Methods and Devices for Evaluating Environmental Remediation Progress and Population Health

by

Erin Michelle Driver

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved October 2018 by the
Graduate Supervisory Committee:

Rolf Halden, Chair
Otakuye Conroy-Ben
Edward Kavazanjian
Rosa Krajmalnik-Brown

ARIZONA STATE UNIVERSITY

December 2018

ABSTRACT

This dissertation critically evaluated methodologies and devices for assessing and protecting the health of human populations, with particular emphasis on groundwater remediation and the use of wastewater-based epidemiology (WBE) to inform population health. A meta-analysis and assessment of laboratory-scale treatability studies for removing chlorinated solvents from groundwater found that sediment microcosms operated as continuous-flow columns are preferable to batch bottles when seeking to emulate with high fidelity the complex conditions prevailing in the subsurface in contaminated aquifers (Chapter 2). Compared to monitoring at the field-scale, use of column microcosms also showed *(i)* improved chemical speciation, and *(ii)* qualitative predictability of field parameters (Chapter 3). Monitoring of glucocorticoid hormones in wastewater of a university campus showed *(i)* elevated stress levels particularly at the start of the semester, *(ii)* on weekdays relative to weekend days ($p = 0.05$) (161 ± 42 μg d^{-1} per person, 122 ± 54 μg d^{-1} per person; $p \leq 0.05$), and *(iii)* a positive association between levels of stress hormones and nicotine (r_s: 0.49) and caffeine (0.63) consumption in this student population (Chapter 4). Also, *(i)* alcohol consumption determined by WBE was in line with literature estimates for this young sub-population (11.3 ± 7.5 g d^{-1} per person vs. 10.1 ± 0.8 g d^{-1} per person), whereas caffeine and nicotine uses were below (114 ± 49 g d^{-1} per person, 178 ± 19 g d^{-1} per person; 627 ± 219 g d^{-1} per person, 927 ± 243 g d^{-1} per person). The introduction of a novel continuous *in situ* sampler to WBE brought noted benefits relative to traditional time-integrated sampling, including *(i)* a higher sample coverage (93% vs. 3%), *(ii)* an ability to captured short-term analyte pulses (e.g., heroin, fentanyl, norbuprenorphine, and methadone), and *(iii)* an overall higher

mass capture for drugs of abuse like morphine, fentanyl, methamphetamine, amphetamine, and the opioid antagonist metabolite norbuprenorphine ($p \leq 0.01$). Methods and devices developed in this work are poised to find applications in the remediation sector and in human health assessments.

DEDICATION

This dissertation is dedicated to every stone along my path to this achievement.

ACKNOWLEDGMENTS

I am honored to have had the opportunity to work with the some of the most dedicated and innovative minds at Arizona State University and beyond. First and foremost I would like to thank my advisor, Dr. Rolf Halden, whom I am grateful to for providing me with diverse and stimulating research opportunities that have kept me engaged and challenged. His unique ability to discern the exceptional in the sea of mundane, inspires me to continually elevate my own perspective. I would also like to thank my committee members, Dr. Rosa Krajmalnik-Brown, Dr. Otak Conroy-Ben, and Dr. Ed Kavazanjian whose continued mentorship have provided invaluable insight and direction to my work.

I would also like to acknowledge my colleagues and classmates that have enriched my time at Arizona State University. In no particular order: Maurissa Charles, Isaac Roll, Sam Supowit, Joshua Steele, Adam Gushgari, Jing Chen, Arjun Venkatesan, Benny Pyke, Steven Hart, Olga Hart, Burcu Yavuz, Justin Kidd, Hansa McGee, Akash Sadaria, Alizée Jenck, Sara Murch, Marcia Spurlock, Zach Smith, Sepideh Taghdiri, Natalia Hoogesteign, Megan Altizer, Isaias Peraza, Charles Rolsky, Varun Kelkar, Megan Mauer, Aly Carlson, Nivi Biyani, Cayla Cook, Dustin Pollard, and Adam Thompson.

Chapters 2 and 3 were made possible by my industry collaborators Jeff Roberts of SiREM laboratories and Peter Dollar from Geosyntec consultants. I am forever grateful for their expertise in bench-scale treatability studies and remediation design, as well as their tireless patience and dedication to my success. I would also like to acknowledge Paul Hurst from Golder Associates for graciously sharing field-generated data from the

pilot-scale study, bringing Chapter 3 to fruition. Chapters 4 and 5 would not have been possible without the help of that city's Public Works Department. I would like to extend my gracious appreciation to Richard Dalton and Chris Garcia, and all participating field technicians, in their tireless commitment to sample collection. Their dedication to this project was nothing short of extraordinary. The *in situ* sampler presented in Chapter 6 was developed and manufactured with assistance from the Arizona State University, Fulton Schools of Engineering, Engineering Technical Services. I would like to thank the Instrument and Prototype Machine Shop and Electronics Shop for their help in fabrication and design. The field work conducted in the Chapter 6 was made possible with the cooperation of John Pinkston and Bhavika Bhakta from the municipal wastewater treatment facility.

This work has been funded in part by the following, to whom I extend my thanks:

- The United States Department of Defense Environmental Technology Certification Program (ESTCP), under project ER-200914
- The National Institute of Environmental Health Sciences, under R01ES015445 and R01ES020889
- The Virginia G. Piper Charitable Trust
- The School of Sustainable Engineering and the Built Environment at Arizona State University, through the Dean's Fellowship
- The Ira A. Fulton Schools of Engineering, through the Ira A. Fulton Fellowship
- Science Foundation Arizona, through the Graduate Research Fellowship
- Philanthropic Education Organization though the Scholar Award

TABLE OF CONTENTS

Page

List of Tables xii

List of Figures xiv

CHAPTER

1- INTRODUCTION 1

1.1 Treatability studies and groundwater remediation 1

1.1.1 Environmental remediation data gaps 2

1.2 Wastewater-based epidemiology and population health 2

1.2.1 Wastewater-based epidemiology data gaps 3

1.4 Primary goals and research strategy of this thesis 4

1.5 Hypotheses 6

1.6 Specific aims 7

TRANSITION 1 8

2 - COMPARATIVE META-ANALYSIS AND EXPERIMENTAL KINETIC INVESTIGATION OF COLUMN AND BATCH BOTTLE MICROCOSM TREATABILITY STUDIES INFORMING IN SITU GROUNDWATER REMEDIAL DESIGN 10

ABSTRACT 10

2.1 Introduction 11

CHAPTER Page

2.2 Materials and methods 14

2.2.1 Literature meta-analysis 14

2.2.2 Experimental investigation 14

2.2.2.1 Site media 14

2.2.3 Microcosm design 15

2.2.4 Chemical analysis 16

2.2.5 Data analysis 16

2.3 Results and discussion 17

2.3.1 Literature meta-analysis 17

2.3.2 Experimental application 19

2.3.2.1 VOC speciation during batch and column incubation 20

2.3.2.2 Transformation kinetics of batch and column microcosms 22

2.3.2.3 Contaminant mass converted in batch and column microcosms 23

2.3.2.4 Comparison of laboratory and meta-analysis data 24

2.3.2.5 Informational value of batch and column microcosms 25

2.3.3 Conceptual comparison of batch and column microcosms 27

2.3.4 Determination of amendment dosages and durability 28

2.3.5 Understanding data inconsistencies between batch and column microcosms 29

CHAPTER Page

2.3.6 Study limitations 31

2.3.7 Applicability of study results 32

2.4 Conclusions 34

TRANSITION 2 36

3 - RECONCILING DATA FROM LABORATORY FEASIBILITY AND PILOT-SCALE IN SITU CHEMICAL AND BIOLOGICAL REDUCTION IN FRACTURED BEDROCK: LESSONS LEARNED FROM PERCHLOROETHYENE-CONTAMINATED SITE 38

ABSTRACT 38

3.1 Introduction 39

3.2 Materials and methods 41

3.2.1 Field site 41

3.2.2 Treatability studies 41

3.3 Results 44

3.4 Discussion 49

3.5 Conclusion 57

TRANSITION 3 59

CHAPTER Page

4 - EXPANDING WASTEWATER-BASED EPIDEMIOLOGY BY USING GLUCOCORTICOID HORMONES IN SEWAGE AS INDICATORS OF POPULATION STRESS 61

ABSTRACT 61

4.1 Introduction 62

4.2 Materials and Methods 64

4.2.1 Chemicals and reagents 64

4.2.2 Study location 65

4.2.3 Sample collection and flow measurements 65

4.2.4 Sample processing 65

4.2.5 Sample analysis 66

4.2.6 Quality assurance and quality control 66

4.2.7 Data analysis 67

4.3 Results and discussion 69

4.3.1 Limitations 79

4.4 Conclusions 80

TRANSITION 4 82

5 - ALCOHOL, NICOTINE, AND CAFFEINE CONSUMPTION ON A PUBLIC U.S. UNIVERSITY CAMPUS DETERMINED BY WASTEWATER METROLOGY 83

CHAPTER Page

ABSTRACT 83

5.1 Introduction 84

5.2 Materials and methods 87

5.2.1 Chemicals and reagents 87

5.2.2 Study location 87

5.2.3 Sample collection and flow measurements 87

5.2.4 Sample processing 88

5.2.5 Sample analysis 89

5.2.6 Quality assurance/quality control 89

5.2.7 Data analysis 90

5.3 Results and discussion 91

5.3.1 Limitations 100

5.4 Conclusions 102

TRANSITION 5 103

6 - IN SITU ACTIVE SAMPLING TO DETERMINE COMMUNITY EXPOSURE USING WASTEWATER METROLOGY 105

ABSTRACT 105

6.1 Introduction 106

6.2 Materials and methods 109

CHAPTER Page

6.2.1 Chemicals and reagents 109

6.2.2 Sample location 110

6.2.3 Sample collection and handling 110

6.2.4 Sample processing 111

6.2.5 Sample analysis 112

6.2.6 Quality assurance and quality control 113

5.2.7 Data analysis 113

6.3 Results and discussion 114

7 - RESEARCH IMPLICATIONS AND RECOMMENDATIONS 124

7.1 Groundwater remediation assessments 124

7.2 Human population assessments 127

REFERENCES 132

APPENDIX

A- SUPPLEMENTAL MATERIAL FOR CHAPTER 2 155

B- SUPPLEMENTAL MATERIAL FOR CHAPTER 4 168

C- SUPPLEMENTAL MATERIAL FOR CHAPTER 5 175

D- SUPPLEMENTAL MATERIAL FOR CHAPTER 6 184

List of Tables

Table Page

1. Characteristics of batch bottle and continuous-flow column microcosms 33

2. Spearman nonparametric statistical analysis of variability for stress hormones 78

3. Average daily mass loads for discrete and continuous samplers..... 120

4. Databases used in treatability study literature review..... 157

5. Batch bottle and continuous-flow column construction details. 158

6. Limits of detection and recoveries for chlroinated solvent analytes..... 159

7. Selected journal articles from treatability study literature review. 160

8. Analyte concentrations in batch bottle and continuous-flow microcosms 164

9. Glucocorticoid mass spectrometry parameters. 169

10. Glucocorticoid analytical method parameters. 169

11. Calculated total mass load of glucocorticoids in literature..... 170

12. Daily wastewater flow on a university campus Fall 2017 171

13. Calculated campus population with biomarkers Fall 2017..... 172

14. Statistical comparisons for Mann-Whitney assessment of glucocorticoids..... 173

15. Measured analyte transitions and method detection limits for alcohol, caffeine, and nicotine..... 176

16. Daily wastewater flow on a university campus Fall 2017 – Spring 2018 177

17. Estimated campus population from biomarkers Fall 2017 – Spring 2018..... 179

18. Published survey data of alcohol, caffeine, and nicotine consumption estimates in college students..... 181

19. List of narcotic labeled standards. 185

Table Page

20. Licit and illicit compound method detection limits and recoveries. 186

21. Licit and illicit ompound excretion values..... 187

List of Figures

Figure Page

1. Ratio of continuous-flow column to batch bottle rate constants (k_{OBS}) from studies using both types of treatability study approaches 18

2. Schematics and photographs of the experimental setup of batch bottle microcosms and continuous-flow column microcosms 19

3. Dechlorination results in control and treatment microcosms operated in batch and continuous-flow mode 21

4. Box plots show first-order rate constants for PCE and its associated degradates in batch and column microcosms 26

5. Illustration of temporal and spatial oxidation-reduction zonation, phases of contaminant removal, and electron donor distribution in batch bottles and continuous-flow columns. 30

6. PCE DNAPL study site location. 45

7. PCE and degradates in sampled wells and continuous-flow columns 47

8. First-order rate plots generated for each monitoring and injection well 48

9. *Dehalococcoides (Dhc)* and reductive dehalogenase-encoding *vcrA* genes in groundwater samples. 49

10. Precipitation, oxidation reduciton potential, and dissolved oxygen at PCE study location 51

11. Mass flux and mass discharge through individual wells at the PCE study location... 56

12. Wastewater-derived glucocorticoid hormone metrics on a major U.S. university campus during the Fall semester of 2017 70

Figure Page

13. Correlation plot of cortisol and cortisone concentrations and rate loss analysis of cortisol and cortisone in wastewater 72

14. Box plot summarizing glucocorticoid occurrences detected in campus wastewater by day of the week, per month, and during weekdays versus weekends 76

15. Excretion profiles of analytes of interest for alcohol, caffeine, and nicotine consumption 92

16. Daily mass loads of indicators of alcohol, caffeine, and nicotine consumption on a major U.S. university campus during the 2017-2018 academic year..... 94

17. Box plot summarizing per capita alcohol, caffeine, and nicotine consumption in campus wastewater during the academic year, organized by month and day. 96

18. Correlation plots showing results of statistical comparisons of alcohol, caffeine, and nicotine consumption between months, days of the week, weekday/weekend, and semesters 99

19. *In Situ* Sampler (IS2) schematic and images. 114

20. Modeled concentrations of target analytes in wastewater and corresponding concentrations in each sampler design 116

21. Mass load comparisons between a discrete and near-continuous *in situ* extraction sampler 119

22. Per-capita consumption rates of licit and illicit drugs of interest generated the discrete and the near- continuous *in situ* sampler..... 122

23. Normal probability plot of column observed rate constants normalized to batch observed rate constants from combined studies captured in meta-analysis 167

Figure Page

24. Liquid chromatography gradient program for glucocorticoids..... 174

25. Concentrations of alcohol, caffeine, and nicotine metabolites on a university campus 182

26. Correlation plot of parent compound and metabolites indicative of alcohol, caffeine, and nicotine consumption in wastewater on a university campus..... 183

27. Flow rates generated by thenear-continuous *in situ* sampler. 188

28. Concentrations measured by the discrete auto-sampler. 189

29. Concentrations measured by the near-continuous auto-sampler. 190

CHAPTER 1

INTRODUCTION

Continued assessment of methodologies and devices for assessing the health of our natural environment and human populations are necessary to continue to innovate in these fields. One important discipline is soil and groundwater remediation, which began in earnest in the late 1970s with the establishment of the Resource Conservation and Recovery Act (1976) and the Comprehensive Environmental Response and Liability Act (1980); laws governing the cleanup of active and legacy hazardous waste sites in the United States (U.S.). The second is the newer discipline termed wastewater-based epidemiology (WBE), a sub-discipline of population of urban metabolism metrology, which focuses on human population health assessments using wastewater. Discussion of these fields are included herein.

1.1 Treatability studies and groundwater remediation

The methodology for hazardous waste remedial determinations includes the use of treatability (feasibility) studies to evaluate the performance, design and cost of potential remediation strategies before implementation [1]. The U.S. Environmental Protection Agency (EPA) offers treatability guidance documents designed to outline basic experimental parameters, however the specific approach taken and data required are not defined [2]. Bench-scale treatability studies commonly use batch bottle or continuous-flow column designs, and contain site geologic materials, groundwater, and the proposed remedial technology [3]. Data obtained from treatability studies include contaminant removal, which is used to develop transformation kinetics [4], often in the form of rate

constants [5]. These variables are subsequently used to populate models [6], which supply cleanup time estimates, and overall remediation costs.

1.1.1 Environmental remediation data gaps

No studies offered information on the use occurrence of batch bottle and continuous-flow column treatability studies, or the proclivity to generate and report reaction kinetics in dual testing situations. Additionally, no study provided an in-depth comparison between bench-scale treatability studies and subsequent field-scale implementation. A critical assessment of this relationship is needed to highlight areas of improvement, whether related to experimental design or data generation and use. These factors have the potential to improve the understanding of how *in situ* remedial activities may unfold and therefore represent an opportunity to improve field-scale remediation. This is especially critical for complex environments, which are defined as those containing recalcitrant or comingled contaminants, complex hydrogeology, or requiring remediation times greater than 100 years.

1.2 Wastewater-based epidemiology and population health

Wastewater-based epidemiology (WBE) provides near real-time information related to health status, lifestyle, and the behaviors of populations contributing to the sampled sewer system [7-9]. This approach measures excretion products of human metabolism in wastewater to estimate consumption patterns of licit and illicit substances (e.g., caffeine, heroin), inadvertent chemical exposure (e.g., antimicrobials), or other indicators of health (e.g., genetic biomarkers) [10-13]. As the field of WBE continues to gain momentum as an effective and trusted tool to assess population health, there is a need to expand and improve upon the basic methodologies. This includes: *(i)* the

expansion of WBE into classes of endogenous compounds that are indicative of certain facets of human health; *(ii)* targeted analysis of specific demographics within a sewershed that may benefit from this type of assessment; and *(iii)* the testing of different sampling methods for collecting analytes of interest from the wastewater.

1.2.1 Wastewater-based epidemiology data gaps

To date, no wastewater-based epidemiology study has assessed glucocorticoids (stress hormones) in wastewater as a method to assess population-level stress. However, these compounds are routinely measured in urine and feces to assess stress in both human behavioral studies and captive and wild animal populations [14-16]. Validating this type of method on the population-scale would be beneficial for human health, particularly because chronic stress is linked to the top six leading causes of death in the US [17].

Currently, WBE studies most often collect wastewater samples from a wastewater treatment plant, because it is a centralized collection point for a community and the sampling infrastructure is already in place for compliance monitoring purposes. Targeted sampling is relatively rare due to limited access points; however, targeted analysis allows for isolation of specific populations that would benefit most from this assessment. One such demographic group is college students, which are often cited for their predilection for over-indulgence. To date, assessments on three campuses have only focused on narcotic consumption [11, 18-20], whereas none have assessed the more common licit compounds that are known to be used and abused, specifically alcohol, nicotine, and caffeine.

As mentioned previously, WBE studies generally collect samples at the wastewater treatment plant, because of accessibly and ease. In almost all circumstances,

this involves a commercially available high-frequency discrete sampler that is designed to capture aliquots of wastewater based on specific time or flow parameters, and these aliquots then are composited over a 24-h time period [21, 22]. There are cited concerns that these samplers may miss pulses of target analytes [23], leading to an underestimation of consumption in the population; however, testing of alternative sample collection methods to date are extremely rare [24].

1.4 Primary goals and research strategy of this thesis

The goal of this dissertation was to critically evaluate the sampling and monitoring methods used to assess the health of the natural environment and human populations. These included bench-scale treatability studies designed to inform on groundwater remediation study design, and the measure of human metabolic excretion products in wastewater to assess human population health. Evaluation of these methods provided the opportunity to improve upon and expand the scope of these fields with respect to the spectrum of analytes and the liquid process streams subject to monitoring.

In the assessment of treatability studies, the strategy was to perform a meta-analysis focused on batch bottle and continuous-flow column treatability studies involving chlorinated solvents, with emphasis on reaction kinetic data generation and use. This dataset was supplemented with data from an experimental study, which involved the same competing treatability studies, executed for three distinct remediation strategies for the common groundwater and drinking water contaminant, perchloroethylene (PCE). Chemical speciation, reaction kinetics and mass removals were assessed and drivers behind variable outcomes were determined. Subsequently, a 6-month bench-scale PCE treatability study was performed, and results were compared to data generated from the

field-scale application of those amendments over a 14-month period. Comparisons were made in regards to chemical speciation, reaction kinetics, microbial counts, and mass fluxes.

In the assessment of methodologies related to population health by wastewater-based epidemiology, the first study moved beyond the conventional biomarkers of consumption commonly targeted by WBE, to monitor compounds endogenous to the human body, the glucocorticoid hormones, cortisol and cortisone. Daily composite samples were collected for seven consecutive days each month on a university campus during the first academic semester, including during the first week of classes and finals week. Statistical assessments were conducted to assess variability between months, days of the week, and weekends, and correlations determined between the hormones and biomarkers of commonly consumed psychoactive substances.

Subsequently, in this target demographic, a WBE study was performed to monitor metabolites of nicotine, alcohol, and caffeine consumption contained in wastewater from a university campus. Published pharmacokinetic data and population estimates were used to determine daily per capita consumption, and these wastewater-generated consumption estimates were compared to self-reported targeted demographic data, and U.S. national average statistics. Trends were assessed statistically within and between consumption behaviors to assess short and long-term consumption variability.

Finally, two competing sampler designs were selected, modified and deployed at a wastewater treatment plant to collect daily composite influent samples for seven consecutive days; this work included the deployment of a novel low-flow, near-continuous active sampler, and a traditional sampler performing time-weighted high

frequency discrete sampling. Captured analytes of interest included common WBE indicators of the consumption of opioids, opioid antagonists, licit and illicit stimulants, and alcohol. Analyte mass loads captured by the two different samplers were compared to assess differences. A modeling exercise was performed to illustrate the relationship between analyte concentrations (static and dynamic events), and the resultant concentration signal in each type of sampler.

1.5 Hypotheses

Hypotheses are outline below:

(i) batch bottle derived kinetic estimates for chlorinated solvent reduction are faster (higher observed rate constant) than those determined in continuous-flow columns;

(ii) bench-scale treatability kinetic estimates are significantly higher ($\alpha = 0.01$) than field-generated kinetic estimates;

(iii) per capita glucocorticoids measured in university-derived wastewater are higher during the first week of classes and finals week ($\alpha = 0.05$) over other sampled weeks;

(iv) per capita estimates of alcohol, caffeine, and nicotine consumption derived from campus-generated wastewater will not be significantly different ($\alpha = 0.05$) from published self-reported data for the target demographic or national average estimates; and

(v) mass load estimates derived from the continuous-sampler were significantly different ($\alpha = 0.01$) than mass loads generated by the discrete sampler.

1.6 Specific aims

Specific aims of this dissertation were to:

(i) Determine common approaches to the use and reporting of derived kinetics in batch bottle and continuous-flow column treatability studies, and the drivers of those differences;

(ii) Discern the relationship between remediation results of a bench-scale treatability study and subsequent field-scale implementation in a chlorinated solvent case study;

(iii) Determine the occurrence of the glucocorticoids cortisol and cortisone in community wastewater and assess trends in hormone levels as a function of known activities;

(iv) Measure indicators of alcohol, caffeine and nicotine consumption directly in wastewater and compare to per capita consumption determined by published self-reported data for the target demographic and U.S. national estimates; and

(v) Measure indicators of licit and illicit drugs in wastewater with two distinct sampler types (near-continuous, high frequency discrete), and compare mass loads and estimated consumption patterns generated by each.

TRANSITION 1

This dissertation is comprised of individual studies that critically evaluate methodologies and devices for environmental and population health assessments, including the selection and implementation of groundwater remediation strategies and human health monitoring using wastewater-based epidemiology.

In groundwater remediation studies, bench-scale treatability studies are a common tool used to determine the efficacy of competing remediation strategies, prior to field-scale implementation, to ensure the chosen technology will create the desired effect with site groundwater and sediments. Two of the most common types of studies include batch bottles and continuous-flow columns. Although treatability study guidelines are provided by government entities, individual practitioners largely define the route to completion. Beginning this research, there were no studies that offered information on the relative percentages of each type of study, the data streams generated, and how these data were being used, particularly kinetic data. Reaction kinetics arguably are the most important data stream, as degradation rates dictate contaminant removal times and with it overall remediation costs. Additionally, no study had assessed the similarities and/or differences between these types of treatability studies, and the implications for data usage degenerated by each.

In Chapter 2, a combined meta-analysis and laboratory experimental study was performed, focusing on batch bottles and continuous-flow columns, and their use in perchloroethylene (PCE) remediation. PCE is one of the most frequently encountered groundwater contaminants in the U.S. and individual site conditions largely drive treatment efficacy. The meta-analysis surveyed combined batch bottle and continuous-

flow column treatability studies, and the data use and generation as it pertained to reaction kinetics. Experimentally, three PCE treatments were evaluated in both treatability study types, and concentrations of PCE and associated downstream degradates, including trichloroethene (TCE), *cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene, 1,1-dichloroethene (1,2-DCE), vinyl chloride (VC), and ethene, were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Subsequent rate analyses and mass conversions were performed.

CHAPTER 2

COMPARATIVE META-ANALYSIS AND EXPERIMENTAL KINETIC INVESTIGATION OF COLUMN AND BATCH BOTTLE MICROCOSM TREATABILITY STUDIES INFORMING IN SITU GROUNDWATER REMEDIAL DESIGN

ABSTRACT

A systematic comparison was performed between batch bottle and continuous-flow column microcosms (BMs and CMs, respectively) commonly used for *in situ* groundwater remedial design. Review of recent literature (2000–2014) showed a preference for reporting batch kinetics, even when corresponding column data were available. Additionally, CMs produced higher observed rate constants, exceeding those of BMs by a factor of 6.1 ± 1.1 standard error. In a subsequent laboratory investigation, 12 equivalent microcosm pairs were constructed from fractured bedrock and perchloroethylene (PCE) impacted groundwater. First-order PCE transformation kinetics of CMs were 8.0 ± 4.8 times faster than BMs (rates: 1.23 ± 0.87 vs. 0.16 ± 0.05 d^{-1}, respectively). Additionally, CMs transformed 16.1 ± 8.0-times more mass than BMs owing to continuous-feed operation. CMs are concluded to yield more reliable kinetic estimates because of much higher data density stemming from long-term, steady-state conditions. Since information from BMs and CMs is valuable and complementary, treatability studies should report kinetic data from both when available. This first systematic investigation of BMs and CMs highlights the need for a more unified framework for data use and reporting in treatability studies informing decision-making for field-scale groundwater remediation.

2.1 Introduction

Despite significant remediation efforts over the last few decades by the United States and other developed nations, the number of hazardous waste sites remains considerable. Assessments conducted by the United States Environmental Protection Agency (U.S. EPA) concluded that 294,000 hazardous waste sites exist across the United States, with projected remediation costs amounting to more than $209 billion [25]. With some of the easiest to remediate sites now closed, a large number of challenging sites remain, estimated to require greater than 100 years for cleanup, and containing recalcitrant or comingled contaminants, typically in hydrogeologically complex environments [26]. In the US, the largest category of recalcitrant contaminants is halogenated volatile organic compounds (VOCs). This contaminant class comprises the highest percentage of sites on the US EPA's National Priorities List [sites eligible for remedial action under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) or Superfund program], and the largest class of organic contaminants detected at Department of Defense installations [25]. Chlorinated solvents, which are the prime contributor to this category, are particularly challenging to remediate because of their pronounced recalcitrance to (bio)transformation and ability to form difficult to locate dense non-aqueous phase liquid (DNAPL) point sources [26].

