, pour définir la fourchette de concentration dans laquelle l'analyte sera utilisé de façon fiable comme indicateur de la contamination fécale d'origine humaine dans l'environnement aquatique. Le calcul de la gamme dynamique permet de déterminer quel compartiment (solide ou liquide) devrait être analysé et quel composé serait le plus fiable à mesurer pour le dépistage des sources de contamination fécale. Par ailleurs, dans les cours d'eau ayant un facteur de dilution élevé et une gamme dynamique faible de PPSP, il faut prendre en considération l'étude de ce dernier dans les sédiments comme dans la colonne d'eau. Les meilleurs indicateurs de contamination fécale d'origine humaine à chercher sont la caféine dans la colonne d'eau et l'acétaminophène dans les sédiments (Figure 4.2).

7.2 Sorption des indicateurs chimiques dans les trop-pleins

Un des critères clés des traceurs de contamination fécale est leur distribution dans les compartiments environnementaux. Connaître leur distribution dans l'environnement aquatique est une donnée essentielle pour établir le programme d'échantillonnage et choisir les techniques d'analyse. Il n'existe pas d'informations dans la littérature sur la sorption et la distribution des indicateurs chimiques ciblés (ACE, CAF, THEO, CBZ et CBZ-DiOH) dans des matrices naturelles provenant des trop-pleins. Récemment, des études de sorption effectuées pour des marqueurs chimiques (citons entre autres la CAF et la CBZ) dans des échantillons dopés des boues usées provenant des STEP ont montré peu ou pas d'adsorption pour ces composés qui ont un faible K_{OW}. Le coefficient octanol-eau était un bon outil pour prédire leur adsorption. L'intensité d'adsorption a augmenté par l'augmentation du contenu organique. La désorption de ces composés était plus lente que leur adsorption et a nécessité plus que trois étapes de rinçage

pour qu'elle soit complète (Morissette et al., 2015). Dans notre étude, tous les composés sélectionnés sont des composés avec une faible affinité pour la phase particulaire. Parmi ces composés, la CBZ semble être le composé pharmaceutique le plus étudié dans la littérature pour déterminer ses coefficients de distribution dans des matrices complexes. Dans des boues primaires et secondaires, les valeurs de K_d de CBZ étaient négligeables, indiquant alors que ce composé n'a pas montré une adsorption de façon appréciable (Ternes et al., 2004). Les molécules de CBZ séquestrées dans les couches de l'argile de type smectite ont montré plus résistantes à la désorption par rapport à celles absorbées par les phases organiques (Zhang et al., 2010). Dans notre cas, l'adsorption de tous les composés était instantanée dans les deux matrices (SS et StS). L'effet de la matière organique du compartiment solide s'est traduit par des concentrations des composés plus élevées dans SS que dans StS (Figure 5.1b).

La désorption s'est fait pour des échantillons dopés et non dopés sous différentes conditions afin d'évaluer l'effet du dopage, de la dilution et de l'agitation sur la distribution et la cinétique de la désorption de ces indicateurs. À ma connaissance, il n'existe pas d'étude publiée sur la désorption des indicateurs chimiques dans des échantillons non dopés et imitant les conditions qui existent dans les trop-pleins. Les résultats obtenus ont montré que la désorption est un processus plus lent que l'adsorption. Les valeurs de $K_{OC,app}$ étaient plus élevées dans les échantillons dopés (Figure 5.2). Peu d'éléments de réponse sont disponibles pour expliquer ces résultats qui n'ont pas été obtenu dans les systèmes préalablement étudiés (Arp et al., 2009). Le dopage pourrait d'une part influencer la solubilité du contaminant ainsi que les sites de sorption par la présence du solvant (le méthanol). D'autre part, cette différence pourrait être expliquée par l'effet de matrice. Ceci est due à la présence de matière organique dissoute (MOD) comme co-soluté complexant et de colloïdes dans l'eau qui à leur tour peuvent affecter le devenir de contaminants hydrophobes, notamment le processus de leur adsorption sur la phase solide. La présence de MOD réduisait la désorption des composés ciblés comme proposé dans la littérature (Navon et al., 2011). Considérons que la matière organique dissoute et les colloïdes sont saturés en contaminants dans le système non dopé, l'addition de ces contaminants par dopage pourrait alors favoriser leur adsorption sur des particules grossières et par la suite les coefficients de distribution dans le système dopé seraient plus élevés.