The methodology for hazardous waste site characterization and remedial determination, known as Remediation Investigation/Feasibility Study (RI/FS), is outlined in CERCLA [27]. Integral to this framework is the use of treatability studies (often referred to as feasibility studies), intended to evaluate the performance, design and cost of potential remediation strategies before implementation [1]. Treatability studies require

site geologic materials and ground-water to be tested with the proposed remedial technology, most commonly at the bench-scale. Although the US EPA offers treatability guidance documents designed to outline basic experimental parameters, a specific roadmap from inception to completion is not explicitly defined [2, 28, 29]. Thus the approach taken and data required to satisfy treatability study goals are open for interpretation.

Bench-scale treatability studies commonly use batch bottle or continuous-flow column designs to characterize and quantify contaminant changes in an experimental system as a proxy for *in situ* site conditions [3, 30, 31]. Batch microcosms (BMs), usually comprised of glass bottles with a narrow neck and orifice, are filled with geologic materials, site groundwater, amendments, and sealed with a gastight septum closure (closed systems). Batch bottle studies are the least expensive alternative in treatability studies and are the simplest to conduct [32-34]. Continuous-flow column microcosms (CMs) are commonly fabricated from glass or plastic cylinders, with sampling ports located at the inlet and outlet, and sometimes along the length of the column [35-37]. Columns are constructed with geologic material, solid amendments (optional), and groundwater is pumped through the column at a specified flow rate, typically in up-flow mode to remove trapped gases (open systems). Continuous-flow column experiments, although more expensive and challenging to operate, are known to be more representative of field conditions, by including the simulation of groundwater flow extant in the subsurface [38].

Data obtained from feasibility studies include the degree of removal (or sequestration) of the contaminant of interest, and are used to develop an understanding of

the transformation kinetics [4, 39, 40]. In studies where the contaminant is chemically or biologically transformed, kinetic data are often presented in the form of rate constants, specifically as first-order rate constants (k) and corresponding half-lives ($t_{1/2}$) [5, 41, 42]. Often, these calculated parameters are directly compared to those of other studies with similar experimental designs, in an effort to further substantiate the feasibility of the tested technology [43-45]. First-order rate constants are often used to populate projection models, which are integral in determining the fate and transport characteristics of the contaminants of interest [6, 46-48]. A kinetic analysis is arguably the most valuable calculated parameter because this approach supplies the time necessary for cleanup, which largely dictates overall remediation costs. However, the experimental design, type of data extracted, calculations completed, and the manner in which data are presented is not stipulated, thereby rendering it subject to considerations of time, money or other issues.

In this study, a meta-analysis of the scientific literature was performed to determine common approaches to the use and reporting of BM- and CM-derived kinetics. In addition, an experimental investigation was conducted to better understand fundamental differences in reaction kinetics derived from batch and column treatability studies. Experimental treatability studies were conducted using bedrock and groundwater impacted by perchloroethylene (PCE), one of the most frequently encountered recalcitrant groundwater contaminants in the US and around the world [49]. The fate of PCE in the environment is a function of prevailing physical, chemical and biological conditions at the cleanup site [50, 51], thus necessitating remedial design that is customized on a case-by-case basis informed by feasibility studies. Literature findings

and original experimental data on combined batch and column BMs and CMs treatability studies were completed to elucidate the benefits and limitations of each.

2.2 Materials and methods

2.2.1 Literature meta-analysis

A literature review was conducted using Arizona State University's OneSearch, which includes Web of Science, JSTOR, RefWorks and other sources (Appendix A: Table 4), to determine the number of combined batch and column chlorinated solvent treatability studies published in peer-reviewed sources. Search criteria included an aggregate of the following keywords and phrases: batch, column, dechlorination, and 'rate constant.' Search results were refined by excluding the following subject terms: atmospheric protection/air quality control/air pollution, limnology, soil science and conservation, waste-water, wastewater treatment, sludge, water purification, and water purification methods. Publication dates included only those articles published from 2000 to 2014. Of the total number of search results (sorted by relevance), 30% of the journal articles were selected randomly for evaluation. Studies captured in search results were excluded from analysis if they only employed analytical columns used in gas and liquid chromatography as opposed to microcosm columns (i.e., containing batch studies only).

2.2.2 Experimental investigation

2.2.2.1 Site media

Bedrock core and groundwater for microcosm experiments were collected from an industrial site in Ontario, Canada. Site groundwater was amended with PCE, trichloroethene (TCE) and *cis*-1,2-dichloroethene (*c*DCE) to concentrations of 57, 43 and 31 mg/L, respectively, for batch experiments and 57 mg/L PCE for columns.

Concentrations of these PCE, TCE, and *c*DCE at this particular site showed concentrations of 58 ± 61 mg/L, 39 ± 9 mg/L, and 17 ± 4 mg/L, respectively, in the most heavily impacted areas. These are similar to concentrations found at other sites contaminated with chlorinated solvents [52, 53].

2.2.3 Microcosm design

Unless otherwise stated, microcosm treatment amendments were sourced from Golder Associates Ltd. (Ottawa, ON), and included common chemical and biological amendments to promote chlorinated solvent reduction. Treatment 1 consisted of a proprietary Controlled-Release Carbon Source (CRCS A). Treatment 2 included a commercially available CRCS B/Microscale Zero Valent Iron (mZVI) blend known as EHC® (Peroxychem, Philadelphia, PA). Treatment 3 consisted of nanoscale ZVI (nZVI) with associated palladium acetate catalyst (TCI America, Boston, MA), and an organic carbon source (soy protein). Further details are included in Appendix A: Table 5.

Microcosms (Treatments 1-3) were bioaugmented with a commercial dechlorinating culture (KB-1®; SiREM, Guelph, ON) [54]. The chemical and biological treatments chosen are common amendments used for *in situ* groundwater remediation [55, 56]. Control microcosms were created in duplicate, and experimental treatments in triplicate, with the exception of Treatment 1 columns (duplicate). CMs were attached to a multi-channel ISMATEC peristaltic pump (IDEX Health and Science, Oak Harbor, WA), and operated with upward flow at a rate of 0.25 pore volumes per day (2.75 mL/day), or a 4-day residence time.

2.2.4 Chemical analysis

Aqueous samples were collected on a weekly to biweekly basis for analysis of PCE, TCE, *c*DCE, *trans*-1,2-dichloroethene (*t*DCE), 1,1-dichloroethene (1,1-DCE), vinyl chloride (VC) and ethene. Batch samples were analyzed using gas chromatography with a flame ionization detector (GC-FID) (Hewlett Packard 7890) (Hewlett Packard, Palo Alto, CA). Column samples were analyzed using headspace solid phase micro-extraction gas chromatography (SPME-GC-2010), with an FID and AOC-5000 auto-sampler (Shimadzu, Columbia, MD), using a previously published method [57]. Limits of detection and analyte recoveries are included in Appendix A: Table 6.

2.2.5 Data analysis

Rate constants were calculated for BMs using the linear regression method [33, 58]. First-order observed rate constants (k_{OBS}) for columns were calculated for each sampling period using the following equation:

$$-k_{OBS} = \frac{\ln\left(\frac{C}{C_0}\right)}{t} \qquad \text{Eq. 1}$$

where, C is effluent concentration, C_0 is influent concentration, and t is time. The statistical relationship between batch and column rate constants were analyzed in unpaired, 2-tailed, homoscedastic Student's t-test.

The total contaminant mass converted to ethene M_{Tot} in batch and column microcosms were calculated using Equation 2.

$$M_{Tot} = \left(\frac{C_{eth}}{C_{eff}}\right)(C_{in})\left(\frac{t}{\tau}\right)(V_{GW}) \qquad \text{Eq. 2}$$

where, C_{eth} is total effluent molar concentration of ethene, $C_{eff}$is effluent molar concentration of total (chloro)ethenes, C_{in} is initial concentration of chloroethenes, t is the study duration, τ is the residence time, and V_{GW} is the groundwater volume in each microcosm. In columns V_{GW} may be further defined as the product of the column volume $\pi r^2 h$ and porosity ϕ, therefore Equation 2 for continuous-flow columns may be written as follows:

$$M_{Tot} = \left(\frac{C_{eth}}{C_{eff}}\right)(C_{in})\left(\frac{t}{\tau}\right)(\pi r^2 h\, \phi) \qquad \text{Eq. 3}$$

2.3 Results and discussion

2.3.1 Literature meta-analysis

A literature search using the criteria presented, yielded 644 publications. Analysis of 30% of these articles (n = 200) revealed that only 20% (n = 40) (Appendix A: Table 7) included a combined study of treatability using both BMs and CMs; the remainder had erroneous hits for analytical rather than microcosm columns and dealt with batch studies only. Of the resultant combined batch and column studies, only 23% (n = 9) performed a kinetic analysis using both datasets (Figure 1). Examination of these studies revealed that in 8 of the 9 studies (89%), the observed rate constants in the columns were greater than those observed in batch systems. On average, column kinetics were greater by a factor of 6.1 ± 1.1 standard error than associated batch studies under similar experimental conditions.

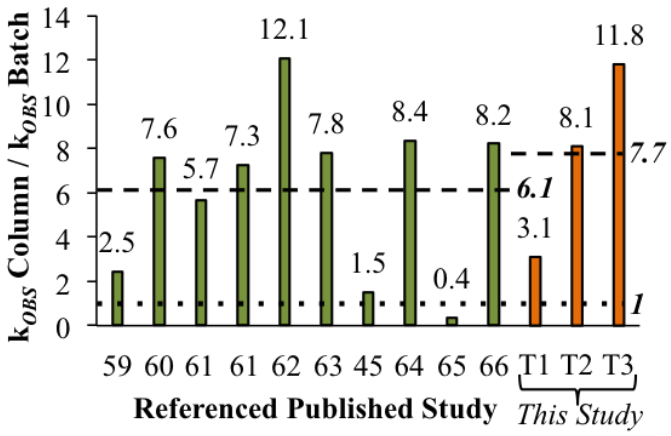


Fig. 1 - Ratio of continuous-flow column to batch bottle rate constants (k_{OBS}) from studies using both types of treatability study approaches. A ratio of unity (1) signals equivalent rates. In 8 of 9 referenced studies (89%), ratios were higher than 1, and published studies averaged a factor of 6.1 ± 1.1 standard error, indicating that columns consistently produced higher rate constants than comparable batch bottles [45, 59-66]. Experimental results show Treatments (T) were within the published range with an average of 7.7 ± 4.4

The literature review revealed batch studies to be more common than column studies, with only a small fraction actually using both BM and CM approaches jointly. This is not surprising, as batch bottles are comparatively simpler systems with fewer moving parts that are easier to manage and replicate [38]. Notably, only 23% of studies with combined use of BMs and CMs conducted, reported kinetic analysis results for both experimental approaches. In other words, in 77% of the studies, available data were not included in the final analysis and reporting. Results show an industry preference to rely on kinetic analyses from batch bottles even when complimentary continuous-flow column results are available. Additionally, only 1 of 9 papers reported both kinetic datasets and discussed the differences between the calculated rate constants. To better understand the benefits and limitations of the two approaches, we conducted an experimental comparison of both systems under standardized conditions.

2.3.2 Experimental application

Paired, matching BM and CM studies were conducted with equivalent experimental conditions for a duration of 146 days (Figure 2), and resultant (chloro)ethene species and associated first-order rate constants determined.

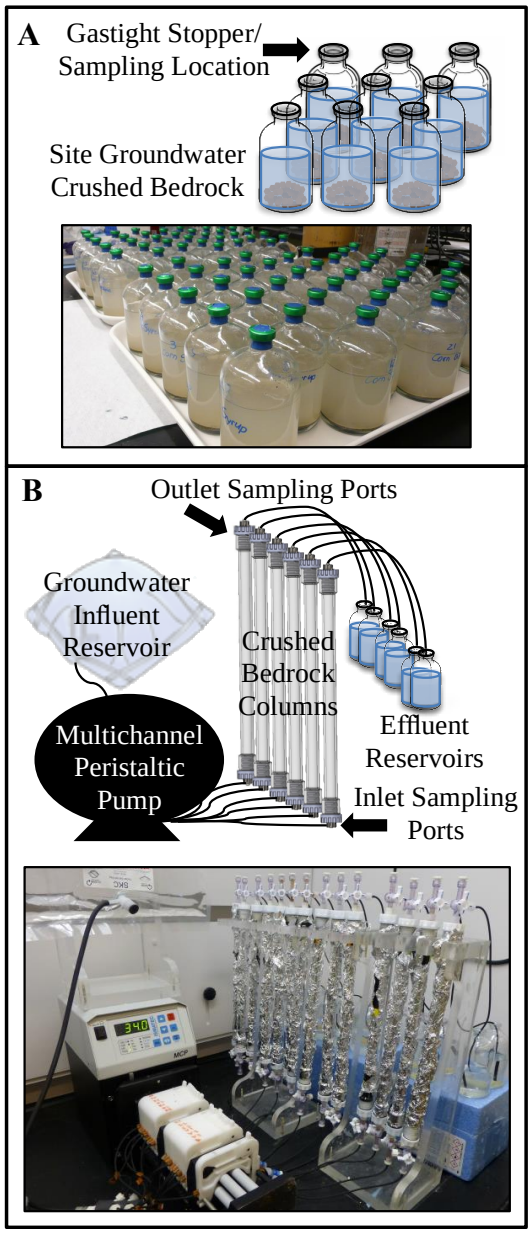


Fig. 2 – Schematics and photographs of the experimental setup of batch bottle microcosms (A) and continuous-flow column microcosms (B).

2.3.2.1 VOC speciation during batch and column incubation

Results from the biotic control and bioaugmentation treatments in BMs and CMs spanned the PCE degradation spectrum from negligible to complete dechlorination, similar to chloroethene treatability studies reported in the literature for laboratory and field investigations [67-69]. Here, chloroethene mole fractions obtained in batch systems showed higher percentages of lighter molecular-weight PCE transformation products compared to the respective column series (Figure 3, Appendix A: Table 8). This pattern relates to differences in chloroethene compound additions and the residence time in each treatability system. In batch, there is a finite input of contaminant into the system at the start of the study, and a residence time equivalent to the study duration (~150 days). In the column system, chloroethenes are continuously being introduced, with a residence time of only 4 days. The primary chloroethene(s) at study conclusion for BMs with Treatment 1 were 75% VC (PCE removed by Day 34), as compared to 45/40% TCE/PCE in the corresponding CMs. In Treatment 2, BMs and CMs displayed ethene production at 70% and 45%, respectively; however, PCE was removed by Day 41 in BMs, while 6% remained in column effluent at the study conclusion on Day 146. In Treatment 3, both microcosm pairs showed ~100% ethene production (PCE removed approximately by Day 75). These findings from equivalent microcosms confirmed data from the literature, indicating a similar pattern of higher production of PCE/TCE degradation products (e.g., *c*DCE, VC and ethene) in batch over column microcosms [61, 66].

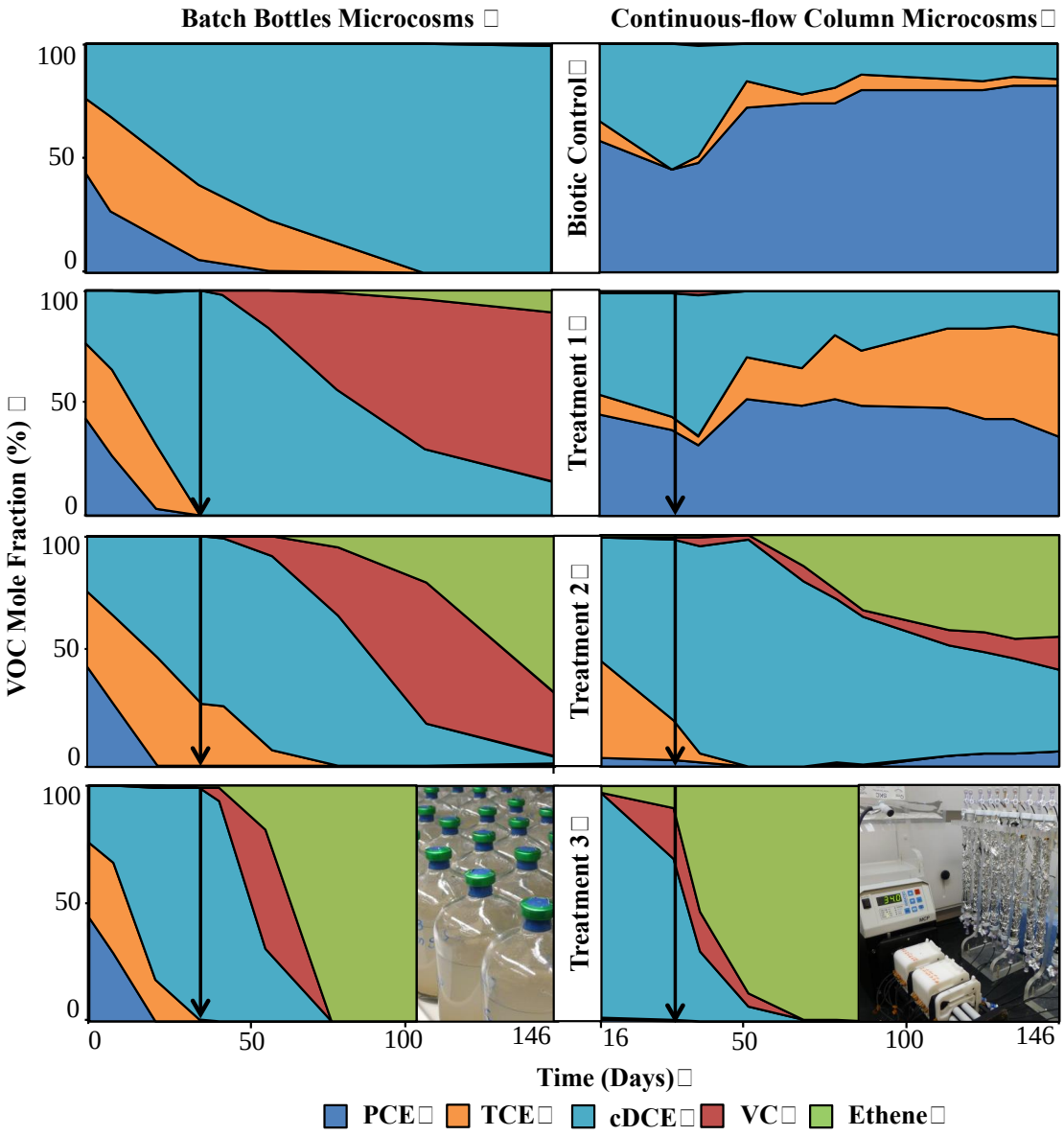


Fig. 3 - Dechlorination results in Control and Treatment microcosms operated in batch and continuous-flow mode. Results are shown as percent volatile organic compound (VOC) mole fraction throughout the study period. Arrows indicate inoculation of microcosms with a dechlorinating culture one month after start of incubation. Batch bottles with Treatment 3 were terminated after complete ethene production was achieved on Day 105. Monitoring of all columns commenced after a short stabilization period on Day 16.

2.3.2.2 Transformation kinetics of batch and column microcosms

Results within analogous experimental series showed CMs to have higher observed rate constants (i.e., faster reaction rates) than BMs for identical dechlorination patterns (Figure 4). First-order rate constants of PCE transformation (removal) in columns were 8.0 ± 4.8 times faster than those of batch bottles, 1.23 ± 0.87 vs. 0.16 ± 0.05 d^{-1}, respectively. On average, CMs in Treatment 1, 2 and 3 were 3.1 ± 1.9 (0.5 vs. 0.2 d^{-1}), 8.1 ± 4.1 (0.9 vs. 0.1 d^{-1}) and 11.8 ± 1.8 (2.1 vs. 0.2 d^{-1}) times faster than associated BMs, respectively. This relationship was statistically significant for each treatment: Treatment 1 ($p < 0.05$), Treatments 2 and 3 ($p < 0.001$).

Box and whisker plots served to inform a detailed, VOC species-resolved comparison of BMs and CMs, showing the 25th, 50th (median), and 75th quartiles with whiskers 1.5x the interquartile (IQR) range (Figure 4). Rate constant variation within column data was greater, as evidenced by the larger quartiles, standard deviations, and the presence of both minimum and maximum outliers. These differences in data variability are explained by the following columns properties: (*i*) greater number of data points; (*ii*) multiple rates per replicate (during steady-state formation); (*iii*) influent samples collected for each sample event, and (*iv*) first-order integrated rate law containing two variables (influent and effluent). These cited differences are a function of the rate calculation method used: linear regression method (batch) versus application of first-order integrated rate law (columns), which stems from inherent differences in physical structure and functionality.

2.3.2.3 Contaminant mass converted in batch and column microcosms

A direct comparison of the two experimental approaches revealed that the total mass of contaminant removed from BMs and CMs is not only dependent on the effective transformation activity, but also on the mass delivered to the system over the study duration. With a residence time equivalent to the experimental duration (here 146 days), each BM received a total VOC mass of 28 mg, or 11.4 mg PCE, 8.6 mg TCE and 6.2 mg *c*DCE (of 0.07; 0.07; and 0.06 millimoles (mmol), respectively). Total contaminant removals in batch Treatments 1 through 3 (determined by percent ethene production) were 6.0 ± 1.3 mg (0.21 ± 0.05 mmol), 23.6 ± 6.9 mg (0.84 ± 0.25 mmol), and 27.7 ± 0.05 mg (0.99 ± 0.002 mmol). The average removal across all treatments for batch bottles was 19 ± 10 mg (0.68 ± 0.36 mmol).

By comparison, CMs showed an average treatment removal of 314 ± 271 mg (11.2 ± 9.7 mmol) as ethene, a 16-fold increase in total contaminant conversion over the associated batch equivalents (19 ± 10 mg). Individually, column Treatments 1, 2 and 3 had total contaminant removals of 0 mg (no ethene production), 204 ± 100 mg (7.3 ± 3.6 mmol), and 632 ± 85 mg (22.5 ± 3.0 mmol), respectively. This increased removal in columns is largely driven by the 4-day residence time. The maximum contaminant mass for the batch systems is the mass supplied at the outset of the experiment, whereas in the column system, the total mass is the mass delivered per pore volume multiplied by the number of pore volumes processed during the study duration. Hence, CMs over time receive a higher overall mass of VOCs and therefore also can transform a comparatively larger mass over time. In this study, the maximum VOC removal per CMs was 656 mg

(23.4 mmol), or 23-times higher than that of its BM analog producing the same experimental outcome (rate) but transforming only a total of 28 mg (1.0 mmol).

2.3.2.4 Comparison of laboratory and meta-analysis data

The experimental data collected here on differences in average and maximum rate constants of PCE transformation (7.7 and 10.6, respectively) fell soundly within the range of values established in the literature meta-analysis (Figure 1). Discerning the inherent variability between batch and column experiments within the literature cited in the meta-analysis is challenging. Reasons for this include: *(i)* one or both microcosm types did not have a replicate [45]; *(ii)* replicates are not truly replicates with variation in an additional parameter (e.g., dissolved oxygen, temperature) [63-65]; *(iii)* replicate results are not presented [60, 61]; *(iv)* replicate results are presented in multiple graphs (instead of a single graph with error bars) [59, 61, 62, 66]; *(v)* replicate results are plotted together without discerning which replicate is which [66]. However in two studies, greater variability in columns data over batch data can qualitatively be discerned [59, 66], which agrees with the experimental results.

In the literature cited in the meta-analysis, the total mass of contaminant removed over the study duration was not included, however relevant metrics, when available, were extracted to estimate the total contaminant mass introduced into or pumped through the two microcosm systems. This number is not indicative of mass removed, but provides a theoretical maximum assuming complete conversion by the selected treatment. In each instance where estimations were possible, results showed that CMs processed greater total contaminant masses than the associated BMs, agreeing with the results of the

present study [59, 61, 62, 66]. A 2011 study showed masses of 11 mg for batch systems and approximately 600 to 2300 mg for the columns (range is a function of flow rate variation) [61]. A 2007 study had calculated masses of 2 to 12 mg for batch as compared to approximately 300 to 3000 mg for columns [59]. Similarly, a 2009 study had calculated masses for batch and columns of 0.2 mg and 75 mg, respectively. Differences in total contaminant mass in the cited treatability studies ranged between 10- and 106-fold. The present study is on the lower range of this spectrum because of the comparatively low flow rates [62].

2.3.2.5 Informational value of batch and column microcosms

The number of data points (*n*) used in kinetic calculations for microcosms varied from 6 to 36 for columns to from 2 to 3 for batch bottles (Figure 4). Data density for BMs were considerably lower than for columns because of inherent design differences, where the number of rate constants is equivalent to the number of bottle replicates in the series. This method of batch rate calculation is common and few rate constants are generated, typically, one rate per bottle [53-55]. In CMs, each discrete sampling point produced a unique rate, calculated based on the delta between influent and effluent concentration; therefore *n* was determined by the number of sampling points occurring after steady-state was achieved. The higher number of data points generated in columns creates what amounts to more reliable estimates for maximum and sustained rates thus may be considered a better predictor of long-term *in situ* kinetic activity.