Les différences entre les coefficients de distribution sous les trois conditions (sans agitation, avec agitation et avec dilution) dans les réacteurs non dopés n'étaient pas significatives pour la CAF,

la THEO et la CBZ (Table 5.12). La répétition de l'étape de rinçage a permis de favoriser un maximum de désorption et la diminution des concentrations des composés dans la phase aqueuse sous l'effet de la dilution était négligeable par rapport aux concentrations ajoutées par désorption. L'effet de la désorption prédomine alors sur celui de la dilution. La caféine et la théophylline ont montré des coefficients de désorption les moins élevés parmi les composés étudiés dans SS et StS, respectivement (Figure 5.15). Par ailleurs, CBZ et son métabolite CBZ-DiOH avaient les vitesses de désorption les plus rapides (Figure 5.3). La masse moléculaire du composé pharmaceutique était un des paramètres potentiels pour prédire sa vitesse de désorption. Plus la masse moléculaire est élevée, plus la désorption du composé est rapide (Figure 5.18).

Ces données nous renseignent sur les potentiels de relargage des composés étudiés à partir des solides de trop-pleins et peuvent être particulièrement intéressantes lorsqu'on s'intéresse au dépistage des sources de ces contaminants émergents. Ces composés peuvent être relargués dans la colonne d'eau s'ils entrent en contact avec une solution aqueuse plus propre, comme il peut être le cas avec les eaux de ruissellement dans les égouts unitaires lors des événements de forte précipitation ou de fonte des neiges. La CAF a été considérée comme un marqueur approprié pour les eaux usées non traitées provenant des débordements d'égouts unitaires ou des rejets directs (Buerge et al., 2006; Daneshvar et al., 2012). La CBZ-DiOH, la CBZ et la CAF étant des composés rapidement désorbés pourraient également être des bons marqueurs pour la contamination provenant de surverses d'égouts unitaires et plus spécifiquement de la remise en suspension des dépôts qui s'y trouvent.

7.3 Combinaison des indicateurs classiques et alternatifs (chimiques et moléculaires) pour le dépistage des sources de contamination fécale

La détection de raccordements inversés s'est faite à l'aide des paramètres indicateurs, citons entre autres, *E. coli* ($> 12 \times 10^3$ MPN 100 mL^{-1}), les streptocoques et les entérocoques ($> 5 \times 10^3$ MPN 100 mL^{-1}), l'ammoniac ($NH_3 > 0.3$ mg L^{-1}), l'orthophosphate (PO_4), le potassium (K) et leurs ratios ainsi que l'ion de bore ($B > 1.0$ mg L^{-1}) (Ellis & Butler, 2015; Panasiuk et al., 2015). À ce jour, il n'existe pas de normes quant au niveau des marqueurs pharmaceutiques et moléculaires acceptables pour l'utilisation de l'eau telles qu'elles existent pour *E. coli*. Sauvé et al. (2012) ont recommandé d'utiliser un seuil de caféine (400 ng L^{-1}) pour identifier les sites qui sont assurément contaminés par des coliformes fécaux (> 200 UFC 100mL^{-1}) (Sauvé et al., 2012). La

caféine toute seule ne peut pas être un outil de dépistage car elle peut être détectée dans l'eau sans qu'il y ait contamination par les égouts. Des déchets associés au café ou à la caféine comme des graines, des moulures, des mégôts de cigarettes, des canettes de boissons gazeuses, etc. peuvent expliquer la présence de la caféine dans l'eau (Seiler et al., 1999).

Notre étude avait pour but de développer une boîte à outils qui pourrait être intégrée à l'approche de gestion des eaux dans les systèmes d'égouts d'assainissement. Parmi le panel de marqueurs de contamination fécale d'origine humaine, les composés pharmaceutiques (ACE, CAF, THEO et CBZ) (Daneshvar et al., 2012; Peeler et al., 2006; Sidhu et al., 2013) ainsi que les marqueurs génétiques spécifiques à l'humain, soit le marqueur *Bacteroides* HF183 et l'ADN mitochondrial (Villemur et al., 2015) semblent être un bon candidat de par leur spécificité pour l'espèce humaine. Par ailleurs, les indicateurs classiques utilisés dans les études de dépistage comme *E. coli* et coliformes fécaux manquent de spécificité. Ils peuvent être d'origine animale, humaine ou même demeurer dans l'environnement par prolifération sous des conditions favorables.

Les résultats obtenus dans notre étude ont montré que la densité d'*E. coli* est plus élevée dans les secteurs résidentiels que dans les régions commerciales, institutionnelles et industrielles (Figure 6.2). De plus, dans les secteurs résidentiels, les indicateurs alternatifs ne sont pas tous corrélés à *E. coli* (Figure 6.3 et Table 6.10). Ce qui est expliqué par la présence des animaux domestiques qui peuvent être une source potentielle d'*E. coli* dans les secteurs résidentiels. Selon l'étude de (Wu, Rees, et al., 2011), les sources humaines étaient fréquemment observées dans les secteurs résidentiels et constituent plus que 30% des sources totales.

L'indice sanitaire développé dans cette étude permettra d'orienter les actions correctives dans les réseaux d'assainissement visant l'amélioration de la qualité de l'eau rejetée sans traitement dans les cours d'eau. Cet indice combine les six indicateurs spécifiques sélectionnés pour cette étude en une échelle de 0 à 1 (Table 6.3). Plus la valeur de l'indice est élevée, plus les chances que le raccordement inversé soit la source de la contamination fécale augmentent. L'application de cet indice sur un secteur problématique du réseau d'assainissement séparé à la l'île de Laval a permis avec succès de repérer les raccordements inversés (étude réalisée et présentée par ma collègue Mounia Hachad (en doctorat) en mars 2016 au *Salon des technologies environnementales du Québec* sous le titre de «*Dépistage de sources de contamination du réseau pluvial par l'emploi de méthodes analytiques innovatrices*»).

CHAPITRE 8 CONCLUSION ET RECOMMANDATIONS

Le dépistage des sources de contamination fécale est une discipline scientifique en émergence qui s'intéresse à la compréhension des sources particulières de contamination fécale ayant des impacts sur une zone donnée. La contamination fécale humaine représente le plus gros risque pour la santé humaine et sa détection est donc prioritaire en comparaison avec la contamination provenant des autres animaux. De nombreux outils de dépistage des sources microbiologiques, chimiques et moléculaires ont été décrits incluant ceux qui reposent sur une banque de matériel de référence et ceux qui sont indépendantes de telle banque de matériel. Aucun des outils actuellement utilisés pour la surveillance régulière de l'eau de surface n'est parfait. À cet égard, des conclusions concernant les outils et les indicateurs utilisés pour la détection de contamination fécale humaine ont été tirées de notre projet. Également, des recommandations ont été proposées au sujet du dépistage des sources de contamination fécale des cours d'eau urbains, afin de les intégrer dans les programmes de surveillance régulière.

8.1 Conclusions

Les conclusions relatives à la détermination simultanée de dix contaminants émergents (ACE, DIC, CBZ, ATL, CAF, THEO, PRO, MedP, APM et DEET) par une méthode multi-résidus basée sur LC/APCI-MS/MS sont les suivantes :

- La méthode développée est simple, sensible, rapide et se prête théoriquement à l'analyse des contaminants émergents provenant de différentes classes thérapeutiques et comprenant des produits pharmaceutiques, des produits de soins personnels et des hormones.
- L'applicabilité de la méthode développée a été confirmée par son application avec succès à des échantillons d'eaux et de sédiments des trop-pleins et des cours d'eau urbains.
- Ces contaminants se retrouvent dans les sédiments à des concentrations de l'ordre du 0.13 jusqu'à 427 ng g^{-1}.
- Ces contaminants proviennent majoritairement des eaux usées et pourraient également provenir de la remise en suspension des dépôts dans les trop-pleins ou des sédiments des cours d'eau.

- L’utilisation de contaminant émergent comme traceur de la contamination provenant d’égouts sanitaires dépend à la fois de la sensibilité analytique (par exemple, le ratio C:LOD) et de sa persistance dans la phase aqueuse (colonne d’eau) et dans la phase solide (sédiments).
- Pour la détection des sources de contamination fécale des cours d’eau urbains, l’analyse du contaminant émergent dans les sédiments est aussi importante que son analyse dans l’eau, surtout dans un cours d’eau ayant un potentiel de dilution élevé et une faible gamme dynamique de contaminant (C : LOD) dans la phase aqueuse.