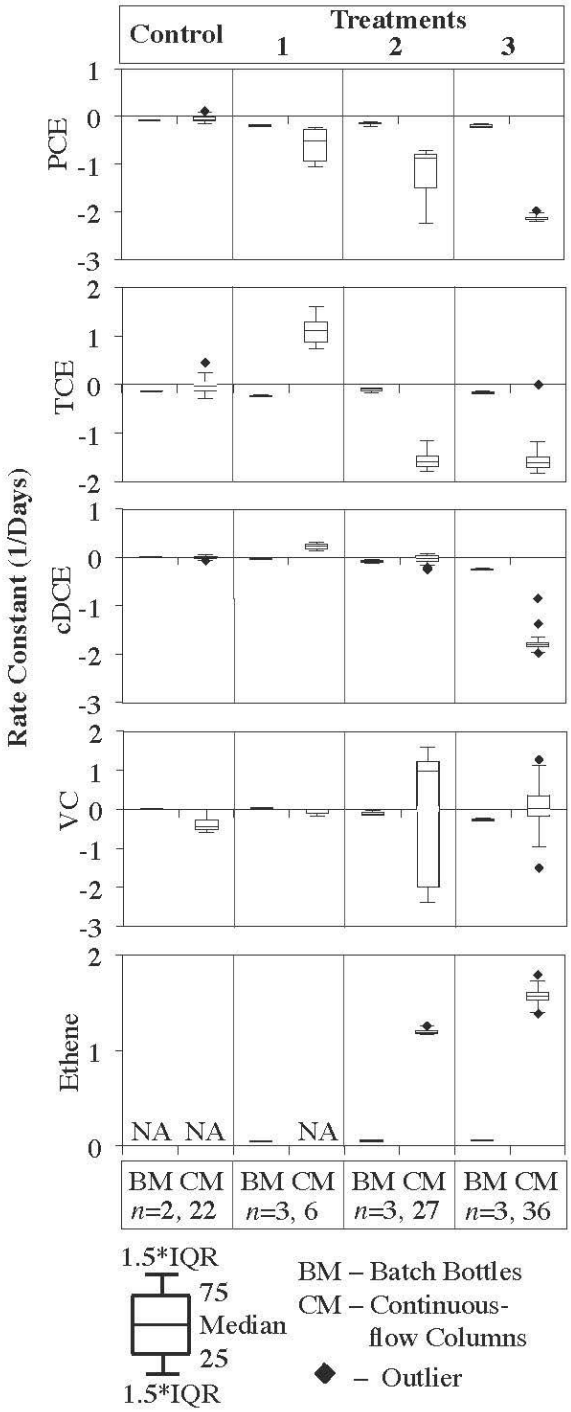


Fig. 4 - Box plots show first-order rate constants for PCE and its associated degradates in batch and column microcosms. Negative rate constants signify removal of the compound whereas positive rate constants signify compound production. Control batch microcosms yielded only the two data points shown here. Note: NA - Not applicable

2.3.3 Conceptual comparison of batch and column microcosms

Batch bottles and continuous-flow columns are inherently different by design and consequently produce not only dynamically different system conditions but also variant kinetic results, as shown in this study and in the literature. In BMs, the system is static (no flow), as compared to the flow-through design of CMs; this difference is often cited as the reason for differences between the two microcosms [70]. We conclude that in addition to differences in flow regime, the closed system/finite contaminant input of the batch bottle versus the open system/continuous contaminant input, drives the variability. Open and closed system designs produce different oxidation-reduction potentials [62, 71], particularly a highly reduced system (over time) in BMs as the finite mass of electron acceptors (O2, NO_2^-, VOCs) are consumed in sequential, hierarchical fashion. This is juxtaposed to the variable reducing conditions in columns (temporally and spatially), which depend on influent storage and flow rate (Figure 5). With the closed system of BMs, the residence time is synonymous with study duration, therefore with analogous amendment concentrations and dechlorination ability, columns passing multiple pore volumes will by default encounter and remove more contaminant mass.

Batch and column microcosms show variation in the ability to illustrate the phases of contaminant removal including: lag, acceleration, steady-state and decline (Figure 5). In both microcosm types, the occurrence of a lag phase is visible. In BMs, the lag phase presents as persistent (near)-initial contaminant concentrations after study inception, and in CMs it is displayed as near-identical influent and effluent concentrations. Discerning the acceleration and steady-state phases in BMs is challenging. Typically, the decline in amendment concentrations as a whole is considered the acceleration phase [72, 73]. Since

the ratio of parent to product compounds is constantly changing in this type of system [74, 75], steady-state can never be attained. In contrast, the acceleration phase in CMs typically shows notable variability in effluent concentrations between adjacent sampling events. As steady-state conditions are achieved, there is a high reproducibility in the delta of influent and effluent concentrations during adjacent sampling periods, which can be observed for extended periods of weeks or months. It is important to note that performing batch microcosm experiments with repeated influent injections in a semi-continuous fashion can create steady-state conditions [76], however, this study was interested in the strict closed system design. The final phase, decline (end of amendment lifetime) cannot usually be separated from an initial degradation sequence in BMs and requires subsequent chemical contaminant additions to the system. In CMs however, end of performance is signaled as a reappearance (break-through) of the contaminant of interest in column effluent.

2.3.4 Determination of amendment dosages and durability

The literature review showed that electron donor dosages for field studies are often selected based on results from batch bottle studies. This may be problematic, however, when considering the addition of controlled-release carbon sources (CRCS). In BMs, the concentration of electron donors from CRCS is a function of the release rate and the length of the lag phase, with the latter allowing for a potential accumulation of donors. In CMs in which the CRCS are packed into the column (as done in this study), electron donor concentrations are expected to be less variable, as they cannot accumulate due to the continuous flushing of the column (Figure 5). With this experimental design, the practitioner can observe the transformation activity resulting from the processing of

larger volumes of fluid, which is expected to be more similar to conditions prevailing at the field-scale during CRCS subsurface injections. Consequently, the effectiveness and durability of slow-release compounds may be best assessed through the use of continuous-flow columns. The differences observed in dechlorination activity between BMs and CMs in the Treatment 1 series (and in a limited fashion in Treatment 2) may have been related to the above phenomenon. Due to the delay of inoculation with the bioaugmentation agent by one month (necessitated by the requirement of the bioaugmentation agent for strictly anaerobic conditions), it is reasonable to expect that the available concentration of electron donors derived from the CRCSs was higher in batch bottles than in continuous-flow systems.

2.3.5 Understanding data inconsistencies between batch and column microcosms

The overall faster rates observed in CMs relative to equivalent BMs are not surprising. From a microbiological standpoint, continuous-flow systems typically yield better overall performance because they result in adaptation and attainment of steady-state conditions that favor mass removal [61, 77]. Moreover, flow through the column does not allow for the accumulation of waste products of microbial metabolism, which could decrease biodegradation performance. Additionally, a spectrum of redox conditions can be established in columns, with different types of microbes co-existing and co-transforming contaminants at the same time but in different physical locations along the length of the column. Therefore, columns harbor a spatially expansive diversity of microbial consortia, all potentially contributing to successful contaminant removal [69, 78]. In batch bottles, the same reactions occur, but they are temporally constrained rather than spatially contiguous [79].

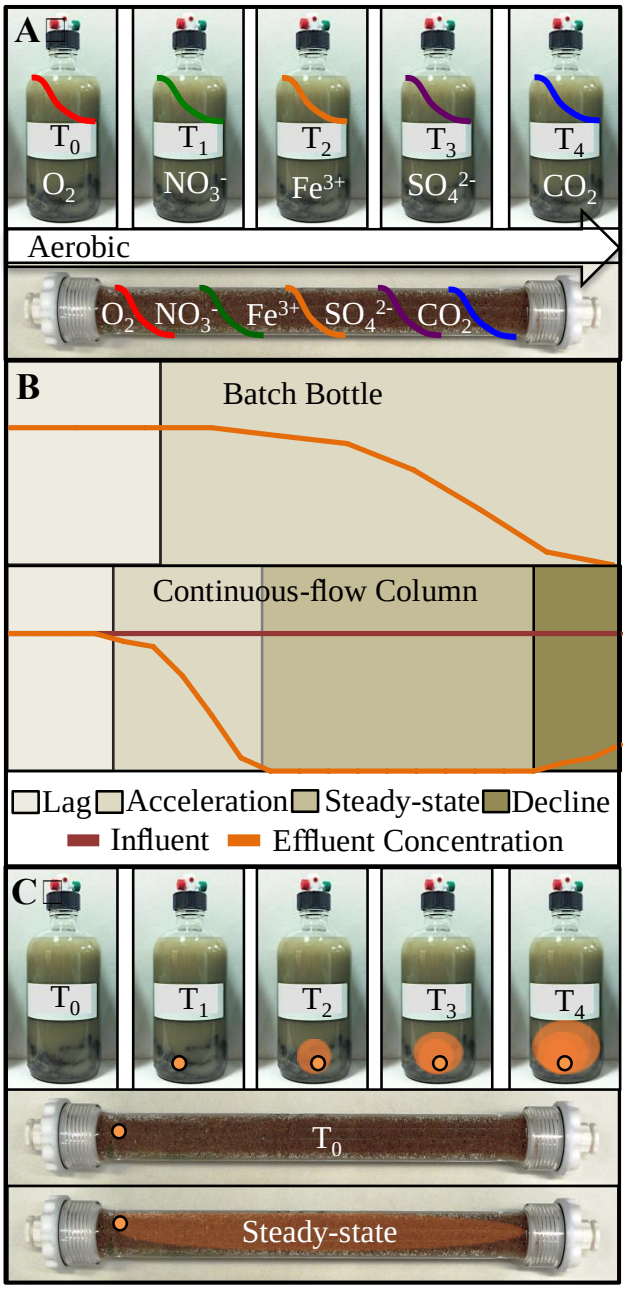


Fig. 5 - Illustration of temporal and spatial oxidation-reduction zonation (A), phases of contaminant removal (B), and electron donor distribution (C) in batch bottles and continuous-flow columns. Modified from McClellan, 2013 [71].

2.3.6 Study limitations

For reasons of practicality, only 30% of the total number of published articles captured by search engine criteria were examined in detail; therefore, the ratio of batch to column degradation rates derived here may differ slightly from the comprehensive, true value representing all studies. However, the data are normally distributed which suggests that extracted values from captured results constitute a representative sample of the entirety of data (Appendix A: Figure 23). The selected field location was a fractured bedrock site and constructing representative microcosms from such materials presents a challenge. Here, we adopted use of crushed bedrock cores, leaving open the possibility of creating reactive surfaces not representative of weathered rock in place *in situ.* However, the goal of the work was to compare rates from batch bottle and column microcosms, and since both were constructed with the same material, no adverse impact from this experimental design is expected. In laboratory experiments, as is typical for long-term operation of column studies, prolonged storage of groundwater was necessary. Error was decreased by bimonthly preparation of PCE-amended influent to achieve the desired cVOC concentration.

Authors focused on a comparison of observed rate constants in this study; alternative approaches can be envisioned, such as normalizing transformation activity by parameters of known importance, e.g., surface area and or biomass. Both approaches potentially can be of value but also are flawed by the fact that neither parameter can be determined with great certainty for *in situ* settings in the field, to which the rates ultimately are applied.

2.3.7 Applicability of study results

Whereas many of the aspects discussed above and captured in Table 1 should be broadly applicable for feasibility determinations, care must be taken when extrapolating the results of this work to other situations, such as different redox conditions (e.g., aerobic conditions) and other contaminants. For cVOC removal under anoxic conditions, we established that BMs tend to produce a higher percentage of lighter molecular weight PCE byproducts relative to CMs, and that columns tend to show faster kinetics than batch. Thus, if only batch bottle kinetics are available, conservative rate estimates result that may be lower than actually attainable rates. Total mass removal in columns is greater than in batch systems under equivalent starting conditions, because in columns, the contaminants are constantly replenished by influent flow. Since CMs can attain and maintain steady-state conditions, they yield a higher number of data points than BMs, which should apply broadly beyond VOC scenarios investigated here. The conclusion concerning the better utility of columns for determining the dosage and durability of slow-release compounds also is expected to extend to other electron donors formulations and contaminant scenarios. In studies examining the impact of highly reducing redox conditions, batch bottles are the methodology of choice because maintaining such conditions in flow-through columns is challenging.

Table 1 - Characteristics of batch bottle and continuous-flow column microcosms

Classification	Parameter	Batch Bottles	Columns
General	**System Design**	Closed	Open
	Reducing Conditions	Unlimited Even When Initially Aerobic	Limited By Residence Time When Using Aerobic Influent Stream
	Groundwater	Static	Flow
	Contaminant Input	Finite or Pulsed	Continuous or Dynamic
Contaminant Transformation	**Lag Phase (if present)**	Visible	Visible
	Acceleration Phase	Visible	Visible
	Steady-State Formation	No	Yes
	Amendment Lifetime Estimate	Limited by Lack of Flow	Reflective of *In Situ* Conditions
Contaminant Transformation Rates	**Rate Constant (Relative)**	Lower	Higher
	Number of Data Points Available	Function of Number of Replicates and Re-spiking Events	Unlimited Sampling Opportunities During Steady-state
Mass Converted	**Total Mass**	Lower than CMs	Higher than BMs

2.4 Conclusions

Given the differences between batch bottle and continuous-flow column studies established herein and summarized above, it is desirable to establish guidelines for treatability studies. It is imperative to evaluate the behavior of both open and closed system treatability studies to understand how the inherent characteristics of each system may affect the overall capability of the amendment to be remediated within the subsurface. This appears to be particularly important for controlled-release carbon sources, whose disparate pattern of activity in batch and column studies (poorer initial performance in columns), suggests reliance on batch data may overestimate *in situ* dechlorination activity if electron and carbon donors are allowed to accumulate over time in bottles, a scenario that does not accurately mimic flow-through, real-world conditions. Determining both the chemical speciation and biotransformation kinetics of both treatability systems are equally important not only because the systems are inherently different, but because resultant rate data may not be initially intuitive from degree of speciation (e.g., higher percentage of downstream degradates does not equate to faster removal rates overall). One neglected parameter, total mass removed over the study duration, should also be included in the analysis because proper estimations of amendment lifetimes are important for field-scale applications to ensure costs are minimized, results maximized, and remediation completed in a timely manner. Additionally, determinations of total mass may elucidate reasons for variability between batch and continuous microcosms (e.g., continuous systems likely processing more mass). When possible, it is also important to elucidate important variables in the field to ensure proper experimental design (e.g., *in situ* analyte concentrations), and important

controls post-treatability study (e.g., amendment dosages, flow rates, etc.) to determine implications for field-scale application. Because the quality of data extracted from treatability studies is the foundation for remedial design associated with thousands of hazardous waste sites across the country, it is necessary to begin the remedial process with a standardized and sound scientific approach.

TRANSITION 2

Comparisons between common bench-scale treatability studies, used to inform decisions on full-scale field remediation activities, were explored in detail in the previous chapter. Critical differences were highlighted, including the inherent drivers behind those differences and the implications of data generation and usage. Similarly to the data gaps between different types of treatability studies, there also exists a deficit of information regarding the relationship between the results of bench-scale treatability studies and those generated by field-scale technology implementation. A critical assessment of this relationship is needed to highlight areas of improvement, whether related to experimental design or the generation and use of data. These factors have the potential to improve a practitioner's understanding of how *in situ* remedial activities may unfold and, therefore, how to improve field-scale remediation. This is especially critical for complex environments, including those with recalcitrant contaminants or complex geology, which represent a large portion of remaining hazardous waste sites in the United States.

In Chapter 3, a treatability study was performed in the laboratory over six months, for a site contaminated with PCE in the form of a dense non-aqueous phase liquid (DNAPL). Practitioners at the site wanted to reduce cleanup times over the currently deployed pump-and-treat system. Results from the treatability study were subsequently compared to data generated over 14 months by groundwater sampling from the field site, where the previously tested chemical and biological amendments were injected into the subsurface multiple times. Groundwater samples were analyzed for chemical species PCE, TCE, *c*DCE, *t*DCE, 1,1-DCE, VC and ethene by gas chromatography; additionally, microbial parameters including *Dehalococcoides* (*Dhc*) and the functional gene encoding

for the vinyl chloride reductase (*vcrA*) were determined by quantitative polymerase chain reaction assays. Subsequent comparisons of speciation, reaction kinetics, microbial counts, and mass fluxes also were performed.

CHAPTER 3

RECONCILING DATA FROM LABORATORY FEASIBILITY AND PILOT-SCALE IN SITU CHEMICAL AND BIOLOGICAL REDUCTION IN FRACTURED BEDROCK: LESSONS LEARNED FROM PERCHLOROETHYENE-CONTAMINATED SITE

ABSTRACT

Dense non-aqueous phase liquid (DNAPL) in fractured bedrock aquifers can be particularly challenging to remediate. Prior to *in situ* field-scale remedial applications, bench-scale laboratory testing is completed to ensure compatibility of amendments with site groundwater chemistry, geology, and microbiology. This study sought to evaluate the relationship between data from laboratory treatability studies and pilot-scale remediation of perchloroethylene (PCE) using i*n situ* chemical and biological reduction of DNAPL source zones, with a particular focus on fractured bedrock sites. For this North American study location, PCE speciation in continuous-flow laboratory columns showed 62% ethene production by the study conclusion (195 days). In the field, multiple field injections showed qualitative similarities in PCE reduction to the laboratory study, with a general reduction of PCE to *cis*-1,2-dichloroethene with observed increases in vinyl chloride and ethene throughout the 433 day sampling campaign. First-order degradation rate constants observed in the lab were 0.71 ± 0.04 d^{-1} for the summation of chlorinated volatile organic compounds (cVOCs) (n = 29), while field data showed spatial and temporal variability with post-injection degradation rate constants from <0.01 to 0.04 d^{-1} (n = 5). In the field, *Dehalococcoides* (*Dhc*) and vinyl chloride reductase (*vcrA*) counts determined by quantitative polymerase chain reaction, were 6E+7 cells L^{-1} (max 2E+8

cells L^{-1}) (n = 8) and 8E+7 cells L^{-1} (max 3E+8 cells L^{-1}) (n = 16), respectively. Interpretation of the study results and literature expounding similar remediation feasibility activities provided more broadly applicable insights and lessons learned. In general, quantitative variability in lab and field results at bedrock sites can be attributed in part to barriers in recreating in the laboratory the *(i)* groundwater chemistry (e.g., field fluctuations in redox conditions and uneven distribution of remediation agents *in situ*); and *(ii)* field geology (i.e., representative bedrock columns) of the remediation site. Generally acceptable metrics in laboratory studies do not translate well to field-scale applications. Possible actions aimed at improving the informational value of laboratory feasibility studies include an assessment of mass flux and/or mass discharge to gauge source strength and remedial progress.

3.1 Introduction

Data from the United States Environmental Protection Agency (U.S. EPA) show that approximately 300,000 hazardous waste sites currently exist in the United States [80]. Of these sites, many are classified as complex, requiring greater than 100 years for successful remediation and site closure [26]. The classification of complex is often based on factors such as the local (hydro)geology (e.g., fractured bedrock) or the variety and distribution of contaminants at the site (e.g., mixed class contaminants). One of the largest and most pervasive contaminant classes found at hazardous waste locations are halogenated volatile organic compounds. Of this class PCE and trichloroethylene (TCE) are two of the most common chemical species based on their ubiquitous industrial applications and relatively low costs [26]. Remediation of sites containing these compounds are often complicated by the solubility and specific gravity of these chemical

species that allow for DNAPL formation [81]. DNAPL generally increases the total time necessary to obtain site closure, and consequently is an impediment to site redevelopment. DNAPL serves as a continuous source of contamination for upgradient pristine groundwater supplies, creating a continuous downgradient dissolved plume until DNAPL dissolution is complete. In an effort to decrease the overall remedial timeframe, *in situ* remediation of the DNAPL source may be attempted. This directed effort typically involves emplacement of high concentrations of amendments into the source, to increase transformation and dissolution of the DNAPL [82]. As with all *in situ* remediation design considerations, extensive bench-scale laboratory testing is first completed to ensure feasibility of the amendments with site groundwater and geologic materials. After lab-scale validation, small-scale pilot remediation applications are implemented as a secondary determination of feasibility, because of the inherent variability in some complex sites. Limited data are available which discuss the results of laboratory studies with field-scale studies. Understanding how lab and field studies correlate may improve the ability to understand complex sites and hasten remediation efforts at decreased costs. The goal of this study is to compare the results of a bench-scale continuous-flow column treatability study to the associated field-scale amendment applications in a PCE DNAPL-impacted fractured bedrock site. Traditional data generation and management will be applied to each dataset, in an effort to critically evaluate our traditional remediation design and testing efforts, and provide recommendations for other similar sites.

3.2 Materials and methods

3.2.1 Field site

The study site is a former aerospace manufacturing facility located in Ontario Canada. Currently the property and adjacent area are commercially zoned and active. The entire city block is bound on three sides by an active containment system (Figure 6). Groundwater at the location is contaminated with chlorinated solvents, principally PCE in DNAPL form. *In situ* chemical and biological reduction were proposed for the source zone to shorten the time required for site remediation. This site is underlain by a shallow fractured Ordovician limestone bedrock aquifer of the Ottawa Formation.

3.2.2 Treatability studies

Bedrock cores were collected following the guidelines outlined in the American Society for Testing and Materials (ASTM) D2113-Standard Practice for Rock Core Drilling and Sampling of Rock for Site Investigation from 9.5 to 10 m depth. Groundwater samples were collected in compliance with Ontario Regulation 154/03, as amended July 1, 2011, Record of the Site Condition Part XV.1 of the Environmental Protection Act, R.S.O. 1990, c. E.19, for preservation of VOCs. Samples were collected in 4 L high-density polyethylene (HDPE) sample collection bottles and stored at 4°C.

Bedrock samples were processed by passing the material through a steel plate rock crusher (Badger Crusher) and disc pulverizer (UA Disc Pulverizer) (Bico Braun International, Burbank, CA). Bedrock fragments were sieved with ASTM standard brass sieves to a desired size of 0.25 mm. Uniform grain size was necessary for reproducible porosities and to sustain equivalent flow rates. Site groundwater was amended to PCE concentrations of 57 mg/L (Fisher Chemical, Waltham, MA). Microcosm treatment

amendments included common chemical and biological amendments, including microscale zero valent iron (mZVI) (Golder and Associates, Kanata, ON) with associated palladium acetate catalyst (TCI America, Boston, MA), emulsified vegetable oil (i.e., LactOil) (JRW Remediation, Lenexa, KS), and xanthan gum (Golder and Associates, Kanata, ON). Zero valent iron is a common reducing agent, and when combined with palladium catalyst, forms a galvanizing couple that increases iron corrosion and the overall dechlorination rate. Emulsified vegetable oil serves as an electron donor for the dechlorinating culture, but also provides a medium to improve dissolution and reduction of DNAPL. Xanthan gum was to be included in the *in situ* injections to produce non-Newtonian flow of the injection slug to improve emplacement.

Individual flow-through glass columns have a length and inside diameter of 25 cm and 1.2 cm, respectively, with a total void volume of 30 mL. Columns were constructed in an anaerobic chamber with 90% nitrogen and 10% hydrogen and sealed with Teflon® caps and O-rings. Each microcosm received approximately 38 g of crushed rock with resultant porosities of approximately 37%. Treatment columns received 1.29 g of mZVI, 1.3 mg of palladium acetate and were initially injected with a mixture of 4% LactOil and 0.01% xanthan gum. Columns remained in batch mode for 40 hours post-injection before attachment to a multi-channel ISMATEC peristaltic pump (IDEX Health and Science, Oak Harbor, WA) via polyvinylidene fluoride fittings and Viton 0.89 mm tubing. Columns were placed in upflow mode at a rate of 0.25 pore volumes per day (2.75 mL), for a residence time of 4 days. Influent was stored in 2 L Tedlar® bags and newly prepared bimonthly. Columns were stored at 20°C and covered to avoid light exposure. After reducing conditions were achieved, treatment columns were inoculated with 2 mL

of KB-1® (SiREM, Guelph, ON) on Day 29. Columns were inoculated at the base using a glass gas-tight luer-lock syringe. The flow rate was reduced to 1 mL/day for 4 days, after which 2.75 mL/day was resumed.

A 1 mL sample was collected from each column and injected via needle into a 2 mL glass vial with gold aluminum magnetic crimp cap and Teflon® septum (Agilent Technologies, Santa Clara, CA). Samples were processed immediately post-collection. VOC samples were analyzed using headspace Solid Phase Micro-Extraction Gas Chromatography (SPME-GC-2010), with an FID and AOC-5000 auto-sampler (Shimadzu, Columbia, MD), using a previously published method [57]. Analytes included: PCE, TCE, *c*DCE, *t*DCE, 1,1-DCE, VC and ethene.

First-order observed rate constants (k_{OBS}) for columns were calculated for each sampling period using the following equation:

$$-k_{OBS} = \frac{\ln\left(\frac{C}{C_0}\right)}{t} \qquad \text{Eq. 4}$$

where, C is effluent concentration, C_0 is influent concentration, and t is time. Advective mass flux into the columns was calculated using the following equation:

$$Mass\ Flux\ (J) = C\frac{Q}{A} \qquad \text{Eq. 5}$$

where, C is the influent concentration, Q is the volumetric flow rate, and A is the column area. The mass discharge is the sum mass flux per day.

3.2.3 Field studies

Collected groundwater samples were sent to a commercial laboratory (SiREM , Guelph, ON) for analysis of (chloro)ethenes including PCE, TCE, *c*DCE, *t*DCE, 1,1-DCE, VC, ethene, total organic carbon, bromide, as well as other available electron donors including iron and sulfate. Environmental molecular testing (quantitative polymerase chain reaction) was also performed to quantify *Dehalococcoides* (*Dhc*) and the vinyl chloride function gene (*vcrA*) (SiREM , Guelph, ON).

First-order observed rate constants (k_{OBS}) were calculated for each well by performing a linear regression analysis of the concentration data, where k_{OBS} was determined by the slope of the line. Rate constants were calculated for individual compounds and summation of cVOCs.

Mass flux at each well location was calculated using the following equation:

$$Mass\ Flux\ (J) = kiC \qquad \text{Eq. 6}$$

where, k is the hydraulic conductivity, i is the hydraulic gradient, and C is concentration. Hydraulic conductivity was calculated from previous field activities at 0.0625 m d^{-1}. Hydraulic gradient was 0.18 m m^{-1}, calculated using the average of three well triangulations. Mass discharge is the sum of these fluxes.

3.3 Results

Triplicate continuous-flow treatment columns were conducted in the laboratory over the course of 195 days, to determine if the selected treatment technology was compatible with site groundwater and geological materials. In the field, two wells were installed, Injection Well 5 and Injection Well 6, for pilot-scale field testing of

amendments (Figure 6). Wells of interest varied from 11 to 12.5 m total depth. Depth to groundwater was approximately 3-5 m and the flow direction was to the north. Injection 1 occurred late October 2014 and Injection 2 occurred mid-September 2015. Groundwater was sampled 8 times over the duration of 433 days.

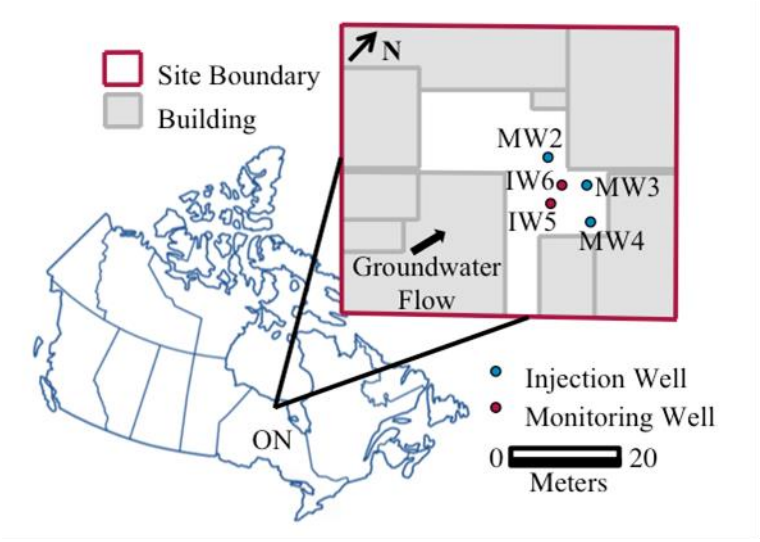


Fig. 6 – PCE DNAPL study site location. Inset shows site boundary, infrastructure, and well spacing of monitoring (MW) and injection (IW) wells.