Les conclusions relatives à la répartition et la dynamique de cinq produits pharmaceutiques (ACE, CAF, THEO, CBZ et CBZ-DiOH) dans les trop-pleins sont les suivantes :

- L’adsorption des produits pharmaceutiques testés (sauf CBZ) dans des matrices dopées était presque instantanée. Cependant, la cinétique de désorption ont montré que l'équilibre a été atteint soit rapidement (de l'ordre des heures) pour CBZ et son métabolite CBZ-DiOH ou lentement (au cours des jours) pour CAF et THEO. Les composés avec les masses moléculaires les plus élevées et les solubilités les plus bas (CBZ et CBZ-DiOH) ont eu les plus forts taux de désorption, contrairement à ce qui a été prévu.
- Les valeurs de $K_{d,app}$ des composés testés dans les sédiments non dopés et dopés étaient significativement différentes ($K_{d,app}$ dans les sédiments non dopés < $K_{d,app}$ dans les sédiments dopés). Ceci revient aux procédures de dopage qui pourraient affecter leur comportement de répartition ou à l’effet de matrice contenant de matière organique naturelle. Par ailleurs, les composés dans des matrices non dopées représentent le mieux leur comportement de répartition dans les systèmes réels.
- À l'échelle de laboratoire, les résultats suggèrent que lorsque des eaux de pluie sont ajoutées à des eaux usées, il y aura un déplacement de l'équilibre et une désorption des produits pharmaceutiques à partir de la phase solide vers la phase aqueuse. Ces résultats confirment ce qui a été observé en pleine échelle dans les débordements d'égouts unitaires par Madoux-Humery et al. (2013). Une augmentation des concentrations de produits pharmaceutiques a été observée dans la colonne d’eau au début des événements de pluie malgré la plus grande dilution par les eaux de ruissellement (Madoux-Humery et al., 2013). Le premier lessivage d’égouts a eu la plus grande influence sur le décrochage de la

pollution présente dans les conduites en comparaison avec la turbulence et la dilution ultérieure des eaux sanitaires par les eaux pluviales. La décharge des composés pharmaceutiques par débordements d'égouts unitaires non traités est plus élevée que prévue si on prend en considération l'effet de dilution. Ceci revient à la désorption des produits pharmaceutiques de la phase solide vers la phase aqueuse pour se trouver par la suite sans traitement dans l'environnement aquatique. Afin de diminuer ces charges en polluants rejetées par les égouts dans les milieux récepteurs, il serait utile de limiter la présence de dépôts dans les conduites par le rinçage des rues d'une part et le nettoyage du réseau d'assainissement d'autre part. Les programmes de suivi concentrés exclusivement sur la phase aqueuse manquent une fraction importante de concentrations totales de pharmaceutiques.

- CBZ-DiOH, CBZ puis CAF ont été désorbés plus rapidement que les autres composés et ils seraient donc des traceurs utiles pour étudier la dynamique des eaux usées des conduites de surverse influencées par les eaux pluviales incluant l'influence de la remise en suspension des sédiments.

Les conclusions relatives à la combinaison des indicateurs microbiologiques (FC et *E. coli*), chimiques (ACE, CAF, THEO et CBZ) et moléculaires (le marqueur 16S de *Bacteroides* HF183 et l'ADN mitochondrial humain Hmt) pour dépister des raccordements inversés dans deux territoires urbains à différentes activités sont les suivantes :

- Ces micropolluants ont été détectés dans tous les échantillons démontrant alors que la contamination fécale humaine est très répandue dans les systèmes d'égouts pluviaux étudiés grâce à des raccordements inversés.
- Des corrélations significatives ont été observées entre la présence de FC et les indicateurs alternatifs (HF183, Hmt, CAF, THEO et ACE), tandis que *E. coli* ont montré des corrélations significatives seulement avec CAF et THEO. Étant donné que la méthode utilisée pour mesurer FC était plus précise que celle utilisée pour *E. coli,* il a été possible de prendre en considération tous les indicateurs corrélés avec FC afin de trouver les meilleurs indicateurs pour l'identification de raccordements inversés.
- Les marqueurs spécifiques aux humains ont également été détectés dans les systèmes d'égouts pluviaux même dans les cas où les concentrations en *E. coli* étaient faibles (en

dessous de 235 MPN 100 mL^{-1}), ce qui illustre l'importance de combiner plusieurs marqueurs associés à des raccordements inversés, et pas seulement ceux qui sont associés avec les apports directs d'égouts qui sont fortement influencés par les modes de défécation humaines.