Results show dechlorination activity in continuous-flow columns reached steady-state at approximately 100 days. Column effluent consisted of approximately 62% ethene, 24% vinyl chloride, with other minor contributions of *c*DCE, TCE, and PCE (Figure 7). In the field, *c*DCE was the prominent cVOC in each well after the first injection, except Injection Well 6, whose concentrations pre- and post-injection remained relatively stable and principally PCE. After the second injection, all well samples were dominantly *c*DCE, however VC concentrations had increased suggesting more favorable dechlorination activity than the previous injection. First-order observed degradation rate constants in the laboratory for the summation of cVOCs were 0.71 ± 0.04 d^{-1} (Figure 8).

PCE, TCE, and *c*DCE showed removal rates of 2.28 ± 0.50 d^{-1}, 1.66 ± 0.27 d^{-1}, and 0.92 ± 0.45 d^{-1}, respectively; Vinyl chloride displayed overall increases. Field data showed fluctuating concentrations for the monitoring and injection wells. First-order rate constants for cVOC sums ranged between <0.01 d^{-1} to a maximum of 0.04 d^{-1}. PCE and TCE showed the most consistent behavior in the well field, generally with immediate declines in concentrations post-injection and rate constants ranging from 0.01 to 0.14 d^{-1} for PCE, and 0.01 to 0.12 d^{-1} for TCE. Field data for the first field injection showed counts of 6E+7 cells L^{-1} (max 2E+8 cells L^{-1}) and 8E+7 cells L^{-1} (max 3E+8 cells L^{-1}) for *Dhc* and *vcrA*, respectively (Figure 9). *Dhc* was quantified at the start of the laboratory treatability study only.

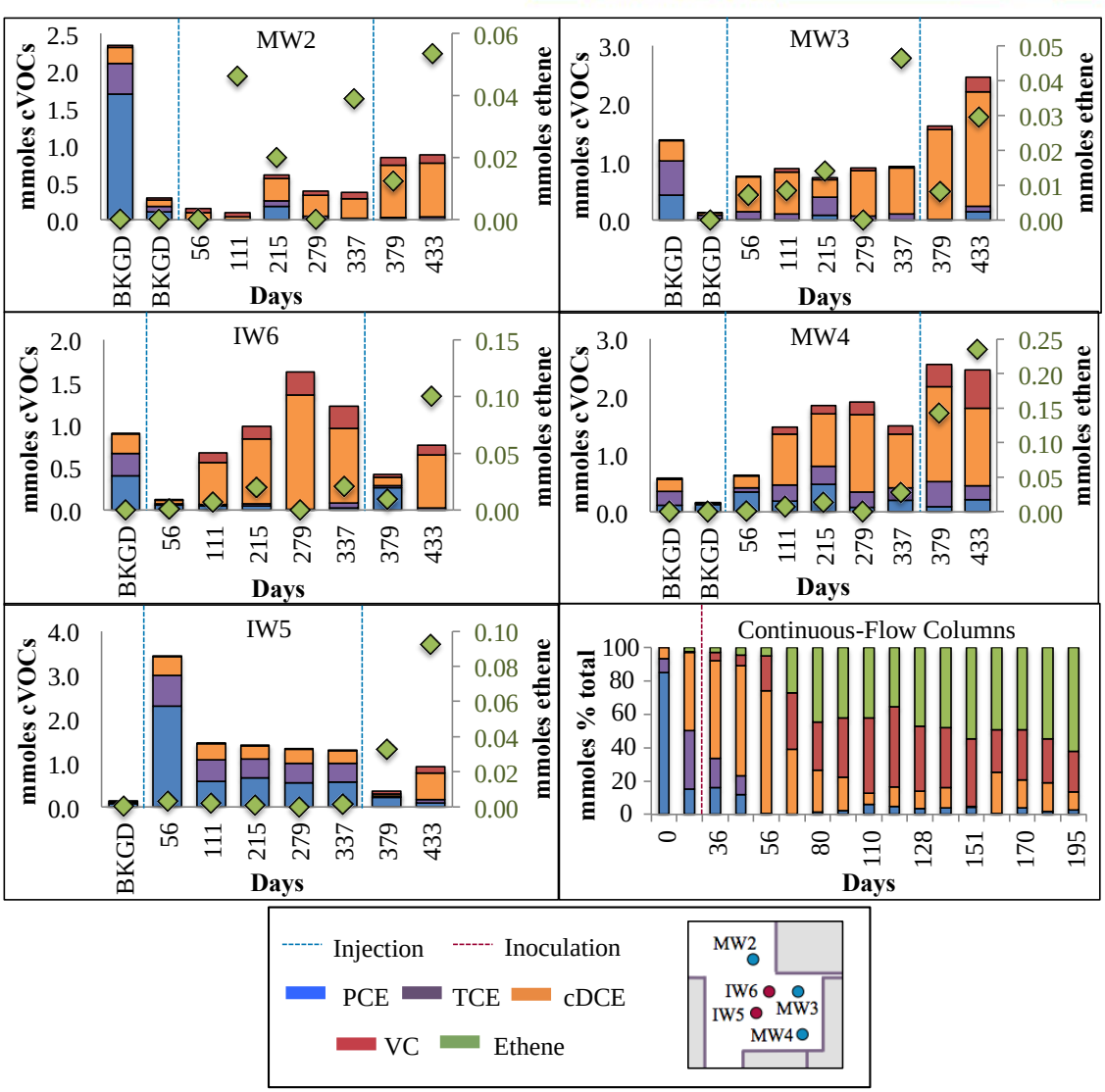
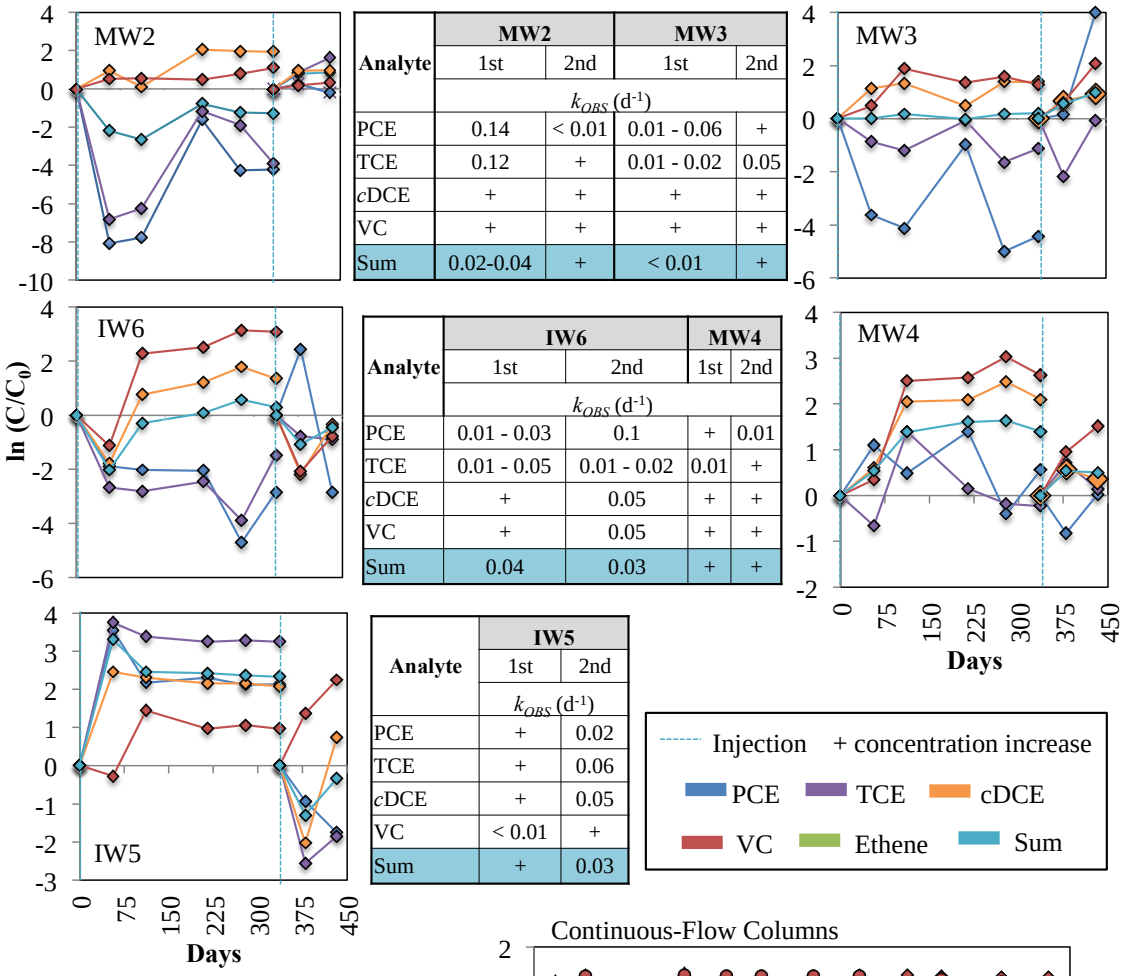


Fig. 7 - PCE and degradates (mmoles) in sampled wells and continuous-flow columns. Note: Newly installed wells have only one background (BKGD) value; Laboratory data are the average of three treatment columns. Inset shows pattern of wells in the field; graph order follows this general pattern. Within approximately 200 days, laboratory columns produced 62% ethene, while the majority of wells were dominated by *c*DCE formation.



Analyte	MW2		MW3	
	1st	2nd	1st	2nd
	k_{OBS} (d^{-1})			
PCE	0.14	< 0.01	0.01 - 0.06	+
TCE	0.12	+	0.01 - 0.02	0.05
*c*DCE	+	+	+	+
VC	+	+	+	+
Sum	0.02-0.04	+	< 0.01	+

Analyte	IW6		MW4	
	1st	2nd	1st	2nd
	k_{OBS} (d^{-1})			
PCE	0.01 - 0.03	0.1	+	0.01
TCE	0.01 - 0.05	0.01 - 0.02	0.01	+
*c*DCE	+	0.05	+	+
VC	+	0.05	+	+
Sum	0.04	0.03	+	+

Analyte	IW5	
	1st	2nd
	k_{OBS} (d^{-1})	
PCE	+	0.02
TCE	+	0.06
*c*DCE	+	0.05
VC	< 0.01	+
Sum	+	0.03

Continuous-Flow Columns			
Analyte	k_{OBS} (d^{-1}) at steady-state		
	max	min	average
PCE	2.28	0.98	1.60
TCE	1.66	0.65	1.36
*c*DCE	0.92	+	0.14
VC	+	+	+
Sum	0.80	0.63	0.71

Fig. 8 - First-order rate plots were generated for each monitoring and injection well and include individual and total cVOCs. Associated observed degradation rate constants are enumerated in the adjacent table. Column data include only data points considered in the steady-state range for each column. Data from triplicate columns are differentiated by symbol with n = 7, 11, 11. Note: Since residence time in the column is 4 days, observed rate constants (k) are plotted here for columns rather than $\ln(C/C_0)$ for field data. Observed rate constants were generally an order of magnitude greater in column systems that those calculated for field data. Positive sign denotes an increase in the rate, or production of the compound.

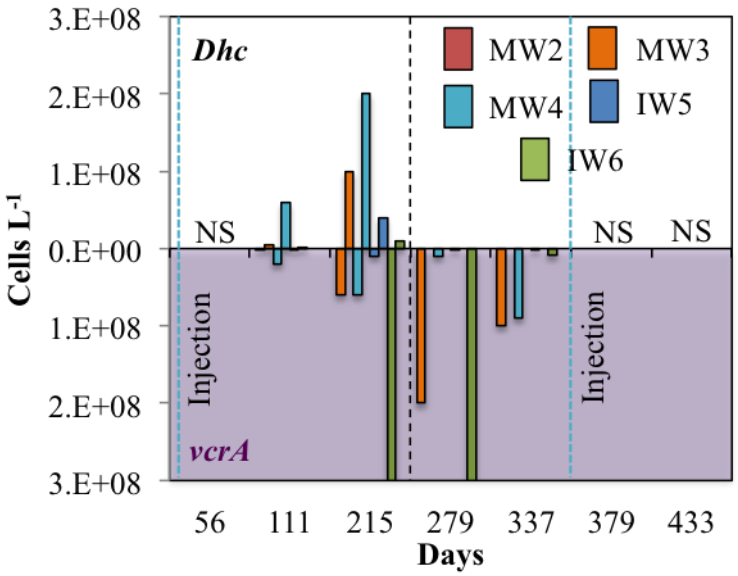


Fig. 9 – *Dehalococcoides (Dhc)* and reductive dehalogenase-encoding *vcrA* genes in groundwater samples. *Dhc* and *vcrA* (cells L^{-1}) are illustrated above and below the 0 line, respectively. After 215 days (denoted by black stippled line) only *vcrA* analyses were conducted. NS- Not Sampled.

3.4 Discussion

Patterns of dechlorination activity were qualitatively similar between the laboratory treatability and field-scale applications. Laboratory columns showed a prevalence of ethene production (62%) and vinyl chloride (24%). Field results showed a general predominance of *c*DCE, with increasing concentrations of vinyl chloride and ethene with subsequent injections. The quantitative differences in PCE speciation between columns and field-scale injections may be related to a variety of factors highlighted herein. In the laboratory study, experiments were designed to determine anaerobic biotic and abiotic dechlorination potentiation of the system, the desired remediation technology. Thus measures were taken to ensure the columns remained anaerobic and highly-reduced throughout the study duration (e.g., columns packed in

anaerobic chamber, influent water sealed in Tedlar® bags). In general, field conditions showed an inherent degree of subsurface heterogeneity, common to a fractured bedrock system, which creates challenges for producing and maintaining anaerobic conditions [83]. Here, field data show that the *in situ* subsurface environment is subject to perturbations that likely disrupt the anaerobic, highly-reduced conditions produced by the organic amendment injections. Dissolved oxygen and oxidation-reduction potential (ORP) fluctuated throughout the study period and cannot be completely explained by post-injection rebound; some of this variability may be a function of high volume precipitation to the shallow aquifer system (Figure 10). Precipitation data points shown are the total accumulated precipitation of the previous 7 days [84]. Data points immediately preceding the sampling event are highlighted in red and numbered 1 through 8 corresponding to the sampling period. On day 337, a high rainfall event (48.4 mm) preceded the sampling period and is temporally concurrent with a substantial increase in ORP in the system. This result suggests that subsurface infiltration may be important to dechlorination capabilities at the site.

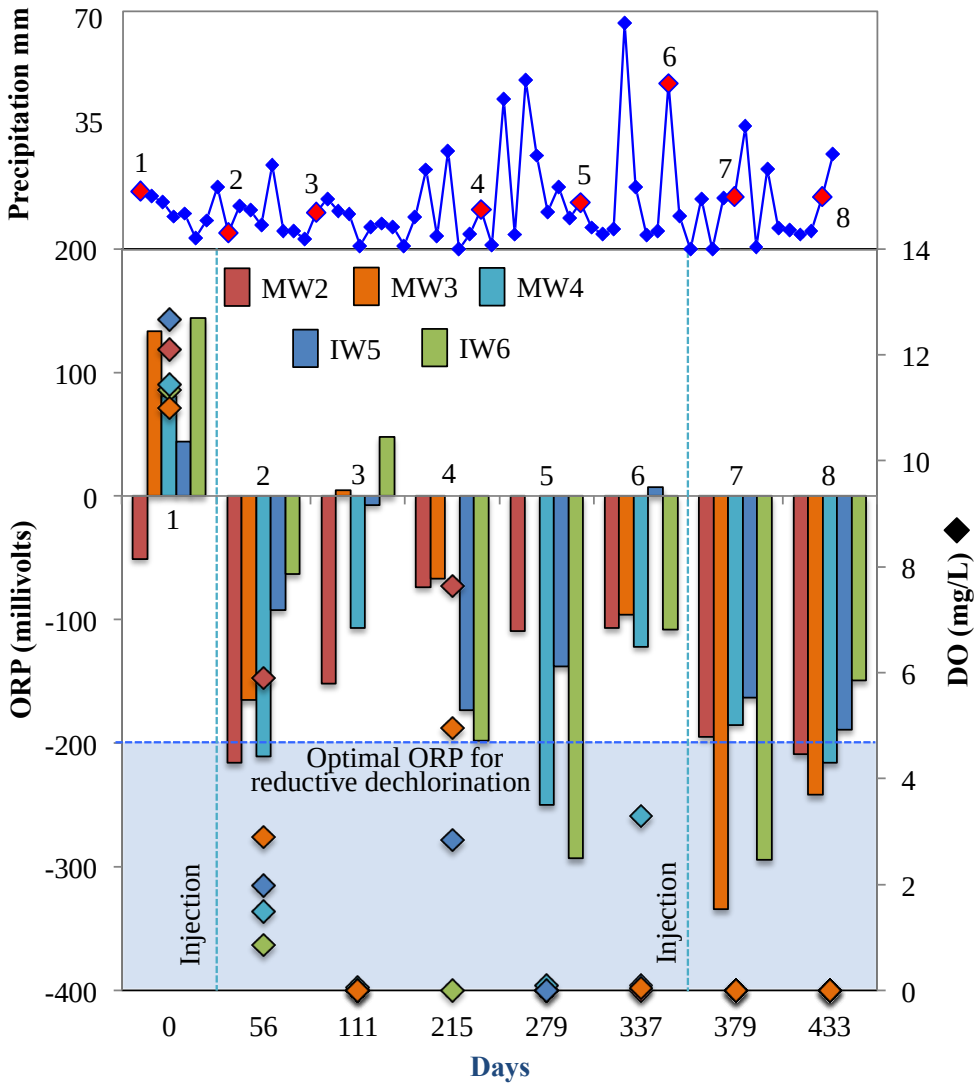


Fig. 10 - Precipitation (mm) at study location. Each point corresponds to the average over the previous 7-day period. Red data points (1-8) correspond to precipitation immediately pre-dating groundwater sampling. ORP (bars) and DO (diamonds) correspond to each of the five wells. The blue shaded region corresponds to optimal ORP conditions for reductive dechlorination

Results of the long-term estimation of shallow groundwater recharge in this Great Lakes Basin by the United States Geological Survey (USGS) show local recharge on average ranging from 20 to 30 cm y^{-1} [85]. The effects of increased ORP and DO in the

field may translate into stalling of PCE dechlorination at *c*DCE. In this case, laboratory columns would overestimate field dechlorination activities. However, less reduced conditions may also result in *c*DCE and VC oxidation and mineralization (carbon dioxide formation), a marked positive benefit [86]. In the latter conditions, ethene may also be oxidized, thus the chemical signature of successful dechlorination may be lost, giving the artificial appearance of less favorable field dechlorination activity. Here, ethene concentrations were generally found to be two orders of magnitude lower than the cVOCs. Low *in situ* ethene concentrations (0.1 ug L^{-1}) may be indicative of much higher dechlorination rates, because of the muted ethene signature due to oxidation [87]. Theoretically, pH (as a surrogate for carbon dioxide production) may be used to discern this oxidative pattern, however many other species in this system have an effect on pH (e.g., organic acids, zero valent iron).

In summary, results from laboratory treatability studies should be viewed as a best-case scenario or upper bounds of remedial success for the specified remedial action for which the study has been optimized. The variability that may occur in dynamic subsurface conditions, such as high precipitation events, should be considered. The best mechanism to understand these perturbations is a thorough conceptual site model and subsequent modeling. Modeling these events will provide a better understanding of how these perturbations may affect implementation of the chosen remediation strategy, providing insights into the lower bounds of efficacy for the strategy.

Beside dynamic subsurface conditions, amendment distribution will also provide variability between laboratory and field-scale results. With subsurface injections, particularly in fractured bedrock, there is the possibility that amendments will not reach

the desired location or emplacement will be delayed, causing less effective subsurface dechlorination [88]. This phenomenon is not captured in laboratory studies. In the lab, emphasis is placed on reproducibility in porosity and flow in the column, as to not introduce additional independent variables that may confound the results. As a byproduct, amendment-contaminant interactions are optimized, and may not always reflect field conditions.

Additionally, differences in laboratory and field results may also be imparted by column study design. Bedrock cores frequently are crushed before packaging into continuous-flow columns, as done in the work described here. However, intact rock cores may provide more realistic *in situ* dechlorination rates [89], as newly created mineral surface of crushed bedrock may exhibit surface chemistries different from *in situ* bedrock through which fluids flow. However, microcosm size and geologic material volume often render the use of intact rock cores an impractical option. It is important to note that during *in situ* injection of amendments, hydraulic fracturing can occur, thereby also exposing newly created surfaces to groundwater and amendments [90]. In these instances crushed rock in laboratory studies would provide a similar environment to subsurface conditions.

Due to the quantitative differences in dechlorination activity in the field as compared to the lab, first-order degradation constants calculated from field data were an order of magnitude lower than those calculated in laboratory columns. The above discussion of higher ORP, ethene oxidation, and amendment distributions all likely influence this result. However, some of the variability in the kinetic results may also be attributed to background data for individual wells and DNAPL presence. Although

background data may exist to establish a baseline concentration, at some sites baseline concentrations may fluctuate due to seasonal variations (e.g., precipitation) or other variables (e.g., groundwater extraction), which can complicate calculations. When determining the baseline concentration necessary for kinetic analysis, a degree of error will exist in any dynamic system. If enough data exists from previous years, it may be possible to determine seasonal variations and correct for this error. However, even if a baseline correction is possible, unforeseen changes in the subsurface related to *in situ* injection may occur (i.e., desorption or changes in DNAPL dissolution rates), which may also impart variability. At this particular location, variability is conveyed by *(i)* changing background concentrations, *(ii)* injection wells located within the DNAPL source zone, and *(iii)* injection wells recently placed, thus having only a single background concentration for each. These results suggest that although rate constants are important in comparisons of laboratory studies, they do not appear to be as important to understanding the relationship between lab and field results.

Microbial parameters illustrated a similar result. Microbial counts quantified in the laboratory are often used to refine degradation kinetics, particularly in competing studies [61, 66]. However with microbial field data, microbial counts instead are used as a second line of evidence for remediation success, so as to ascertain that reactions are microbially-mediated rather than a byproduct of other factors such as dilution or volatilization [91, 92]. In continuous-flow treatment columns, *Dhc* were quantified in initial inoculations but were not subsequently sampled because conditions were known to be optimized for this consortium in the controlled setting. In the field, sampling was

conducted to confirm biological reduction was occurring, and to assess *in situ* activity in this non-ideal, real-world environment.

It is important here to restate that two of the most important parameters in laboratory bioremediation treatability studies, reaction kinetics and microbial counts, are either much less important or used for alternative purposes when evaluating field data. In an effort to rectify this disparity, a more suitable parameter in evaluating field remedial activities is mass flux. In 2010, the Interstate Technology Regulatory Council (ITRC) published guidelines for using mass flux and mass discharge to evaluate a contaminated system, particularly when DNAPL was present, where evaluation of groundwater concentrations alone may fall short in characterizing the system [93]. Mass flux may be used to *(i)* better understand *in situ* remedial technology when variations in mass and complex degradation cascades exist, *(ii)* determine the amount of DNAPL left in a system (assuming source volumes known), or determine feasible removal at a site. Mass flux in the field was variable between wells, with an average of 92 ± 39 mmoles m^{-2} d^{-1} by the study conclusion (Figure 11). Mass discharge ranged between 1900 and 7000 mmoles m^{-2} d^{-1}. It is important to note that the change in slope of the monitoring well mass flux curves after the second injection suggests mass migration from the source to receptor wells, highlighting injection effectiveness. By comparison, mass flux into the continuous-flow columns was much lower, with a constant flux of 0.084 mmoles m^{-2} d^{-1}. On similar timescales this equates to a mass discharge into the columns of approximately 36 mmoles m^{-2} d^{-1} by the study conclusion (Day 433).

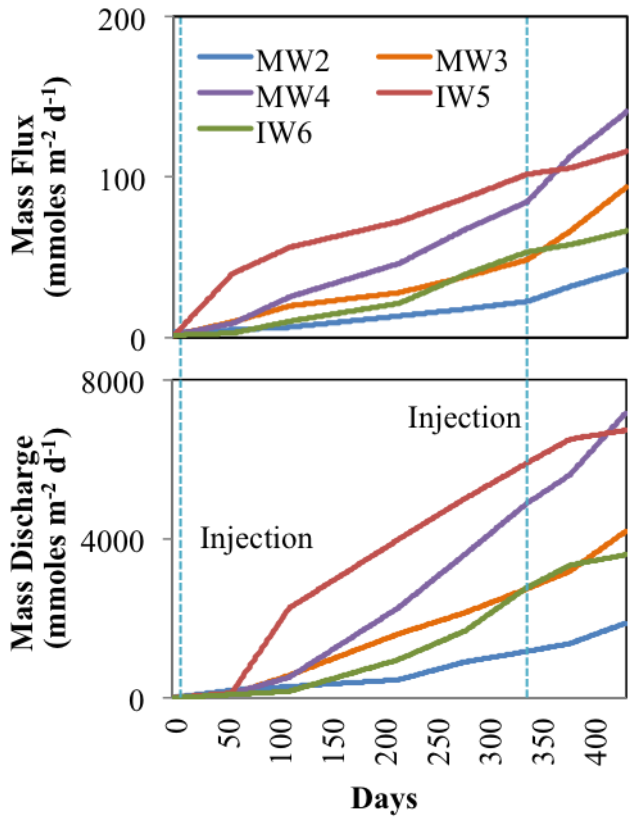


Fig. 11 - Mass flux and mass discharge through individual wells. Field data show wells with a mass flux of 92 ± 39 mmoles m^{-2} d^{-1} by the study conclusion and mass discharge ranging between 1900 and 7000 mmoles m^{-2} d^{-1}. Mass flux and discharge make visible the increase in mass removal from the DNAPL source zone into receptor wells (MW), particularly visible after the second injection. Columns had a significantly lower constant influent mass flux of 0.08 mmoles m^{-2} d^{-1} and a mass discharge of 36 mmoles m^{-2} d^{-1} (not shown).

Mass determinations in treatability studies are rarely discussed likely because mass in the system is highly controlled (constant influent concentrations), the customary approach involves elucidating speciation and the associated kinetics, and DNAPL addition is rare. Although column studies were aligned with average field contaminant concentrations and flow rates, the disparity in mass between the field and the lab suggests that the importance of this parameter should be evaluated in future studies to determine if

this metric should be more similar. Additionally, *in situ* DNAPL source zone remediation involves mass removal by dechlorination (e.g., ethene formation) as well as enhanced dissolution/desorption and subsequent downstream capture in receptor wells [94]. The traditional column study design only assesses the ability of the amendments to transform aqueous concentration of cVOCs to ethene in the presence of site bedrock and groundwater. Previous studies have evaluated dissolution in DNAPL amended columns; however, this is typically not practical in commercial treatability studies [95]. Discerning true inhibitory effects and competitive utilization via emplacement may provide more realistic depiction of field activities and thus does represent a research approach of potential merit.