- Le marqueur de HF183 (100% le taux d'exactitude de classification) et la CAF (75% le taux d'exactitude de classification) sont les indicateurs les plus sensibles pour prédire les concentrations d'*E. coli* dans des échantillons d'égouts prélevés en temps sec qui sont au-dessous et au-dessus du seuil (235 CFU or MPN 100 mL^{-1}), respectivement.
- Des concentrations supérieures à 3 Log pour HF183, 2 Log pour Hmt, CAF, et Theo, et 1 Log pour ACE sont les seuils proposés pour identifier des raccordements inversés (les concentrations d'*E. coli* sont supérieures à 235 *E. coli* CFU 100 mL^{-1}). Donc, la valeur de référence standard de la caféine (400 ng L^{-1}) proposée par Sauvé et al. (2012) a été validée dans notre étude.
- L'utilisation d'un index, pour classer les sites pour la réhabilitation des raccordements inversés en combinant des marqueurs alternatifs, est plus précise que le seuil d'*E. coli* de 12000 UFC 100 mL^{-1} proposé par (Pitt, 2004) qui peut manquer de nombreux raccordements inversés, en particulier si l'échantillonnage est effectué à tout moment sauf au début de la journée. En outre, une contamination fécale mixte a été mise en évidence dans des sites ayant des concentrations en *E. coli* au-dessus de 12000 CFU 100 mL^{-1}.
- Des différences significatives entre les secteurs résidentiels et les secteurs ICI suggèrent que différentes stratégies devraient être utilisées lors de la surveillance des secteurs affectés par des raccordements inversés. Le moment de la journée de l'échantillonnage et les habitudes de défécation humaines pourraient être plus importants en ce qui concerne les concentrations d'*E. coli* dans les zones résidentielles. Ainsi des raccordements inversés seraient plus apparents dans les secteurs ICI toute la journée en utilisant des traceurs chimiques en comparaison avec les zones résidentielles où les intrants seraient plus transitoires. L'échantillonnage passif des indicateurs chimiques serait utile dans les zones résidentielles, alors qu'il ne semble pas être nécessaire dans les secteurs ICI.
- Les égouts pluviaux analysés ne représentent qu'une partie de l'ensemble du système d'égout pluvial étudié. 16/30 (53%) des égouts étudiés (8/16 (50%) de sites (moyen à

degré élevé de contamination fécale) avaient une contamination fécale humaine (forte concentration d'*E. coli*). Les méthodes et les seuils proposés sont pour la réalisation d'une analyse préliminaire de la contamination fécale humaine dans les réseaux d'égouts pluviaux. Une fois que les secteurs sont identifiés et priorisés, des enquêtes supplémentaires impliquant les propriétaires pourraient être menées pour identifier des connexions problématiques spécifiques.

- L'utilisation des indicateurs fécaux spécifiques aux animaux pourrait être utile dans les réseaux d'égouts urbains des zones résidentielles où se trouvent des animaux domestiques, mais ne semble pas être nécessaire dans les secteurs ICI.

8.2 Recommandations

Les recherches menées, à travers cette thèse, sur le dépistage des sources de contamination fécale dans les cours d'eau urbains permettent d'émettre des recommandations pour des futures recherches.

Les axes de recherches recommandés sont :

- Bien cerner le problème de contamination fécale avant d'envisager d'entreprendre une étude de dépistage des sources de pollution fécale.
- Les décisions concernant la conception d'un programme donné de surveillance des eaux de surface doivent prendre en compte les besoins propres et la situation particulière de la zone concernée ainsi que toute information passée pertinente.
- Attirer l'attention sur l'analyse des compartiments solides étant donné que l'analyse des indicateurs DSPM dans les compartiments aqueux n'est pas toujours évidente pour bien définir le problème de contamination fécale. Les compartiments solides risquent d'influer sur la qualité de l'eau ou permettent d'identifier des sources inattendues de pollution fécale.
- Limiter la remise en suspension des dépôts contaminés dans les systèmes d'égouts par des travaux de curage et de nettoyage. Plusieurs méthodes de nettoyage sont disponibles. Parmi ces méthodes citons entre autres, les méthodes de nettoyage hydraulique, nettoyage

mécanique et nettoyage par raclage. Des considérations et des précautions particulières sont à tenir en compte pour chaque méthode, selon l'état du réseau.

- Ajouter des valeurs de recommandation pour certains paramètres (comme des indicateurs chimiques et moléculaires spécifiques aux humains) aux valeurs déjà établies dans les programmes de suivi de la qualité des cours d'eau urbains.
- Combiner différents marqueurs spécifiques aux humains et aux animaux (surtout dans les zones résidentielles) pour discriminer de nombreuses sources potentielles de pollutions fécales

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