3.4.1 Limitations

Data interpretation was limited to data shown since full access to site conceptual models, including plume mass and architecture, bedrock fracture patterns, and details on groundwater flow were unknown. Little historic data was available for some monitoring wells, which may impart error to baseline conditions used in kinetic analyses. Additionally, a byproduct of ZVI reduction for chloroethenes is ethane. The compound was not monitored in bench-scales studies so field-scale results are not included here.

3.5 Conclusion

Challenges arise in reconciling laboratory treatability study and field-scale remedial data. Qualitative comparisons between the two systems may often align, whereas quantitative differences become evident. Many of these differences stem from goals of the laboratory study (e.g., testing of a specific remediation strategy under ideal

conditions), and the ability to achieve those conditions in an *in situ* dynamic system. Additionally, other barriers include the inability to recreate field-identical subsurface conditions in the lab (e.g., subsurface flow in fractured bedrock). Interestingly, two common metrics important to column treatability studies, i.e., reaction kinetics and microbial counts, are less useful in the field, and are not employed in the same manner. This result suggests that moving towards alternative means of evaluating field data, particularly mass flux and mass discharge, may prove beneficial in future studies. Of particular promise are approaches that align the contaminant flux in influent of lab studies to the baseline fluxes extant in the field. This would ensure that results obtained in the laboratory are more representative of the rate and extent of *in situ* activities.

TRANSITION 3

In previous chapters, assessments of methodologies designed to improve or recover the health of the natural environment were performed, which illustrated the utility of the obtained information for assessing the success of bench-scale treatability studies and limitations when translating these approaches to the field-scale. The following chapter shifts the focus of these studies from the natural environment to more urban settings and exposures incurred by human populations. Wastewater-based epidemiology (WBE) is a methodology used to assess population health by measuring human metabolic excretion products in wastewater. The majority of WBE studies have focused on consumption behaviors, particularly those of illicit drugs. The need exists to expand WBE methods to include endogenous compounds; one such group of amenable compounds is glucocorticoids, also known as stress hormones. Chronic stress has been linked to the top leading causes of death in the U.S., including heart disease, and the economic costs of excessive stress are estimated to figure in the billions of dollars annually.

In Chapter 4, a WBE study was performed on a major U.S. university campus to monitor two glucocorticoid hormones, cortisol and cortisone, with the goal of assessing changes in stress experienced by students relative to the semester schedule and to educational testing. Twenty-four hour, flow-weighted daily composite samples were collected seven consecutive days each month, including the first week of classes and finals week; collected samples were analyzed by LC-MS/MS. Wastewater flow data and population estimates were used to determine daily per-capita glucocorticoid production, and statistical assessments were performed to assess variability between months, days of

the week, and weekends. Concurrently, biomarkers of alcohol, nicotine, and caffeine consumption were also monitored and correlations assessed between the associated behaviors and glucocorticoid production.

CHAPTER 4

EXPANDING WASTEWATER-BASED EPIDEMIOLOGY BY USING GLUCOCORTICOID HORMONES IN SEWAGE AS INDICATORS OF POPULATION STRESS

ABSTRACT

Wastewater-based epidemiology (WBE) enables monitoring of biomarkers of human health in wastewater, including endogenous substances such as stress hormones. We tracked the concentrations of two principal glucocorticoids, cortisol and cortisone, in wastewater by liquid chromatography tandem mass spectrometry (LC-MS/MS), to assess changes in physiological markers of stress in a student population (n = 60,000) undergoing educational testing. Daily composite samples were collected on a major U.S. university campus for seven consecutive days each month during the Fall 2017 academic semester. Results showed reproducible weekly patterns in stress hormone excretion, with the highest levels occurring on Mondays (149 ± 61 μg d^{-1} per person), and the lowest on Saturdays (72 ± 18 μg d^{-1} per person) and Sundays (71 ± 22 μg d^{-1} per person). Weekdays were significantly different from weekend trends (p = 0.05). Results also showed significantly higher stress levels during the beginning of the semester (August and September, 161 ± 42 μg d^{-1} per person and 122 ± 54 μg d^{-1} per person) as compared to the remaining months (p = 0.05, 0.01). A Spearman rank order statistical analysis showed a positive correlation between total glucocorticoids and nicotine (r_s = 0.49) and caffeine (r_s = 0.63) consumption, but not alcohol intake. Whereas hormones have been analyzed in wastewater before, this study constitutes the first WBE study to assess population stress level via determination of glucocorticoids in community wastewater.

4.1 Introduction

Wastewater-based epidemiology (WBE), a sub-discipline of population metabolism metrology, provides near real-time information related to health status, lifestyle, and the behaviors of populations contributing to the sampled sewer system [7-9]. This approach utilizes the detection in (raw) wastewater of excretion products of human metabolism to estimate the consumption of chemicals of concern, including illicit drugs [11, 96, 97], alcoholic beverages [98, 99], tobacco [100, 101], and caffeine [12, 102]. A recent study also monitored isoprostanes as a measure of oxidative stress [103] and genetic biomarkers for assessing population-wide carcinogenesis [13]. Wastewater-based epidemiology and urban metabolism metrology also have been utilized to determine population exposure to various contaminants of concern, such as antimicrobials and preservatives found in personal care products [10, 104]. A further expansion of the types of analytes targeted by WBE is desirable to improve our understanding of community health. One potential target group are glucocorticoids, a class of natural steroid hormones commonly referred to as stress hormones. Cortisol is the major glucocorticoid driving the stress response in the human body and is transformed to cortisone by the enzyme 11-beta-hydroxy steroid dehydrogenase (11- ß HSD), to moderate the stress response in the body [105].

Stress has been shown to affect an individual's mood, sense of well-being, and overall health. Studies to quantify the economic cost of stress in the United States have focused solely on workplace stress. The most recent estimate of costs associated with stress-related job absence and staff turnover is on the order of US$430 billion (2018-adjusted) annually for the U.S. [106]. The American Psychological Association (APA)

has linked chronic stress to the top six leading causes of death in the US, including heart disease, cancer, lung ailments, accidents, cirrhosis of the liver, and suicide, [17] creating a societal burden and hardship not fully captured by economic estimates.

Clinical monitoring of stress hormones in patients' blood serum, urine, or saliva is widely practiced to diagnose diseases such as adrenal insufficiency, hypercortisolism (Cushing syndrome), and abnormalities in 11-ß HSD production [105, 107]. With personal healthcare, glucocorticoids are not measured to understand an individual's level of stress due to specific stimuli, or to understand the ability of an individual to mitigate the negative effects of perceived stressful events. However in academic research, cortisol and its metabolite cortisone have been measured in different demographic groups (e.g., sex, age, race), to determine the type and duration of stressors, and to assess physiological and psychological disorders [108-110]. Monitoring stress hormones is also used extensively to assess stress levels in wild or captive animal populations [14, 15]. Human studies have established glucocorticoids detectable in urine [16], saliva [111], hair [112, 113], and blood [114] as biomarkers informative on acute and chronic stress.

Glucocorticoids have previously been measured in surface waters and in wastewater, but not for the purpose of stress assessment of human populations. Much of this work focused on the detection of these hormones after introduction to natural environments by wastewater treatment plant effluent, and the resultant environmental distribution and potential impacts to ecosystems [115-118]. Additional studies have examined the occurrence and fate of stress hormones during conventional wastewater treatment. Concentrations of cortisol and cortisone in wastewater ranged from non-detect to 100s of ng L^{-1} [119-121]. Measuring cortisol and cortisone for wastewater-based

epidemiology purposes has not been attempted to date. Only one published study is related to WBE, and attempted to employ detectable concentrations of cortisol as a variable informing an estimation of the number of individuals represented in pooled community wastewater [122]. Steroid hormones in sewage as indicators of human health status thus currently remain an untapped informational resource.

The goal of the present study was to *(i)* determine the occurrence of cortisol and cortisone in community wastewater, and *(ii)* assess trends in detectable stress hormone levels as a function of known activities and potential social stressors on a major university campus in the United States. Concurrently, a determination of additional biomarkers was performed to estimate population size, and assess the consumption of common psychoactive substances, including alcohol, nicotine, and caffeine.

4.2 Materials and Methods

4.2.1 Chemicals and reagents

Native and labeled glucocorticoid standards including cortisol, cortisol-*d4*, cortisone, and cortisone-*d8* were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). HPLC-grade methanol, acetone, and water were from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA), and formic acid was purchased from Fluka Chemical Corp (Milwaukee, WI). Stock solutions of native and isotopically-labeled compounds were prepared in methanol and stored in glass vials with polytetrafluoroethylene septa at −20 °C (or 4 °C).

4.2.2 Study location

The study was conducted from August through December 2017 at a large public university campus in the southwestern United States. The study location has 52,000 enrolled students, and a total campus population of approximately 60,000.

4.2.3 Sample collection and flow measurements

Daily composite samples were collected for 7-consequative days, by an Avalanche® refrigerated sampler (Teledyne ISCO, Lincoln, NE, USA), with 9 mm inner diameter (ID) silicon tubing for the suction line and the pump head. Collected volumes ranged from 50 to 100 mL for each of the two sampling locations. Prior to each aliquot collection, the instrument was programed to rinse the sample lines with fresh wastewater and to purge that water before the actual sample collection. Samples were collected in acid-washed 10 L glass bottles, transferred to high-density polyethylene (HDPE) bottles for transport, and processed on the same day to limit degradative losses. Flow was monitored by ISCO LaserFlow flow meters (Teledyne ISCO, Lincoln, NE, USA), located within each adjacent manhole.

4.2.4 Sample processing

Aliquots of 200 milliliter of each sample were split into equal fractions and extracted separately using a Dionex™ AutoTrace™ 280 Solid-Phase Extraction Instrument (Thermo Scientific, Waltham, MA). Prior to extraction, deuterated standards were added to the aliquots for a final concentration of 50 µg L^{-1}. Wastewater was not filtered or centrifuged prior to processing. Samples were extracted using Hydrophilic-Lipophilic Balance (HLB) cartridges (6 cc, 150 mg, 30 um particle size) manufactured by

Waters (Milford, MA). Each cartridge was conditioned with 5 mL of methanol followed by 5 mL of water, and then extracted with 1:1 (v/v) methanol and acetone containing 0.5% formic acid, into a final volume of 4 mL. Aliquots of the final extract were blown-down under a gentle stream of nitrogen at ambient temperature using a Reacti-Therm TM Heating and Stirring Module TS-18821 (Thermo Scientific, Waltham, MA). Samples were reconstituted in 1+1 (v/v) methanol and water mixture for analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

4.2.5 Sample analysis

Organic extracts of wastewater were analyzed for glucocorticoids using a Shimadzu 2100 high performance liquid chromatographer (HPLC), coupled to an AB Sciex APE 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA). Analytes were separated on a Symmetry C_8 column (4.6 × 150 mm, 3.5 μm particle size) preceded by a Symmetry VanGuard Cartridge (3.9 × 5 mm, 3.5 μm particle size) (Waters, Milford, MA). Methanol was used as mobile phase A, and water containing 0.1% formic acid used as mobile phase B. The injection volume was 10 μl. Details regarding gradient program and mass spectrometer parameters are provided in the supplemental information (Appendix B: Table 9, Figure 24).

4.2.6 Quality assurance and quality control

Reported concentrations were determined based on a 9-point standard curve, with concentrations ranging from 0.1 μg L^{-1} to 100 μg L^{-1}; the minimum coefficient of determination was $r^2 = 0.99$. Average recoveries for analytes were determined based on spike-recovery experiments with four replicates. Relative recoveries, calculated with data

from labeled standard recoveries, were 88 ± 5% for cortisol and 94 ± 5% for cortisone. Absolute recoveries of labeled standards cortisol-*d4, and* cortisone-*d8* were 69 ± 4% and 70 ± 4%, respectively. Each extraction was performed with method blanks (DI water), and instrument blanks (1:1 methanol and water) to enable the detection of potential contamination and analyte carryover in the instrument.

Method detection limit (MDL), limit of detection (LOD), and limit of quantification (LOQ), were determined using methods outline here [123-125], and are provided in the supplemental information (Appendix B: Table 10). Precision was expressed as Relative Percentage Differences (RPD) and calculated using the following equation:

$$RPD\ (\%) = ABS\left(\frac{C_{S1} - C_{S2}}{C_{S1} + C_{S2}}/2\right) x\ 100 \qquad \text{Eq. 7}$$

where C_{S1} and C_{S2} are the measured concentrations in the sample and its associated duplicate. RPDs ranged between 0 and 20%.

4.2.7 Data analysis

LC-MS/MS data were acquired with Analyst 1.5 software (Applied Biosystem, Foster City, CA). Concentrations were calculated using the isotope-dilution method and were reported as a concentration (ng L^{-1}). Concentrations of glucocorticoids were reported when the analyte peak height to background signal (signal-to-noise ratio) was greater than 3, and the concentrations were above the MDL. Measured concentrations were converted to mass loads (g d^{-1}), and mass per person (µg d^{-1} per person) using flow volume and population size data.

Per person consumption was determined by estimating daily population estimates using the average population size estimate obtained from population biomarker monitoring and from daily wastewater volumes using the following equation:

$$Population = \left(\sum \left(\frac{C_n * Q_{Tot} * \left(\frac{\mathrm{MW_P}}{\mathrm{MW_M}}\right)}{EF_n * 0.00125 * 14.1 * 0.14} \right) + \sum \left(\frac{C_c * Q_{Tot} * \left(\frac{\mathrm{MW_P}}{\mathrm{MW_M}}\right)}{EF_c * 0.3} \right) + \left(\frac{C_e * Q_{Tot}}{0.0011}\right) + \left(\frac{Q_{Tot}}{189}\right) \right) \Big/ n \quad \text{Eq. 8}$$

where Q_{Tot} is the total volumetric flow rate, C_n is the measured nicotine-related analyte concentration, $\left(\frac{\mathrm{MW_P}}{\mathrm{MW_M}}\right)$ is the ratio of parent to metabolite molecular weights, EF_n is the nicotine-related excretion factor (0.09, nicotine; 0.125, cotinine; 0.365 *trans*-3'-hydroxycotinine) [126], 0.00125 is the nicotine absorbance factor (1.25 mg) [126], 14.1 is the number of cigarettes smoked by the average smoker [127], 0.14 relates to Arizona's percentage of smokers at 14% [128], C_C is the measured caffeine-related analyte concentration, EF_c is the caffeine-related excretion factor (0.017, caffeine; 0.046, paraxanthine) [102], 0.3 is the average amount of caffeine consumed (in grams per person) in the US [129], C_e is the measured concentration of enterolactone, 0.0011 is the average g/day enterolactone excretion of this analyte [130], 189 is the estimated liter per person per day for water consumption on the mixed-used campus (approximately 50 gallons per day), and n is the number of population size biomarkers used in the assessment.

Statistical analyses, including Mann-Whitney and Spearman non-parametric tests, were performed using Microsoft Excel 2010. The Benjamini-Hochberg (BH) correction below was performed for multiple tests to control for Type I errors.

$$BH = \frac{i}{m} Q \qquad \text{Eq. 9}$$

where i is the rank assigned to the *p*-value in the array, m is the number of comparisons, and Q is the false discovery rate, 0.1.

4.3 Results and discussion

This study sought to measure the major human stress hormone cortisol and its hormonally inactive counterpart cortisone in wastewater, to assess temporal changes in stress levels of a university campus population as a function of semester schedule and educational testing. Consecutive 24-h composite samples were collected Monday through Sunday for each month of the semester (n = 35) including the first week of classes and finals week. Measured concentrations determined in campus wastewater are shown in Figure 12.

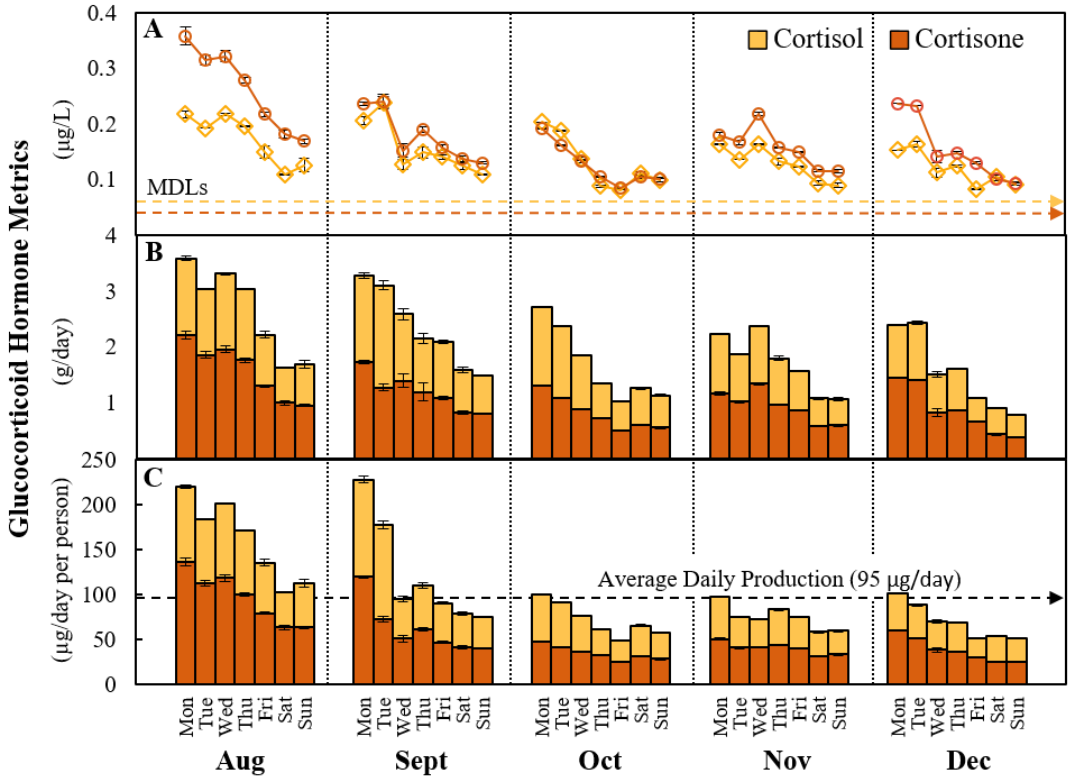


Fig. 12. Wastewater-derived glucocorticoid hormone metrics. Concentrations in wastewater (µg L^{-1}) (A), daily mass loading (g d^{-1}) (B), and hormone occurrence normalized to per person excretion (µg d^{-1} per person) (C) on a major U.S. university campus during the Fall semester of 2017. Error bars represent minimum and maximum results of duplicate samples. The value of average daily production (95 µg d^{-1} per person) of both compounds in healthy individuals [131].

In the current 5-month study (n = 35), the average cortisol concentration 140 ± 40 ng L^{-1}, while cortisone was 160 ± 60 ng L^{-1}. In a Spanish study of two treatment plants, detectable concentrations of cortisol and cortisone averaged 203 ± 67 ng L^{-1} and 204 ± 82 ng L^{-1} [132]. In China, a study of seven WWTPs found cortisol and cortisone at 39 ± 26 ng L^{-1} and 30 ± 21 ng L^{-1}, respectively [120], and in the Netherlands, hospital wastewater had measured cortisol and cortisone at 275 ± 27 ng L^{-1} and 381 ± 44 ng L^{-1}, respectively [121]. In order to interpret these concentrations as indicators of population stress, the

concentrations would need to be normalized to hormone mass loading per standardized population size per unit time. In studies that supplied wastewater treatment plant flow and population data for twelve treatment plants, average cortisol and cortisone were 18.6 ± 14.1 and 15.4 ± 12.3 μg d^{-1} per person, respectively [119, 120, 132, 133] (Appendix B: Table 11).

In this campus study, raw cortisol and cortisone concentrations displayed a distinct pattern of elevated levels during the earlier portion of the week, followed by a general decline through the remainder of the week to terminate at minimal levels on the weekend. Cortisone consistently occurred at higher concentrations than cortisol; a correlation plot exhibited a slope of 1.15 between the two compounds, with a coefficient of determination (R^2) value of 0.89 (Figure 13). The occurrence of cortisone to cortisol in urine from clinical studies, suggests a 3:1 to 2:1 ratio reported for [105]. The difference in the observed values likely is caused by multiple factors, including the presence of glucocorticoids in fecal matter and potential differences in the stability of both glucocorticoids during passage through the sewer pipe after excretion. In the present study, the severity of potential in-sewer degradation was limited because the maximum residence time in the sewer prior to sampling was calculated to be no greater than 2 hours and less than one hour on average. A study of cortisol and cortisone stability in human urine over 24 hours at room temperature showed no significant changes [134]. However, urine of healthy individuals has few microorganisms, whereas domestic wastewater contains very high microbial counts and is in contact with active biofilms within the sewer system. A stability test with untreated wastewater showed cortisol experienced a first-order rate loss with approximately 90% loss after 2 days at room temperature [122].

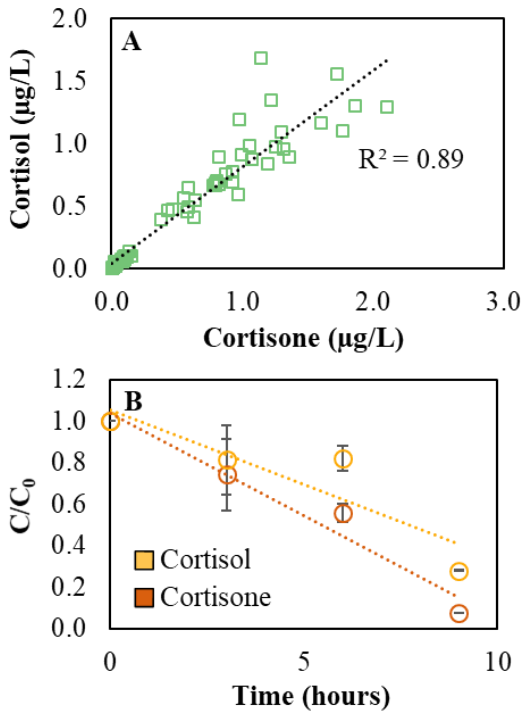


Fig. 13. Correlation plot of cortisol and cortisone concentrations detected in 67 raw wastewater samples collected on a major campus of a southwestern US university (A); Rate loss analysis of cortisol and cortisone in wastewater at ambient temperatures (B).

With the literature lacking relevant data of cortisone stability, we determined the stability of each in raw wastewater (Figure 13). Results from the study showed a roughly zero-order rate loss for both compounds. Cortisone exhibited a faster degradation rate than cortisol, which is in agreement with lower ratios observed in campus wastewater. Whereas the occurrence of glucocorticoid hormones in the stool of mammals is well established, literature values of cortisol and cortisone in human feces are lacking. Animal studies suggest an abundance of these compounds in feces of various other species [135, 136], including chimpanzees [135, 136], one of the genetically closest species to humans.

Measured concentrations of glucocorticoids were converted to mass per day values, using total flow data captured by flow meters at each of the two sampling locations (Appendix B: Table 12). In-sewer mass fluxes of glucocorticoids showed a nearly identical pattern to that of measured concentrations, with higher mass loads extant on weekdays (2.3 ± 0.5 g d^{-1}) when compared to weekends (1.3 ± 0.3 g d^{-1}; $p = 0.01$). The maximum value occurred on the first Monday of the first week of the semester, with a total glucocorticoid mass of 3.6 ± 0.1 g d^{-1}, while the minimum occurred on the Sunday of December, after the completion of final exams (Figure 12). The total mass of stress hormones measured over the duration of the study was 69 ± 0.7 g, with the daily average of 2.0 ± 0.8 g d^{-1}.

Per person daily stress was calculated by estimating the contributing population on the campus for each day sampled. It is important to take this information into account because of the inherent variability in the campus population: almost 80% of students commute to campus. Additionally, as a National Collegiate Athletic Association Division I school, the campus population is also known to vary with sporting events, particularly during football season. Population was estimated from a series of chemical biomarkers commonly used to estimate population [122, 137-140], as well as the estimated daily per-person water usage for mixed residential [303 liters per day (80 gallons per day)] and commercial use [e.g., office and school; 76 liters per day (20 gallons per day)]. Additionally a novel biomarker, enterolactone, was also used to estimate population. This compound is a product of microbial gut flora during the breakdown of lignin, and the intraspecies excretion variability is low making it a potentially good marker for population. Population estimates using this compound fell soundly within the range of the

other proven biomarkers of interest. Of the five measured compounds used to estimate population, including caffeine, paraxanthine, nicotine, cotinine, *trans*-3'-hydroxycotinine, and enterolactone, only the latter five were used to estimate population (Appendix B: Table 13). Caffeine was not included because it was determined that population estimates were consistently too high (175,000 ± 54,000 individuals), well above the campus maximum of 60,000 based on known student enrollment, and number of employees and faculty. Thus, population size was calculated as an average of six population size metrics, the five remaining biomarkers plus the estimate derived from observed water usage.

Per person glucocorticoid hormone levels displayed a relatively similar overall pattern to that apparent in the mass data; however, the differences between weekday and weekend glucocorticoids levels were less pronounced when population size was considered in the analysis, particularly for the months of October through December (Figure 12). The maximum per person glucocorticoid excretion occurred on a Monday in September (228 ± 2 μg d^{-1} per person), while the excretion minimum of 50 ± 0.7 μg d^{-1} per person was observed on a Friday of October. Only one day in the sampling campaign corresponded with a significant campus event. The Saturday in October was the University's annual Homecoming, with an on-campus football game. Not surprisingly, this Saturday showed elevated levels of total glucocorticoids compared to the traditional weekly pattern. Compared to the average that we calculated from literature (cortisol, 18.6 ± 14.1 μg d^{-1} per person; cortisone 15.4 ± 12.3 μg d^{-1} per person), per-person estimates for total glucocorticoids in this study population were higher on all days.

Per person levels of total glucocorticoid hormones showed elevated excretion on Mondays (149 ± 61 μg d^{-1} per person), with consecutive decreases throughout the week,

culminating in the lowest averages on Saturdays (72 ± 18 µg d^{-1} per person) and Sundays (71 ± 22 µg d^{-1} per person). Weekday stress hormones were significantly higher than weekends (p = 0.05), as established by a Mann-Whitney non-parametric assessment with Benjamini-Hochberg correlation for multiple comparisons (Figure 14, Appendix B: Table 14). Based on a monthly comparison, stress levels were highest during the first month of the semester, which corresponded to the first week, and showed a monthly decline in average glucocorticoid levels as the semester progressed. This decline was rather stepwise, with higher recorded per person glucocorticoid levels during August (161 ± 42 µg d^{-1} per person) and September (122 ± 54 µg d^{-1} per person), followed by an approximate 35 to 55% decrease during the remaining three months. Mann-Whitney statistical assessments showed measured glucocorticoids in the month of August were significantly different from October, November, and December (p = 0.01), while September was significantly different from October, November and December (p = 0.05).

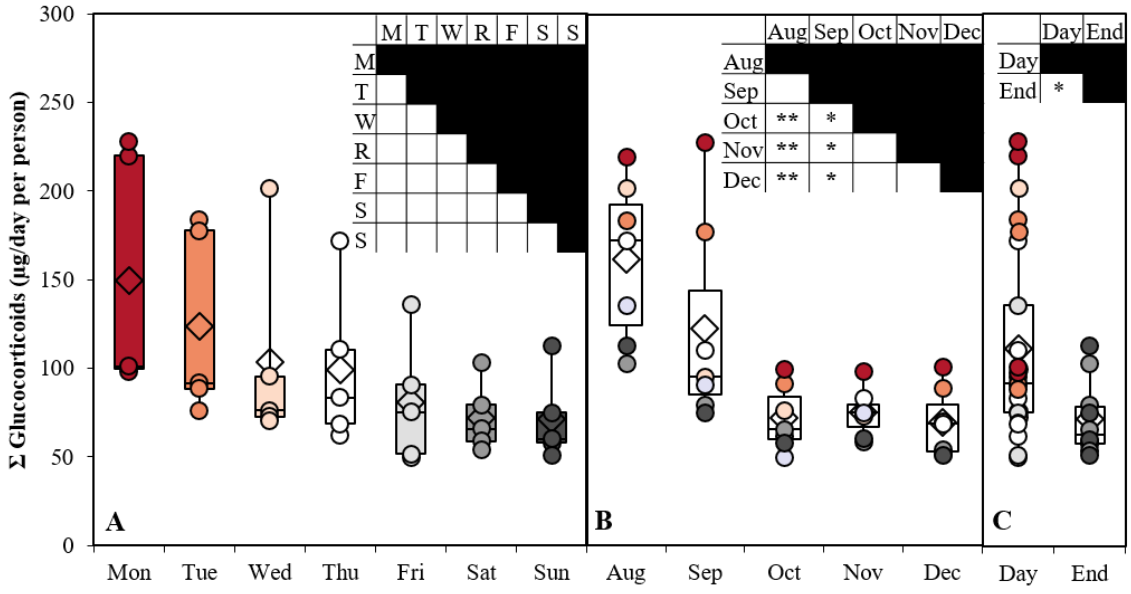


Fig. 14. Box plot summarizing glucocorticoid occurrences detected in campus wastewater by day of the week (A), per month (B), and during weekdays versus weekends (C). Plots show the 25th, 50th (median) and 75th quartiles, minimum/maximum error bars, mean (diamond), and all contributing data points (circles). Colors correspond to day of week. Results of Mann-Whitney nonparametric statistical analyses of variability performed with the Benjamini-Hochberg (BH) correction for multiple tests are shown adjacent to the corresponding box plot. Identified significant differences are shown within the matrices at the 95% (p = 0.05)* and 99% (p = 0.01),** confidence level, respectively.

The daily excretion ranges in urine (reference values) of glucocorticoids for an individual greater than 18 years of age, is 3.5 – 45 µg d^{-1} per person for cortisol, and 17 – 129 µg d^{-1} per person for cortisone [105]. However, laboratory research has shown that these ranges vary in healthy individuals, as well as those with diagnosed conditions (e.g., depression) [141, 142]. Additionally, reference ranges also vary by type of quantitation method employed, thus alternative commercial laboratories have different 24-h urinary reference values [143]. A Mayo Clinic report citing an LC-MS/MS study of 24-h urine samples, suggested a cortisol maximum of 43 to 60 45 µg d^{-1} per person, and a cortisone

maximum of 122 to 141 45 µg d^{-1} per person, with both values being gender dependent and higher in males [144]. Cortisol surpassed the cortisol 60 45 µg d^{-1} per person threshold on Monday through Thursday (August) and Monday and Tuesday (September); cortisone did not breach the 141 45 µg d^{-1} per person threshold value. Reference ranges provide a coarse assessment to gauge stress levels; however, it is important to remember that campus per person glucocorticoid measurements are average estimates applied to the total contributing population. Since this average is at the very high end of the reference value range (regardless of source), some individuals in the population will have had much higher glucocorticoid levels than the average value.

To estimate an average hormone production by healthy individuals to compare with measured averages in wastewater, rather than the characteristic reference range, we turned to the literature. A study of urinary daily cortisol and cortisone measurements in healthy individuals (n = 60) found average values of 23 ± 8 45 µg d^{-1} per person and 72 ± 22 45 µg d^{-1} per person for cortisol and cortisone in urine, respectively [131]. Two additional studies using cortisol as a single metric, agree with the above average estimate, 20 µg/day per person (median, interquartile range [IQR] 15, 29; n = 82) [145], and 17 µg/day (median, IQR 14, 22; n = 19, age range 18.6 - 24.9) [146]. An average production estimate was calculated and applied to per person results in Figure 12 for comparison.

Results show high levels of stress associated with this campus population during the workweek when the bulk of classes are offered, and during the earlier months in the semester. The weekday/weekend trend agreed with previous studies, where populations with traditional work schedules (Monday through Friday) showed higher cortisol levels on weekdays versus weekends [147, 148]. Intuitively, it seems reasonable that at the start

of the academic semester, the level of stress would be higher, followed by a progressive decline due to acclimation to student life. Surprisingly, no increase in glucocorticoid levels was observed during finals week. Studies related to academic stress have found increases in cortisol levels in the individual, associated with exams [149-151]; however, no such change in glucocorticoids was measured on the campus during finals week.

Metabolites of nicotine, caffeine, and ethanol intake, including the summation of cotinine and *trans*-3'-hydroxycotinine, paraxanthine, and ethyl sulfate were also measured during this period, concomitant with stress. A Spearman rank correlation analysis showed strong associations between the wastewater-determined per person excretion rate of glucocorticoids, and the excretion of caffeine (r_s = 0.63) and nicotine (r_s = 0.49), but not of alcohol (r_s = 0.03) (Table 2).

Table 2 - Spearman nonparametric statistical analysis of variability between the sum of concentrations of two glucocorticoid hormones measured (cortisol and cortisone) and individual concentrations of alcohol and two stimulants detected in campus wastewater.

	Parameter	**Ethanol**	**Nicotine**	**Caffeine**
Σ Glucocorticoids	Coefficient r_s	0.03	0.49	0.63
	z-score	1.8	2.9	4.1
	p value	0.86	0.002	0.00005

Strength of Association |Coefficient r_s Range|	None; Very Weak 0 - |0.09|	Weak |0.10| - |0.29|	Moderate |0.30| - |0.49|	Strong |0.50| - |1.00|

These correlations are not surprising because caffeine is often used as a study aid, and to combat tiredness and/or low energy by university students, and faculty and staff [127, 152, 153]. Additionally, some studies have also showed that caffeine may increase

cortisol production and delay its deactivation in the body [154, 155]. Nicotine was also strongly correlated with measured stress. This result agrees with literature where nicotine consumption has been shown to serve as a coping strategy by smokers experiencing stress [156, 157]. However, the reasons behind why individuals smoke is complex (e.g., habit, addiction, stimulation), which is likely why the glucocorticoid-nicotine relationship is less robust than caffeine. Per person alcohol consumption showed no correlation with per person glucocorticoid excretion rates on campus, although alcohol consumption is known to increase cortisol production in the body [158]. However, alcohol consumption is also commonly employed as a means to relax or de-stress [159], thus it is likely that any kind of increase in cortisol production associated with the alcohol intake itself would be mitigated by the therapeutic use.

4.3.1 Limitations

Sampling occurred for seven consecutive days each month and was considered indicative of that entire month. It is possible that the reported minima, maxima and average values reported here would be subject to change if the sample coverage had been higher than 25% (1 of 4 weeks), as chosen in this study.

There is not a universally agreed upon analyte for the purposes of estimating population; different studies cite the same compounds with varying levels of success. This study included five of the most commonly sampled population biomarkers, and estimated population with previously published equations [137, 138, 160]. Equation parameters were determined based on the larger geographic location in which the campus is located. As these parameters are broad estimates, they may not exactly match the actual

values of this campus population. To help mitigate any biases, population estimates from individual compounds were averaged to obtain a final population estimate for each day.

Unlike WBE studies that monitor consumption of compounds that are consumed (e.g., marijuana), this study measured compounds that are produced endogenously and continuously by the human body. Consequently, longer-term monitoring was necessary, so that baselines estimates could be established, trends assessed, and perturbations identified. Glucocorticoid variability occurs both as a function of the stress response, and also due to the natural diurnal rhythm of cortisol production in the body [107]. To safeguard against over- or under-estimating glucocorticoid levels, aliquots of wastewater were collected throughout the entire day, rather than by a single "grab" sample. This should be considered a study strength rather than limitation, and this strategy also is employed in clinical settings, where it is a common practice to composite urine or blood samples for a 24-hour period to obtain robust estimates of daily excretion for cortisol and other biomarkers of interest [161].

4.4 Conclusions

The data presented here identify glucocorticoid monitoring in wastewater as a practical and promising tool for assessing population-level stress through anonymous and inexpensive means. As expected, the study found a rather pronounced modulation of glucocorticoid hormone levels over the 5-month study period. This, and the fact that glucocorticoid levels vary by gender, person, and over the course of the day, renders them interesting for studying population well-being and unreliable for the purpose of measuring population size. Measuring stress hormones in hair and fluids in non-human species is being performed extensively to assess stress levels; however, currently the only

human assessments occur in academic clinical research. With the largely deleterious impacts of stress on human health, monitoring glucocorticoids in wastewater represents a practical, economical and informative means of assessing stress at the community level. With the successes illustrated here, other endogenous compounds commonly measured in clinical settings, such as aldosterone (obesity-related hypertension) may also be evaluated for use in assessing population health via WBE approach [7].

TRANSITION 4

In the previous chapter, the utility of WBE was illustrated in an assessment of stress levels in a student population by way of tracking wastewater-borne glucocorticoid hormones, specifically cortisol and cortisone. Elevated stress levels are only one of many health threats in this demographic group, alcohol, nicotine and caffeine consumption are also included. This phenomenon is well documented in self-reported data from behavioral science studies, indicating higher incidences of binge drinking (> 4-5 drinks per event), cigarette consumption, and excess caffeine consumption (> 400 mg/day), often in energy drinks and co-occurring with the other behaviors. Since behavioral assessments based on self-reporting have well documented limitations, the WBE approach represents an alternative and complementary way of assessing these behaviors anonymously and at a low-cost, while side-stepping potential risks of data bias from self-reporting.

In Chapter 4, a WBE study was performed on a major U.S. university campus during the 2017-2018 academic year, with the goal of monitoring major metabolites of nicotine, alcohol, and caffeine consumption, including ethyl sulfate, nicotine, cotinine, *trans*-3'-hydroxycotinine, caffeine, and paraxanthine. Twenty-four hour daily composite samples were collected for seven consecutive days each month, at two access points on the campus. Samples were analyzed by LC-MS/MS, pharmacokinetic and population estimates used to determine daily per capita consumption for each sampled day. Wastewater-generated consumption estimates were compared to self-reported targeted demographic data, and U.S. national statistics. Trends were assessed statistically within and between consumption behaviors to assess short and long-term consumption variability.

CHAPTER 5

ALCOHOL, NICOTINE, AND CAFFEINE CONSUMPTION ON A PUBLIC U.S. UNIVERSITY CAMPUS DETERMINED BY WASTEWATER METROLOGY

ABSTRACT

Stimulant and depressant use in at-risk subpopulations, including college students, is a public health concern typically assessed intermittently only via self-reporting in surveys of limited sample size. We employed an alternative approach of directly measuring levels of three psychotropic substances (alcohol, caffeine, and nicotine) and their metabolites in wastewater derived from a large Southwestern U.S. university campus, during the 2017-2018 academic year. Per-capita alcohol consumption determined from ethyl sulfate levels in wastewater of the 'alcohol- and smoke-free' campus were 11.3 ± 7.5 g d^{-1} per person or 0.8 ± 0.5 drinks d^{-1} per person, similar to nationwide estimates from self-reporting of this subpopulation aged 18 - 25 years (10.1 ± 0.8 g d^{-1} per person or 0.7 ± 0.06 drinks d^{-1} per person).Wastewater-based rates of caffeine and nicotine consumption were significantly lower (p = 0.05) than nationwide estimates from self-reporting (caffeine: 114 ± 49 vs. 178 ± 19 mg d^{-1} per person; nicotine: 627 ± 219 vs. 927 ± 243 µg d^{-1} per person). Positive correlations were found for consumption of alcohol and nicotine (Spearman r_s: 0.71), nicotine and caffeine (0.59) and, more loosely associated, alcohol and caffeine (0.17). Alcohol and nicotine consumption were significantly higher on weekends over weekdays ($p < 0.0001$, $p = 0.01$), while caffeine consumption was higher during the week (p = 0.05). Between academic semesters, consumption patterns decreased for caffeine and nicotine consumption, and remained the same for alcohol. This

first U.S. wastewater-based epidemiology study on alcohol, caffeine and nicotine use among American college students demonstrated the feasibility and practicability of longitudinally tracking the behavior of an entire campus population of some 60,000 students directly, repeatedly, and inexpensively, when compared to traditional surveys that rely on memory, limited sample size, and geospatial extrapolation of survey results to locales of interest.

5.1 Introduction

Alcohol, caffeine, and nicotine are commonly consumed psychotropic substances, exhibiting varying levels of addictiveness while lacking the social stigma associated with consumption of illicit drugs. Excess alcohol consumption is estimated to lead to 88,000 deaths y^{-1} in the U.S. [162], with alcohol abuse costs (2018-adjusted) of US$289 billion annually [163]. Deaths related to nicotine consumption are predominately associated with cigarette consumption, including those from secondhand smoke, reach approximately 480,000 deaths annually [164]. Costs from lost productivity and health impacts of tobacco consumption range between $317.5 and $354 B annually [165]. Comparable economic data on the impact of caffeine consumption are currently unavailable, however to date, caffeine-related deaths are estimated at 92 individuals [166].

Consumption of legal compounds is generally assessed by self-reporting in behavioral studies, medical questionnaires, through clinical identification, or interpretation of consumer spending data. Unfortunately, these data sources may be subject to biases, incomplete coverage, delays, and frequently are lacking for demographically and geographically distinct subpopulations and settings. Wastewater-based epidemiology (WBE) is a complimentary approach of data gathering, whereby

consumption patterns are determined by measuring human biomarkers in sewage. Data can be collected and interpreted in near real-time and are population specific. Alcohol consumption is commonly assessed by the ethanol metabolite ethyl sulfate (EtS), with some studies including the less stable ethyl glucuronide [167, 168]. Nicotine consumption is commonly monitored with the parent compound as well as the two major metabolites, cotinine (COT) and *trans*-3'-hydroxycotinine (3-OH-COT) [169]. The minor metabolite 3-OH-COT is formed directly from COT rather than nicotine (NIC), and the relationship between the metabolites is an indicator of nicotine metabolism [170]. Caffeine consumption is assessed from caffeine, and one or more of the following metabolites including: paraxanthine, 7-methylxanthine, 1-methylxanthine, 1-methyluric acid, and/or 1,7-dimethyluric acid [160].

WBE has been used extensively in Europe to monitor alcohol, nicotine and caffeine consumption, as well as in China, Vietnam, Canada and Australia [98, 160, 169, 171-173]. By comparison, use of WBE in the U.S. is still in its infancy, with a notable lack of information on baseline concentrations of parental stimulants and metabolites interpreted in the context of the local geographic and demographic setting and health challenges faced by local communities.

An optimal demographic for WBE are institutions of higher education. Self-reported alcohol consumption for college students collected by the Substance Abuse and Mental Health Services Administration (SAMHSA) in 2016 (n = 3,544) showed 57.2% of students consumed alcohol in the past month, with 38% reporting binge drinking (4 - 5 drinks per event), and 10.5% reporting heavy alcohol use (binge drinking > 5 days per month) [174]. This study also reported that 21.1% of students consumed tobacco

products, predominantly cigarettes. More recently, nicotine consumption by vaporizers has increased, which has shown to carry less stigma than cigarette smoking and allows consumption in smoking-prohibited areas (e.g., smoke-free campuses, although often not permitted in these settings) [175]. Currently there are concerns about vaporizer use being a gateway to new or increased cigarette consumption.

Caffeine is the most highly consumed of the psychotropic compounds evaluated in this study, with 85% of the U.S. population reporting daily consumption [176]. In the college student population, there is concern of overuse (> 400 mg caffeine per day FDA recommendation), particularly because of the increased popularity of energy drinks (75 to 174 mg caffeine per serving) [177]. Additionally, self-reporting by college students showed that 25% of respondents reported consuming ≥ 3 energy drinks when mixing with alcohol (at a single event) [178]. Mixing caffeine with alcohol, masks the depressant effects of the latter, leading to increased alcohol consumption and the risk of alcohol-attributed injury or death [179, 180].

The goal of the present study was to *(i)* measure indicators of alcohol, caffeine, nicotine consumption directly in wastewater of an entire university campus population, *(ii)* compare per-capita consumption determined in wastewater to published self-reported data from the target demographic as well as U.S. national consumption averages, and (*iii)* assess consumption variability between months, days of the week, and semesters to identify patterns.

5.2 Materials and methods

5.2.1 Chemicals and reagents

Standards of ethyl sulfate (EtS) and sodium ethyl sulfate-d_5 (EtS-d_5) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Nicotine (NIC), cotinine (COT), trans-3'-hydroxycotinine (3-OH-COT), caffeine (CAF), paraxanthine (PAR), isotopically-labeled cotinine-d_3 (COT-d_3) and $^{13}C_3$-Caffeine ($^{13}C_3$-CAF), were purchased from Sigma-Aldrich (St. Louis, MO). LC-MS (liquid chromatography-mass spectrometry) grade methanol, acetone, and water were purchased from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA); formic acid was purchased from Fluka Chemical Corp (Milwaukee, WI). Stock solutions of native and isotopically-labeled compounds were prepared in methanol, except EtS and EtS-d_5 which were prepared in water. Stock solutions were stored in glass vials with polytetrafluoroethylene septa at −20 °C, except aqueous stock solutions which were stored at the 4°C.

5.2.2 Study location

The study was conducted at an undisclosed public university (population 60,000) in the United States during the 2017 - 2018 academic year (August 2017 to May 2018). Wastewater exiting the campus was captured at two collection points (i.e., North, South), with relative wastewater volumes of >95%, and <5%, respectively.

5.2.3 Sample collection and flow measurements

Each sample location was equipped with an Avalanche® refrigerated sampler (Teledyne ISCO, Lincoln, NE, USA), with 9 mm inner diameter (ID) silicon tubing on the pump head and suction line. Flow was monitored by an ISCO LaserFlow Flow meters

(Teledyne ISCO, Lincoln, NE, USA), located within each adjacent manhole. Flow-weighted daily composite samples were collected at each sampling location for 7-consequative days each month, with aliquot volumes ranging from 50 to 100 mL. Prior to each collection, the instrument was programmed to wash and purge the suction line before collection. Composite samples were collected in acid-washed glass bottles, transferred to high-density polyethylene (HDPE) sample bottles for transport and storage. Samples were processed same day to limit degradative losses.

5.2.4 Sample processing

Two-hundred milliliter aliquots of wastewater sample (raw) were spiked with 20 ng of COT-d_3 and $^{13}C_3$-CAF, and extracted separately using a Dionex™ AutoTrace™ 280 Solid-Phase Extraction Instrument (Thermo Scientific, Waltham, MA) with Hydrophilic-Lipophilic Balance (HLB) cartridges (6 cc, 150 mg, 30 um particle size) manufactured be Waters (Milford, MA). Cartridges were conditioned with 5 mL of methanol, then 5 mL of water, and after loading, were rinsed with 5 mL of water, and dried with nitrogen for 10 minutes. Target analytes were extracted with 1:1 (v/v) methanol and acetone containing 0.5% formic acid, into a final volume of 4 mL. Finally, 200 µL of the final extract was blown-down under a gentle stream of nitrogen at ambient temperature using a Reacti-Therm TM Heating and Stirring Module TS-18821 (Thermo Scientific, Waltham, MA), and reconstituted in 1+1 (v/v) methanol and water mixture for analysis. For EtS, 10 mL of raw wastewater was centrifuged at 4,000 *g* for 10 min (Eppendorf Centrifuge 5810 R, Hamburg, Germany). To 500 µL of the supernatant, 490 µL of water and 1 ng of EtS-d_5 were added. Analyses were performed by liquid

chromatography tandem mass spectrometry (LC-MS/MS); each sample was prepared and analyzed in duplicate.

5.2.5 Sample analysis

Wastewater extracts were analyzed using a Shimadzu 2100 high performance liquid chromatographer (HPLC), coupled to an AB Sciex APE 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA). Analytes were separated on a Symmetry C_8 column (4.6 × 150 mm, 3.5 μm particle size) preceded by a Symmetry VanGuard Cartridge (3.9 × 5 mm, 3.5 μm particle size) (Waters, Milford, MA). Methanol was used as mobile phase A and water containing 0.1% formic acid used as mobile phase B (0.2% formic acid for EtS). Detailed analytical method information is provided in supplemental information (Appendix C: Table 15).

5.2.6 Quality assurance/quality control

Reported concentrations were determined based on a 7-point (minimum) standard curve, with a minimum coefficient of determination was $r^2 = 0.99$. Average recoveries for analytes were determined based on spike-recovery experiments. Relative recoveries ranged from 73% to 109%. Each extraction was performed with method blanks (deionized water), and instrument blanks (1:1 methanol and water) to enable the detection of potential contamination and analyte carryover in the instrument. Method detection limits (MDL) were determined using methods outline here [123], and are provided in the supplemental information (Appendix C: Table 16). Precision was expressed as Relative Percentage Differences (RPD) and calculated using the following equation:

$$RPD\ (\%) = ABS\left(\frac{C_{S1} - C_{S2}}{C_{S1} + C_{S2}}/2\right) x\ 100 \qquad \text{Eq. 10}$$

where C_{S1} and C_{S2} are the measured concentrations in the sample and its associated duplicate.

5.2.7 Data analysis

LC-MS/MS data were acquired with Analyst 1.5 software (Applied Biosystem, Foster City, CA). Concentrations were calculated using the isotope-dilution method when analyte peak signal-to-noise ratio was greater than 3, and the concentrations were above the MDL. Per capita daily Alcohol Consumption (AC) was calculated using the following equation:

$$AC = \frac{C_{EtS} * Q_{Tot} * \left(\frac{MW_{EtOH}}{MW_{EtS}}\right)}{EF_{EtS} * Pop} \quad \text{Eq. 11}$$

where C_{EtS} is the measured ethyl sulfate concentration, Q_{Tot} is the total daily volumetric flow rate, $\frac{MW_{EtOH}}{MW_{EtS}}$ is the ratio of molecular weights of the parent [ethanol (EtOH)] and metabolite (EtS), EF_{EtS} is the excretion factor of ethyl sulfate (0.012%) [98, 99, 181], and Pop is the estimated population (Eq. 14). Daily Nicotine Consumption (NC) was calculated using the following equation:

$$NC = \frac{(C_{COT} + C_{3\text{-}OH\text{-}COT}) * Q_{Tot} * CF_{NIC}}{Pop} \quad \text{Eq. 12}$$

where C_{COT} and $C_{3\text{-}OH\text{-}COT}$ are the measured concentrations of COT and 3-OH-COT, and CF_N is the correction factor 1.35 (74% excretion, weight-basis) [101]. Daily Caffeine Consumption was calculated using the equation:

$$CC = \frac{C_{PAR} * Q_{Tot} * \left(\frac{MW_{CAF}}{MW_{PAR}}\right)}{EF_C * Pop} \quad \text{Eq. 13}$$

where C_{PAR} is the measured paraxanthine concentration, $\frac{MW_{CAF}}{MW_{PAR}}$ is the ratio of molecular weights of the parent (CAF) and metabolite (PAR), and EF_{PAR} is excretion factor of paraxanthine (4.6%) [102].

Per-person consumption was determined by estimating daily population using the average population size indicated by sewage-borne population biomarkers and by daily wastewater volumes using the following equation:

$$Population = \left(\frac{C_{HIAA} * Q_{Tot}}{EF_{HIAA}} + \frac{C_{ENT} * Q_{Tot}}{EF_{ENT}} + \frac{Q_{Tot}}{189}\right)/n \quad \text{Eq. 14}$$

where C_{HIAA} is the measured concentration of 5-Hydroxyindoleacetic acid (5-HIAA), EF_{HIAA} is 4.16 mg/day per capita excretion [122], C_e is the measured concentration of enterolactone, EF_{ENT} 1.1 is the average mg/day enterolactone excretion [130], 189 is the estimated liter per person per day for water consumption on the mixed-used campus (approximately 50 gallons per day), and n is the number of population size biomarkers used in the assessment.

Statistical assessments were performed using Mann-Whitney U-Tests for inter-species variability. A Benjamini-Hochberg (BH) correction factor was applied with a false discovery rate of 0.1 to avoid false positives with replicate comparisons. Spearman rank-order correlations were performed to assess correlations between nicotine, caffeine, and alcohol consumption.

5.3 Results and discussion

This study sought to employ WBE to assess alcohol, caffeine, and nicotine consumption on a university campus to provide an alternative and comparative metric to

surveys from behavioral studies that targeted the college-age demographic. Daily composite wastewater samples were collected for seven consecutive days (Monday through Sunday) during each month of the semester, from August 2017 through May 2018 (n = 70). Measured concentrations in campus wastewater from the north and south sample points are shown in Appendix C: Figure 25. Excretion profiles of selected analytes are shown in Figure 15, with half-lives ranging from 3.1 h to 16 h.

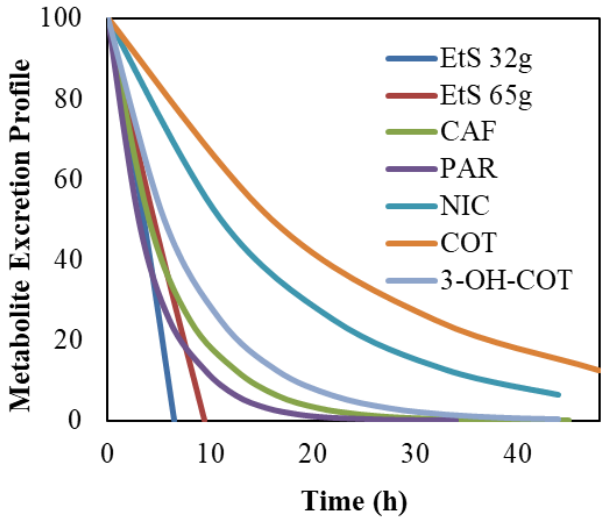


Fig. 15. Excretion profiles of analytes of interest for alcohol, caffeine, and nicotine consumption. Half-lives of these compounds include 3.3 h [Ethyl Sulfate (EtS) 32 g], 4.7 h (EtS 64 g), 4.1 h [Caffeine (CAF)], 3.1h [Paraxanthine (PAR)], 2 h [Nicotine (NIC)], 16 h [Cotinine (COT)], and 5.5 h *trans*-3'-hydroxycotinine (3-OH-COT)]. EtS shows corresponding dose of alcohol consumed. [126, 182-184]. Excretion profiles follow zero- and first-order kinetics [185-187]

Concentrations of EtS, PAR, COT, and 3-OH-COT were converted to daily mass loads using total flow data captured by flow meters at each of the two sampling locations (Appendix C: Table 17). Only metabolite data were used in consumption assessments due to suspected non-excretion inputs into wastewater (Appendix C: Figure 26). Mass loads

of EtS were higher on weekends (130 ± 39 g d^{-1}) compared to weekdays (68 ± 19 g d^{-1}, $p < 0.00001$), with maximums occurring on the day of the annual homecoming football game in October (Saturday, 233 ± 1.4 g d^{-1}) and St. Patrick's Day (Saturday, 124 ± 6.3 g d^{-1}; Figure 16). An opposing trend was observed for PAR, with elevated mass loads on weekdays (3.4 ± 1.3 kg d^{-1}) over weekends (1.8 ± 0.4 kg d^{-1}, $p < 0.00001$). The highest loading occurred during the Monday of October (7.3 ± 0.004 kg d^{-1}), but higher masses were also recorded during various weekdays in September, October, and February. COT and 3-OH-COT showed variable patterns through the academic year, with some months showing a decline in weekend loads (e.g., October), while others did not change between weekdays and weekends (e.g., January).

Per-person consumption of alcohol, caffeine, and nicotine were estimated using the measured metabolite concentrations, combined with previously published pharmacokinetic excretion factors. Population estimates were generated using the biomarkers 5-HIAA (major metabolite of serotonin), ENT (byproduct of lignin breakdown in gut), and estimated daily per-person water usage for the campus (189 liters per day per person) (Appendix C: Table 18). Alcohol, nicotine, and caffeine consumption for the entire academic year illustrated a similar trend for all analytes, where consumption was elevated at the start and end of the academic year, with a mid-year trough (Figure 16).

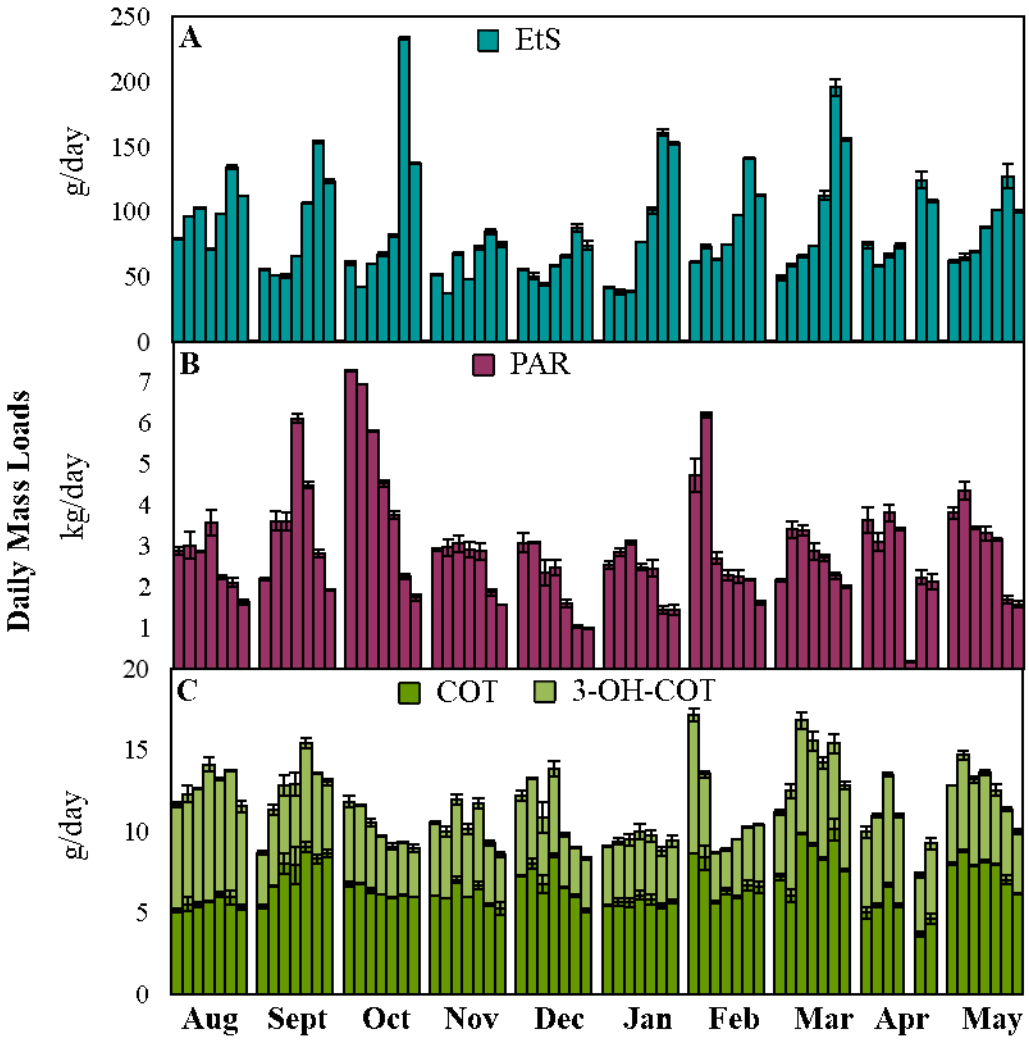


Fig. 16. Daily mass loading of ethyl sulfate (EtS) (g d^{-1}) (A), paraxanthine (PAR) (kg d^{-1}) (B), cotinine (COT) and *trans*-3'-hydroxycotinine (3-OH-COT) (g d^{-1}) (C) on a major U.S. university campus during the 2017-2018 academic year. The corresponding bars for each month include Monday through Sunday mass loads. Error bars represent the minimum and maximum results of duplicate samples.

Per-capita consumption of alcohol displayed a pattern similar to EtS daily mass loads, however, weekday/weekend differences were more prominent ($p < 0.00001$) because of the weekend decrease in campus population. Highest per-person consumption remained the Saturday of the university's annual homecoming football game (41 ± 0.2 g

d^{-1}), which also coincided with the Saturday immediately preceding Halloween. Consumption here was 1.5-times higher than any other day of the academic year and equated to 3 drinks per person per day. A standard drink is defined as 14 g of pure ethanol or more generally, 355 mL of beer (5% alcohol by volume), 148 mL of wine (12%), or 15 mL of distilled spirits alcohol (40%) [188]. Targeted demographic data of alcohol consumption from multiple studies that monitored alcohol consumption over the academic year showed average consumption at 10.1 ± 0.8 g d^{-1} per person (0.7 ± 0.1 drinks d^{-1} per person) [189-191]. The annual consumption average in this study was similar at 11.3 ± 7.5 g d^{-1} per person (0.8 ± 0.5 drinks d^{-1} per person). For 6 of the 10 months of monitoring, the monthly per-person average for alcohol consumption obtained in this study was at or below literature values. A comparison to the U.S. national average (19 g d^{-1}, 1.4 drinks per day per person) [192], proved higher than the targeted demographic data estimated from wastewater and from survey-based data. The campus population only surpassed this US national average on 10 sampled days during the academic year, all of which occurred on a Saturday or Sunday. Statistically, the month of August had the highest average consumption (17.4 ± 5.2 g d^{-1}), and was statistically different from November (p = 0.05), December, February and April (p = 0.01) (Figure 17). Over the entire academic year, there was no evidence of increased alcohol consumption in campus wastewater as the fall (1^{st}) and spring (2^{nd}) semesters were not significantly different. These results agree with available demographic survey data [189, 190, 193].

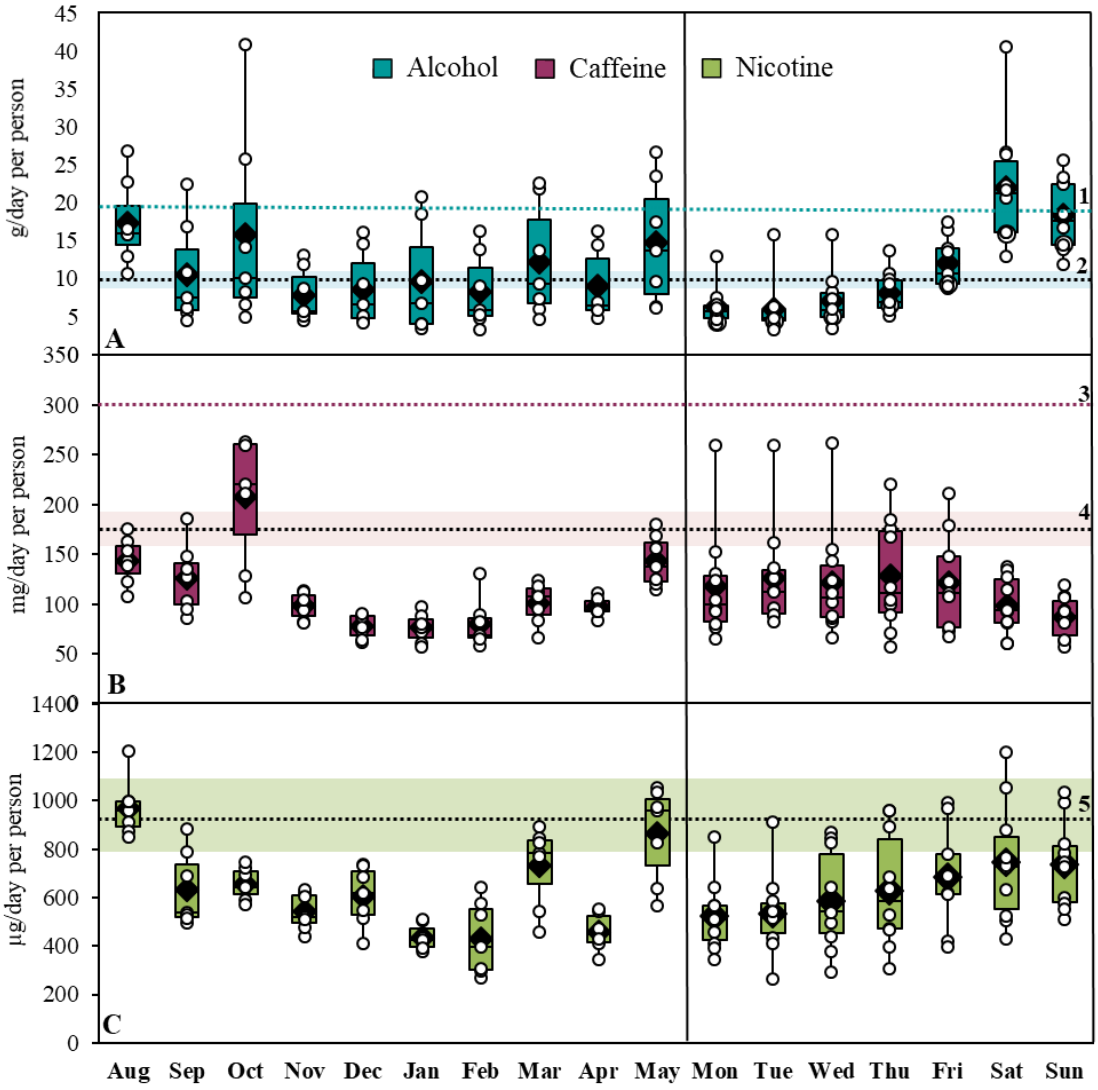


Fig. 17. Box plot summarizing per capita alcohol (A), caffeine (B), and nicotine (C) consumption in campus wastewater during the academic year, organized by month and day. Plots show the 25th, 50th (median) and 75th quartiles, minimum/maximum error bars, mean (diamond), and all contributing data points (circles). U.S. college student average per capita consumption are illustrated by lines 2, 4, and 5, standard errors shown in color. U.S. per capita consumption is included for alcohol (1) and caffeine (3). U.S. per capita nicotine consumption (not shown) is 4,147 µg d^{-1} per person.

Per-person caffeine consumption also displayed a similar pattern to daily mass loads, however, weekday/weekend differences were dampened when population was included in the analysis (p = 0.05). Monthly comparisons show elevated levels of caffeine

consumption in October, driven by surging weekday consumption, which were the five highest consumption days of the entire year (243 ± 22.4 mg d^{-1}). Complimentary student-specific caffeine consumption data showed an average annual consumption at 196 ± 19 mg d^{-1} per person [194, 195], whereas in this study the average was lower at 114 ± 49 mg d^{-1} per person. The majority of daily campus consumption estimates were roughly comparable to the average amount of caffeine found in standard 150 mL home-brewed coffee (100 mg) [196]. The first and last months of the academic year (August, May), and October were found to be significantly different from December and January (p = 0.01). There were no statistical differences between days of the week, however the fall semester was significantly higher than the spring semester (p = 0.05) (Figure 17). During no point of the academic year did the per-person caffeine consumption average surpass the Food and Drug Administration (FDA) U.S. average caffeine intake estimate of 300 mg d^{-1} [129] or the recommended threshold value of 400 mg caffeine d^{-1} [197].

Per-capita nicotine consumption showed a more pronounced pattern of elevated weekend consumption than mass loads (Figure 16), with weekends being statistically higher (p = 0.01). The highest consumption occurred during the first month of the semester (970 ± 109 µg d^{-1} per person), which was significantly different from all other months except September, April, and May (p = 0.01) (Figure 18). This highest consumption occurred on the Saturday in August, with a per-capita estimate at 1205 ± 44 µg d^{-1} per person, equivalent to 1 standard cigarette per day per person. Targeted demographic survey data from published sources show average nicotine consumption at 927 ± 243 µg d^{-1} per person [198, 199]. In this wastewater study, nicotine consumption was only 627 ± 219 µg d^{-1} per person. The lower estimate here is in agreement with a

2017 campus survey study that found only 10% of students smoke, lower than the other published studies of campus nicotine consumption [200, 201]. The U.S. estimate of average per capita nicotine consumption can be expressed as approximately 3.3 cigarettes per day per person or 4147 μg d^{-1} per person [202], assuming 1.25 mg nicotine absorbance form a standard US cigarette [126]. Consumption patterns on the campus were well below this value. Comparisons between days of the week, showed no statistical difference, however the fall semester was significantly higher than the spring semester ($p = 0.05$), driven by August consumption.

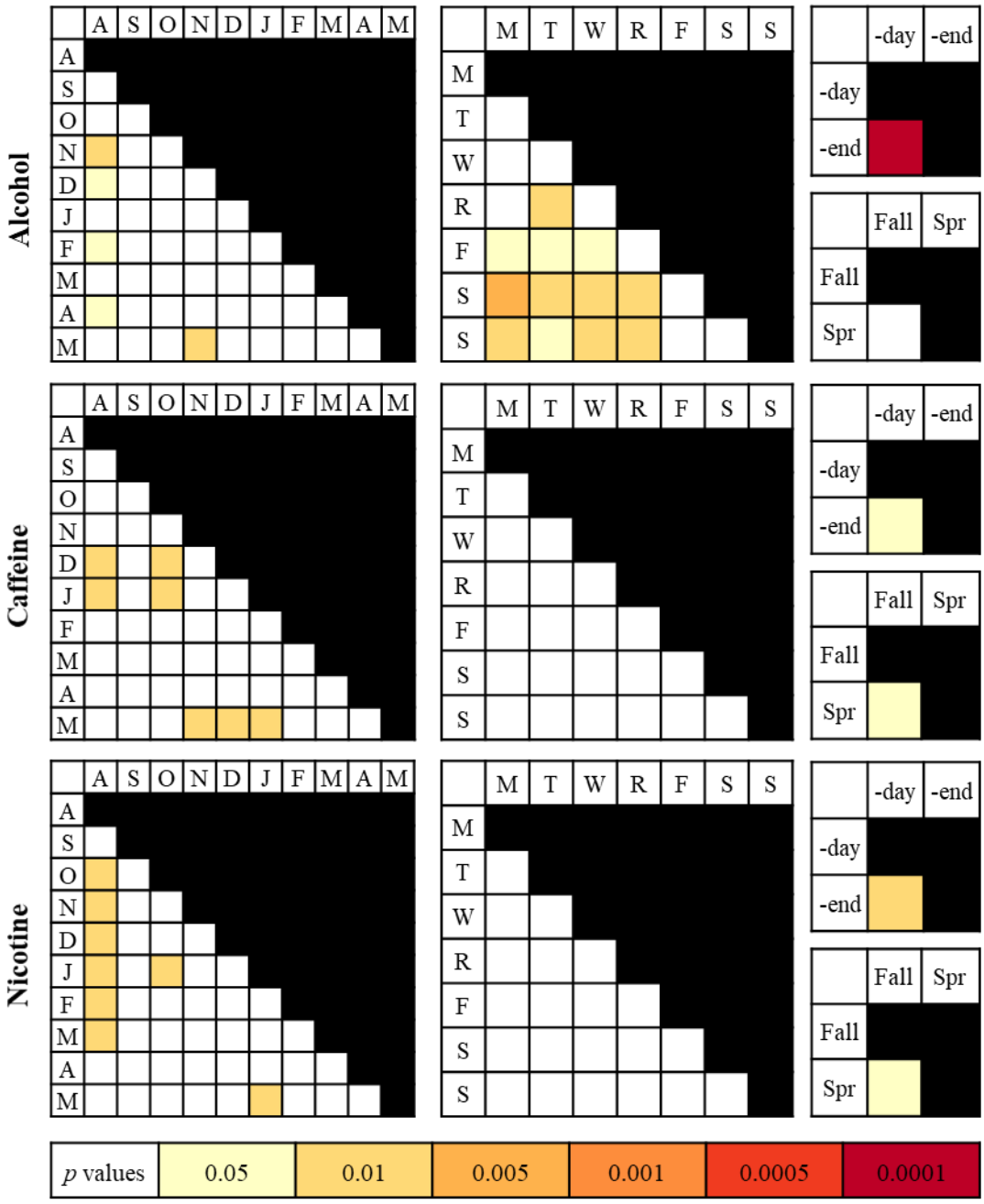


Fig. 18. Correlation plots showing results of statistical comparisons of alcohol, caffeine, and nicotine consumption between months, days of the week, weekday/weekend, and semesters. Colors denote statistical significance.

A Spearman rank correlation analysis was performed to assess the relationship between these three consumption behaviors. Strong correlations were found between alcohol and nicotine consumption (r_s = 0.71, p < 0.0001), and nicotine and caffeine consumption (r_s = 0.59, p = 0.001), and a weak correlation between alcohol and caffeine (r_s = 0.17). Co-occurrence of alcohol and nicotine is well documented, with studies citing increases in the number of cigarettes smoked by a smoker when alcohol is being consumed [203, 204]. Additionally a subset of the college-age population is reported to only consume nicotine during alcoholic beverage consumption [205]. This co-occurrence is largely driven by pleasure-seeking and reportedly increased the craving for each [206]. Similarly, the relationship between nicotine and caffeine has also been shown to co-occur, with caffeine used to enhance the effects of the nicotine [207]. Consequently, nicotine consumers have been shown to have higher daily caffeine consumption than those who abstain [208]. In this study, only a weak correlation was observed between the consumption of alcohol and caffeine. However, recent literature on caffeine consumption in college students has specifically focused on the increasing trends of energy drink and alcohol consumption. Studies have found a correlation between daily energy drink intake and increased alcohol consumption, and reported that co-consumption increased the total quantity of alcohol ingested during an episode [179, 209-211]. Since this co-occurring behavior is a small percentage of the total caffeine consumption pattern [194], the correlation may not be visible in the wastewater assessment.

5.3.1 Limitations

The measured signal in wastewater is not only dependent on the duration and quantity of the consumption behavior studied, but also on the excretion half-life profiles

of the measured analyte and on the timing of campus attendance. The interplay between these variables drives the measured signal and becomes particularly important during weekend consumption by the non-resident population, and afternoon/evenings consumption of this non-resident demographic, when excretion rates are fast. Single excretion factors were used to back-calculate alcohol, nicotine, and caffeine consumption in the population, however individual demographics and behaviors will vary excretion rates for a specific individual. Selected excretion factors fell within accepted ranges and were previously used in WBE studies.

Comparisons between wastewater data generated here and complimentary datasets were limited largely due to the lack of granularity in the self-reported data. Often data were presented as consumption ranges, which were not directly amendable to direct comparisons. Data dense self-reported studies (e.g., diary studies) that are best for comparison to WBE studies, are infrequent, as these are highly cost prohibitive. Wastewater sampling here occurred for seven consecutive days each month and was considered indicative of that entire month. Reported values may change if sample coverage was > 25%, particularly if winter or spring breaks were included in the analysis.

Analyte losses during travel time in the sewer pipes were not accounted for in this study. Previously stability studies have shown that these compounds are relatively stable in wastewater [138, 212]. Here, in-pipe travel times were on average 50 min, shorter than most published studies. However, any degradative losses in wastewater-derived estimates would be systematic, and would not affect trends in the data. Additionally, there are no universally agreed upon analytes to assess population numbers in WBE. Many of the common compounds used in the field are related to nicotine and caffeine consumption,

which could not be included here. Therefore, fewer markers were used in this study, however the selected analytes showed previous success, and required fewer assumptions in the estimate equations.

5.4 Conclusions

This study is the first in the U.S. to assess alcohol, caffeine, nicotine consumption by WBE, on a university campus for the entire academic year and demonstrates the feasibility of longitudinally tracking the consumption behaviors of an entire university campus of a population of 60,000 students. Traditional surveys are generally much more limited in sample size. The published self-reported university studies cited herein for comparisons, surveyed approximately 1,000 people each. Larger governmental surveys like those conducted by the Substance Abuse and Mental Health Services Administration, sampled approximately 3,500 college students to determine alcohol and caffeine consumption patterns for the nation [174], while the Bureau of Labor Statistics consumer expenditure surveys sample 12,000 households per quarter [213]. Estimates of survey costs range from $0.50 to $4.50 per completely answered questionnaire (depending on dissemination method) [214]. WBE surpasses the sample size of traditional self-reported data collection methodologies, and at a fraction of the cost associated with those studies.

TRANSITION 5

In previous chapters, steroid hormones and common licit substances were monitored in a student population of a major U.S. university campus, to assess population health and consumption patterns throughout the academic year. These studies illustrated the utility in these assessments for directly measuring population stress via glucocorticoid hormones; an interesting result was that this particularly academic population apparently did not indulge in overconsumption of licit drugs, reported in complementary studies of this age group.

As wastewater-based epidemiology studies become increasingly more common for use in population health assessments, an evaluation of frequently employed WBE methodologies is warranted to continuously improve the robustness of data collected on health metrics. Wastewater sampling methodologies are one such component of the methodology requiring scrutiny and optimization, as errors imparted in sampling techniques implemented upstream will propagated through to the final estimates of per-capita consumption. Wastewater sampling methodologies traditionally employ high frequency discrete samplers to collect aliquots of wastewater at intervals defined by a time- or flow- regime. With these sampling methods, there have been concerns regarding the potential to miss pulses of analytes in the sewer system.

In Chapter 5, two different sampling strategies, a low-flow, near-continuous active sampler and a traditional, commercially available time-weighted discrete sampler, were deployed at a wastewater treatment plant. The samplers were programmed to collected daily composite influent samples over the course of 7 consecutive days. Analytes of interest measured by LC-MS/MS included the following opioids, opioid

antagonists, stimulants and one benzodiazepine anxiolytic (parent/ metabolite): morphine/ morphine-3-glucuronide, oxycodone/ noroxycodone, codeine/ norcodeine, heroin/ 6-acetylmorphine, fentanyl/ norfentanyl, methadone/ 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, buprenorphine/ norbuprenorphine, methamphetamine, cocaine/ benzoylecgonine, 3,4-methylenedioxy-methamphetamine (MDMA), amphetamine (Adderall), methylphenidate (Ritalin), and alprazolam/ alpha-hydroxyalprazolam (Xanax). Additionally, ethyl sulfate, nicotine, cotinine, *trans*-3'-hydroxycotinine, caffeine, and paraxanthine were also monitored. Analyte mass loads were compared and statistically assessed for comparison of the two distinct sampler types. Consumption behaviors were estimated using pharmacokinetic and population data. A modeling study also was performed to illustrate how static and dynamic events in analyte concentrations are recorded differently by each type of sampler used.

CHAPTER 6

IN SITU ACTIVE SAMPLING TO DETERMINE COMMUNITY EXPOSURE USING WASTEWATER METROLOGY

ABSTRACT

Accurately assessing community health by wastewater-based epidemiology requires the acquisition of representative samples and a preservation of the biomarkers contained therein. Traditional discrete liquid capture methods may fail to capture pulse loads of biomarkers in wastewater, potentially underestimating consumption. This study compared the capture of wastewater-borne biomarkers of licit and illicit drug consumption using two different devices, a traditional time-integrated liquid capture sampler and a low-flow, near-continuous active sampler performing *in situ* solid phase extraction. Results from modeling and field studies showed that the continuous sampler *(i)* drew samples for 93% of the total sampling duration as compared to the 3% for the conventional sampler that operated in 15-min intervals only; *(ii)* captured pulse events when the discrete sample did not, including for the opioids heroin and fentanyl, and the opioid antagonists norbuprenorphine and methadone; and *(iii)* consistently provided statistically significantly higher mass loads for morphine, heroin, fentanyl, norfentanyl, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine [EDDP, (methadone metabolite), norbuprenorphine, methamphetamine, amphetamine, and caffeine ($p \leq 0.01$). This study represents the first reported use of *in situ* solid phase extraction for capture of analytes of interest contained in sewage for health monitoring using wastewater-based epidemiology.

6.1 Introduction

Domestic wastewater from centralized sewerage systems is a useful matrix to monitor the health, lifestyle, and behaviors of populations, including licit and illicit drug consumption, and inadvertent chemical exposures [98, 215-217]. Wastewater-based epidemiology (WBE) may be performed in parallel to traditional forms of public health monitoring to enrich and complement available information [169, 218, 219]. As these methods become increasingly integral to the analysis of public health, researchers are beginning to improve and standardize WBE studies [23, 220, 221].

The method of sample acquisition plays an important role in the representativeness and quality of WBE data, affecting results obtained later during analysis and estimation of population exposure rates. Sampling frequency is one such parameter under assessment. Sampling to date has generally focused on weeklong sampling campaigns to estimate monthly or annual consumption [13, 222]. Efforts are underway to determine the optimal collection frequency to accurately characterize exposure in a sewershed based on known analyte consumption patterns (e.g., known differences between weekend and weekday consumption) [223]. A recent study suggested 56 stratified random samples are necessary to obtain reliable results for annual drug consumption estimates [224]. To increase the number of days sampled, alternative sampling techniques have been employed, including the use of passive samplers [225, 226].

Another facet of sampling that necessitates evaluation is the manner in which the sewage is collected for a given day. Wastewater sampling methodologies traditionally included single discrete (grab) samples or 24-hour composite samples [227]; the majority

of recent WBE studies collect daily composite samples [21, 22]. These daily composite samples are collected at discrete intervals based on time (time-integrated) or flow (flow-weighted), and collected aliquots are combined into a single vessel. With these methods, there have been concerns regarding the potential to miss pulses of analytes flowing through the sewer system [23]. For compounds with rare use and short elimination half-lives, consumption estimates may be driven largely by chance capture, resulting in an under- or over-estimation of substance use and exposure in the population. Although samplers that collect daily composite samples are capable of pumping continuously, the lowest flow rates possible still produce a large total volume of sample, creating challenges for sample storage, handling, processing, and disposal of resultant hazardous waste [226].

Relatively little attention has been paid thus far to identifying and evaluating different sampling methodologies for collection of daily composite samples. Guo et al., 2017 designed a device employing organic diffusive gradients in thin films (o-DGT) for the capture of three substances of abuse, including ketamine, methamphetamine, and amphetamine [24]. Deployed o-DGT samples were extracted daily and compared to simultaneously collected grab samples. When using passive samplers, the intent generally is to maximize deployment time, although short-term deployments are a viable option [24]. With any environment, passive samplers face challenges related to compound uptake rates, which are sensitive to factors such as turbulent flow, boundary layer development, changes in temperature, and fouling [228]. A principal challenge is to convert mass-per-sampler results to concentration data, a challenge typically addressed by employing performance reference compounds as done in polar organic chemical

integrative samplers [229]. The high amount of suspended particulate matter in wastewater poses additional challenges to the use of passive samplers. First, monitoring of wastewater necessitates sampler removal and cleaning to allow for continued efficiency [226]. Additionally, analytes of interest with an affinity for sorption to particulates will not be as readily captured by passive samplers, which only capture dissolved-phase species.

A promising alternative solution to this predicament is the use of active samplers operating at low flow rates to limit the total sample volume obtained, while still capturing the chemistry for each time increment of interest. Active sampling devices meter water in a controlled manner, removing uncertainty in compound uptake rates and have the ability to capture the total mass of analytes regardless of whether they are truly dissolved or partially/completely sorbed to small, suspended particles. Low-flow active samplers offer a variety of designs including, submersible, *in situ* extraction, on- or off-board power, and functionality for surface water, porewater, groundwater, or ocean deployment [230-234].

The goal of this study was to compare a novel low-flow, near-continuous active sampling device with solid phase extraction technology to a traditional 24-hour time-weighted sampling strategy employing a traditional high frequency discrete sampler, to evaluate how mass loads and apparent consumption patterns changed between sampling methodologies. The underlying hypothesis was that near-continuous sampling at low flow rates may increase the overall mass of analyte recovered, due to better capture of short-term biomarker pulses and due to *in situ* extraction and preservation of labile biomarkers on solid phase extraction resin beds. Analytes of interest included common

compounds measured for WBE purposes including indicators of alcohol, caffeine, nicotine consumption, and various licit and illicit narcotics.

6.2 Materials and methods

6.2.1 Chemicals and reagents

Standards of ethyl sulfate (EtS) and sodium ethyl sulfate-d_5 (EtS-d_5) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Nicotine (NIC), cotinine (COT), *trans*-3'-hydroxycotinine (3-OH-COT), caffeine (CAF), paraxanthine (PAR), isotopically-labeled cotinine-d_3 (COT-d_3) and $^{13}C_3$-Caffeine ($^{13}C_3$-CAF), were purchased from Sigma-Aldrich (St. Louis, MO). Narcotics, including labeled standards, were purchased from Cerilliant (Sigma, Aldrich, Louis, MO) and included parent/ metabolite of morphine (MOR)/ morphine-3-glucuronide (M3G), oxycodone (OXY)/ noroxycodone (NOXY), codeine (COD)/ norcodeine (NCOD), heroin (HER)/ 6-acetylmorphine (6-AM), fentanyl (FENT)/ norfentanyl (NFENT), methadone (MDONE)/2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine (BUP)/ norbuprenorphine (NBUP), methamphetamine (METH), cocaine (COC)/ benzoylecgonine (BZYL), 3,4-methylenedioxy-methamphetamine (MDMA), amphetamine (AMP), methylphenidate (MPH), and alprazolam (ALP)/ alpha-hydroxyalprazolam (OH-ALP). Narcotic labeled standards are listed in Appendix D: Table 19. LC-MS (liquid chromatography-mass spectrometry) grade methanol, acetone, and water were purchased from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA); formic acid was purchased from Fluka Chemical Corp (Milwaukee, WI). Stock solutions of native and isotopically-labeled compounds were prepared in methanol, except EtS and EtS-d_5 which were prepared in water. Stock solutions were stored in glass

vials with polytetrafluoroethylene septa at −20°C, except aqueous stock solutions which were stored at the 4°C.

6.2.2 Sample location

The study was conducted at an undisclosed wastewater treatment plant (WWTP) in the southwestern U.S. The facility processes approximately 57 million liters per day (MLD) (15 million gallons per day) of wastewater from domestic and industrial sources. The WWTP serves a population of approximately 125,000 people.

6.2.3 Sample collection and handling

Daily composite samples were collected by two sampling technologies from wastewater influent at the treatment plant. An ISCO 4700 refrigerated sampler (Teledyne ISCO, Lincoln, NE, USA), with 1.27 cm (½ in.) diameter vinyl tubing (suction line), and silicon tubing (pump head), was used to collect aliquots of wastewater influent at 15-minute intervals, equating to approximately 40 min d^{-1} of analyte capture. Instrument lines were purged and rinsed with wastewater prior to sample collection. Aliquots were collected in a 10-L LDPE bottle, and transferred to high-density polyethylene (HDPE) sample bottles (on ice) for transport to the laboratory. Samples were processed on the same day to limit degradative losses. Concurrently, daily composite samples were collected by a sampler designed and fabricated at the Biodesign Center for Environmental Health Engineering at Arizona State University [230, 233]. The sampler consists of a positive displacement pump, which meters liquid through an inert fluid train, to replicate solid phase extraction (SPE) cartridges. Daily composite samples were collected from

approximately 7:30 am to 7:30 am Monday through Sunday during the Month of April 2018.

6.2.4 Sample processing

Wastewater collected by the high-frequency discrete sampler was separated into duplicate two-hundred milliliter aliquots and spiked with 20 ng of labeled standard. Wastewater was extracted using Hydrophilic-Lipophilic Balance (HLB) cartridges (6 cc, 150 mg, 30 um particle size) manufactured by Waters (Milford, MA) on a Dionex™ AutoTrace™ 280 Solid-Phase Extraction Instrument (Thermo Scientific, Waltham, MA). Cartridges were first conditioned with 5 mL of methanol, then 5 mL of water before sample loading, then rinsed with 5 mL of water, and dried with nitrogen for 10 minutes. Analytes were extracted with 1:1 (v/v) methanol and acetone containing 0.5% formic acid, into a final volume of 4 mL. Finally, 200 µL of the final extract was evaporated under nitrogen at ambient temperatures using a Reacti-Therm TM Heating and Stirring Module TS-18821 (Thermo Scientific, Waltham, MA). Samples were reconstituted in 1+1 (v/v) methanol and water mixture for analysis of caffeine- and nicotine-related compounds, and 100% water for narcotics. For EtS, 10 mL of raw wastewater was centrifuged at 4,000 *g* for 10 min (Eppendorf Centrifuge 5810 R, Hamburg, Germany). Supernatant (500 µL) was collected and mixed with 490 µL of water and 10 µL of 100 µL mL^{-1} of EtS-*d5*.

The near-continuous sampler extracted wastewater *in situ* throughout the 24-h time period when the instrument was deployed, at an average flow rate of 160 µL min^{-1}. Prior to deployment, 20 ng of labeled standard were spiked into ~200 mL of deionized water and loaded onto SPE cartridges; the conditioning steps were the same as outlined

above, however the cartridges were not dried. Cartridges were exchanged daily on the in the instrument. Loaded cartridge were capped and upon return to the lab, the remaining wastewater volume was vacuum-pulled through the cartridge using a Visiprep™ Solid Phase Extraction (SPE) Vacuum Manifold [Sigma-Aldrich (St. Louis, MO)], followed by 5 mL of liquid chromatography grade water. The cartridges were then dried for approximately 10 minutes under vacuum. Cartridge extraction and sample preparation were the same as outlined above for nicotine- and caffeine- related compounds, and for narcotics. The processed wastewater from each SPE cartridge was captured and later used to quantify EtS in the same manner as outlined above for the raw wastewater samples. All analyses were performed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

6.2.5 Sample analysis

Wastewater extracts were analyzed using a Shimadzu 2100 high performance liquid chromatographer (HPLC), coupled to an AB Sciex APE 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA). CAF, PAR, NIC, COT, 3-OH-COT, and EtS were separated on a Symmetry C_8 column (4.6 × 150 mm, 3.5 μm particle size) preceded by a Symmetry VanGuard Cartridge (3.9 × 5 mm, 3.5 μm particle size) (Waters, Milford, MA). For narcotics, a Symmetry C_{18} column (4.6 × 75 mm, 3.5 μm particle size) preceded by a Symmetry VanGuard Cartridge (Waters, Milford, MA), was used. Methanol was used as mobile phase A for all methods, and water containing 0.2% formic acid used as mobile phase B for EtS and narcotics; 0.1% formic acid was used for remaining compounds. Detailed analytical method information has been published previously [11].

6.2.6 Quality assurance and quality control

Concentrations of biomarkers were determined using a 7-point (minimum) standard curve, and a coefficient of determination of ≥ 0.99. Each extraction was performed with method blanks (deionized water), and instrument blanks (1:1 methanol and water) to enable the detection of potential contamination and analyte carryover in the instrument. Method detection limits (MDL) were determined according to national protocols [123]. MDLs and analyte recoveries are shown in Appendix D: Table 20. Precision was expressed as Relative Percentage Differences (RPD) and calculated using the following equation:

$$RPD\ (\%) = ABS\left(\frac{C_{S1} - C_{S2}}{C_{S1} + C_{S2}}/2\right) x\ 100 \qquad \text{Eq. 15}$$

where C_{S1} and C_{S2} are the measured concentrations in the sample and its associated duplicate.

5.2.7 Data analysis

LC-MS/MS data were acquired with Analyst 1.5 software (Applied Biosystem, Foster City, CA). Concentrations were calculated using the isotope-dilution method when analyte peak signal-to-noise ratio was greater than 3, and the concentrations were above the MDL. Per-capita daily consumption was calculated using the following equation:

$$Consumption = \frac{Conc\ x\ \ 57\ MLD\ x\left(\frac{MW_{Parent}}{MW_{Metabolite}}\right)}{EF\ x\ 125{,}000} \qquad \text{Eq. 16}$$

where $Conc$ is the measured concentration of the analyte of interest, $57\ MLD$ is the total daily volumetric flow rate, $\frac{MW_{Parent}}{MW_{Metabolite}}$ is the ratio of molecular weights of the parent and metabolite (used where applicable), EF is the excretion factor of the analyte [11]

(Appendix D: Table 21), and 125,000 is the population served by the WWTP. Statistical analyses, including Student's *t*-test, were performed using Microsoft Excel 2010.

6.3 Results and discussion

A modified near-continuous *In Situ* Sampler (C-IS2) was outfitted with a glass syringe pump assembly, fluid train consisting of polytetrafluoroethylene (PTFE) intake lines and an influent manifold, combinations of PTFE and nylon fitting connections, styrene-acrylonitrile resin/silicon check values to control flow direction, and Viton® tubing for solid phase extraction (SPE) effluent capture (Figure 19).

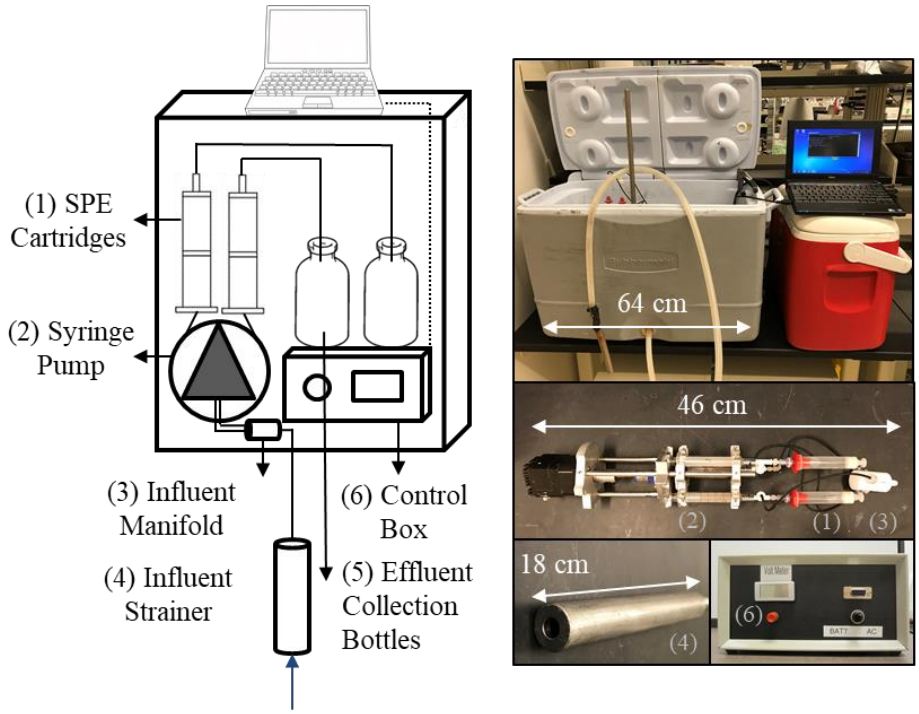


Fig. 19. *In Situ* Sampler (IS2) schematic and images.

The instrument's water intake was modified to include a commercially available stainless steel strainer, which served to protect the influent line from clogging by large suspended solids. Wastewater moved through the instrument as followed: intake, influent

manifold, glass syringes of pump, internal standard-loaded SPE cartridges. SPE cartridges were inverted in the instrument to help prevent clogging in the resin by allowing suspended solids to settle away from sorbent frits. For evaluation purposes, effluent was recovered from each cartridge to determine pump efficacy, and for the analysis of EtS, which is not amendable to SPE capture with the chosen cartridge type. The pump flow rate was 160 µL min^{-1}, translating to approximately 93% sample coverage for the day (Figure 20).Wastewater volumes processed per day per operating channel are shown in Appendix D: Figure 27. On average, the C-IS2 pumped 191 ± 24 mL for the 7-day sampling period (n = 14). The pump program was stopped and restarted during daily SPE collection, and the influent line purged from the influent manifold. The commercially available high frequency sampler was programed to collected sample aliquots every 15 minutes (~ 30 second collection time), equating to approximately 3% coverage of the sampling period (Figure 20). This sampler is in common use at WWTPs for compliance sampling purposes, and when set in time-integrated sampling mode, it is commonly programed to sample at 15-min. intervals, a strategy that was implemented and evaluated here.

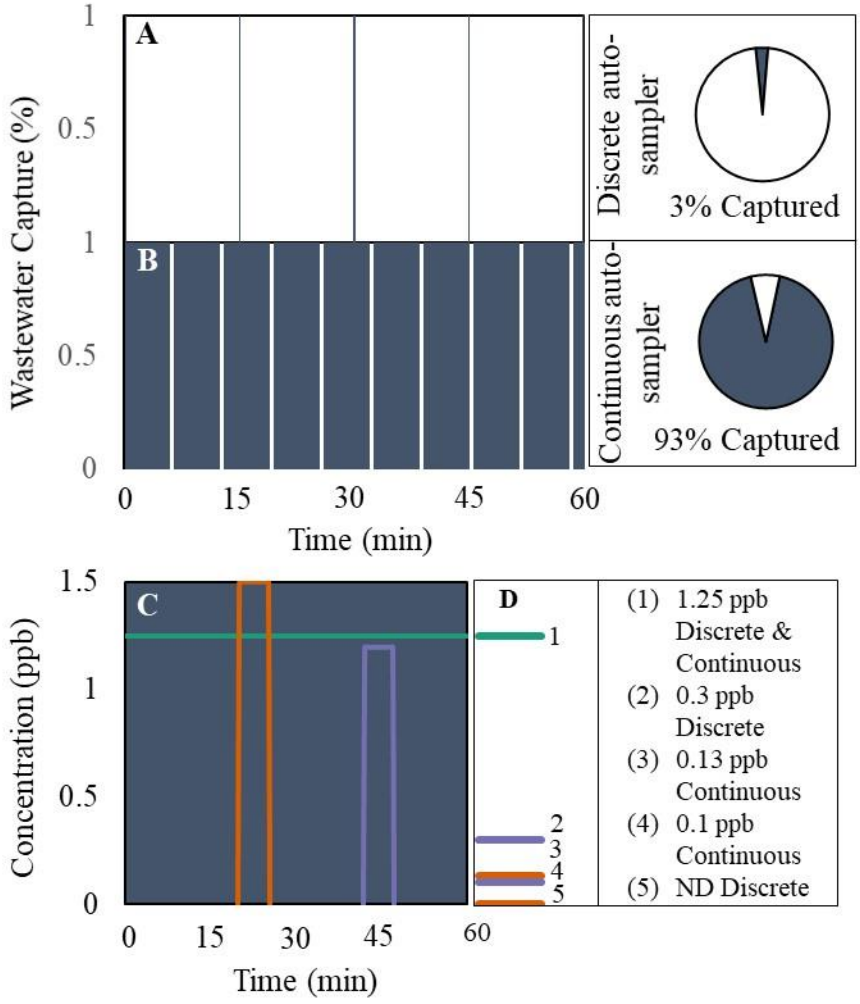


Fig. 20. Modeled concentrations of target analytes in wastewater. Blue intervals denote when sampler is collecting wastewater and the % captured during a 1-hour timeframe in the discrete (A) and continuous (B) samplers. Constant and variable concentrations in wastewater (C) and in the corresponding inferred concentration estimates returned by the two respective samplers for a given analyte mass flow (D).

The concentrations and subsequent masses recorded by the discrete and continuous (C-IS2) samplers vary depending on whether the analyte concentration is constant or occurs as a pulse event. Figure 20 models three types of distinct inputs scenarios, including a constant analyte input, and two pulse events occurring at varying times throughout the hypothetical sampling period. The constant input in wastewater (1.25 ppb)

produced identical concentrations in both the discrete and continuous samplers, reporting the conditions in wastewater with high fidelity. However, for pulse events, the timing, and duration of sample acquisition have a large impact on the data quality of the estimated concentrations recorded by the two samplers. In the event of pulse inputs into wastewater, the C-IS2 has a greater likelihood of capturing the event because the instrument is collecting wastewater for approximately 93% of the monitoring period. Whereas the C-IS2 data are anticipated to be robust, this device cannot inform on the duration of a pulse and the peak concentrations during the event (Figure 20). The 1.5 ppb spike in concentration from t = 20 to t = 25 minutes was captured by the C-IS2; however, the average concentration recorded by the sampler was 0.13 ppb. A similar result was seen with the 1.20 ppb pulse scenario that produced a corresponding C-IS2 concentration of 0.1 ppb.

With pulse events in the discrete sampler, the likelihood of capture is lower, as only ~3% of the wastewater during the sampling period is captured. If the sampler does not capture the pulse in those 100 seconds of pumping, non-detect values will result, as was the case with the 1.5 ppb pulsed input. And, if fortuitously captured as shown with the 1.20 ppb pulse, the recorded concentration estimate will represent only 25% of the actual average concentration, decreased by the other three 15-minute interval collections that occurred during that sampled hour (e.g., 1.2 ppb in wastewater, 0.3 ppb in the sampler).

During a field study at a full-scale wastewater treatment plant, under conditions where the actual concentration profiles of analytes in sewage were unknown, the C-IS2 and discrete samplers indeed returned different average concentration estimates, as shown in Appendix D: Figure 28, Figure 29. Twenty-six of the analytes were consistently

detected and quantifiable in each duplicate daily composite sample collected by each sampler. Notable exceptions included the analytes morphine-3-glucuronide, heroin, norcodeine, alprazolam, and alpha-alprazolam. Morphine-3-glucuronide was only detected in four samples, including in duplicates collected by the discrete sampler and in those collected by the C-IS2 on Thursday. Similarly, heroin had non-detects (ND) for all days except for the duplicate C-IS2 samples collected on Monday. Norcodeine, alprazolam, and alpha-alprazolam were generally detected except in select duplicate samples in the C-IS2 samples near the end of the week/weekend (Appendix D: Figure 28).

Concentrations were converted to daily mass loads using provided flow data by the WWTP. Of the 26 analytes showing consistent detection, the average daily mass loads of 9 of the analytes (36%) were significantly higher ($p < 0.01$) in the C-IS2 (Figure 21, Table 3). These included morphine, 6-acetylmorphine, fentanyl, norfentanyl, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), norbuprenorphine, methamphetamine, amphetamine (Adderall), and caffeine. Only one compound, the metabolite of alprazolam, recorded higher masses in the discrete sampler over the C-IS2 ($p < 0.001$). The low mass load of this compound in the C-IS2 were driven by its susceptibility to degradation. Instances of increased masses captured by the C-IS2 were apparent throughout the duration of the study. Most noteworthy included *(i)* a +100% in fentanyl on Tuesday concurrently with a *(ii)* +55% in the opioid antagonist metabolite norbuprenorphine on the same day, and a 218% increase in methadone on Thursday (Figure 21).

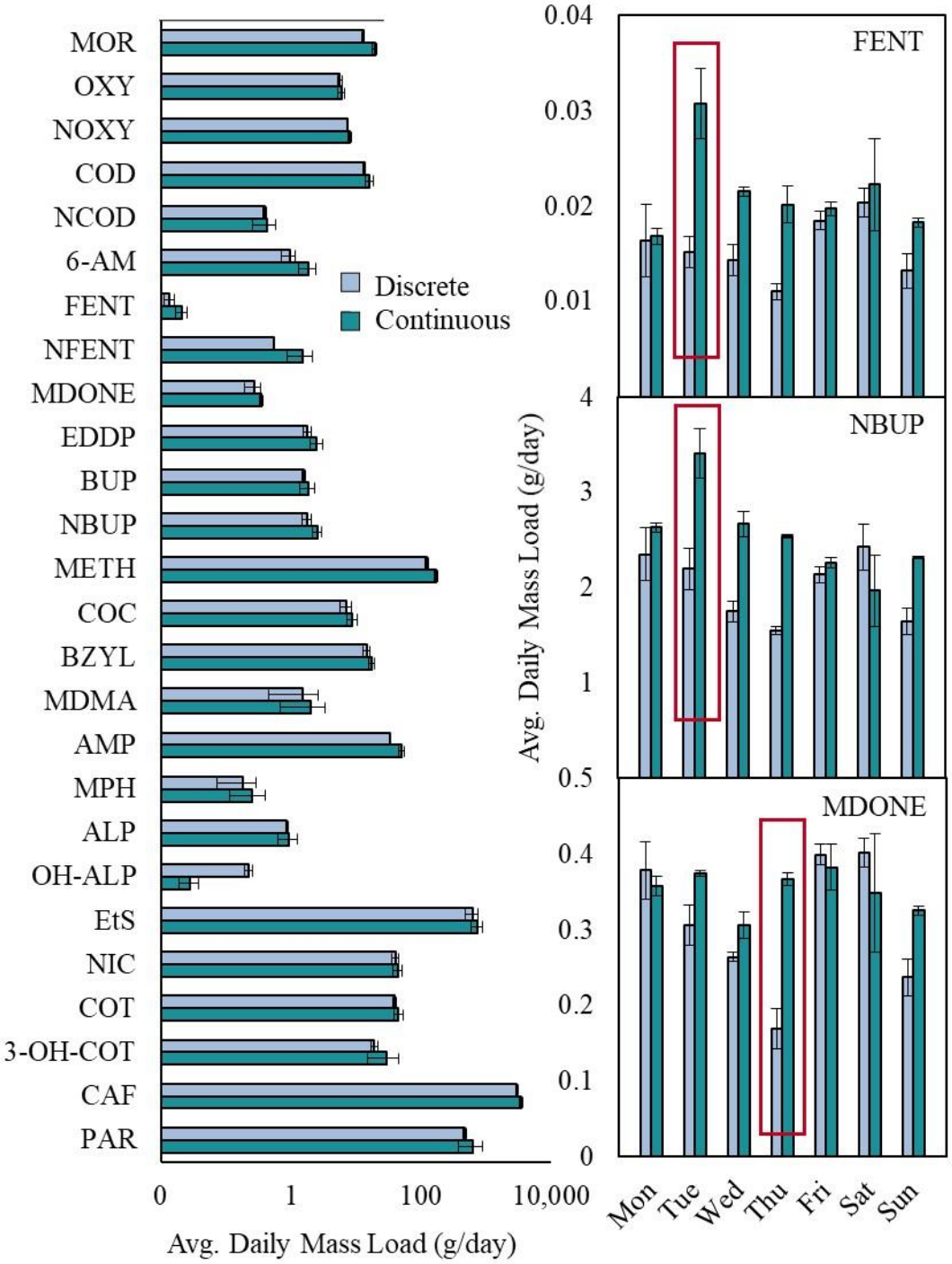


Fig. 21. Mass loading comparisons between a discrete and near-continuous *in situ* extraction sampler. Inset shows daily mass load comparisons for specific compounds including fentanyl (FENT), norbuprenorphine (NBUP), and methadone (MDONE). Boxed portions denote divergences between samplers suggesting captured events by the C-IS2.

Table 3 - Average daily mass loads and standard deviations (SD) for the discrete and continuous samplers, including associated *p* values where applicable ($p \leq 0.01$). Shaded values delineated the sampler with the higher mass loading. In all but a single case, the continuous sampler provided higher mass loads.

Analyte	Discrete	Continuous	*p* value
	mg/day (SD)		
Morphine	13 (0.5)	19 (1.7)	<0.0001
Oxycodone	5.7 (0.5)	6.0 (0.8)	--
Noroxycodone	7.4 (0.18)	8.0 (0.5)	--
Codeine	14 (0.6)	16 (2.5)	--
Norcodeine	0.40 (0.02)	0.42 (0.17)	--
6-acetylmorphine	0.95 (0.23)	1.9 (0.56)	0.005
Fentanyl	0.01 (0.003)	0.02 (0.004)	0.001
Norfentanyl	0.55 (0.01)	1.5 (0.65)	0.01
Methadone	0.27 (0.07)	0.35 (0.03)	--
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	1.8 (0.24)	2.5 (0.42)	0.01
Buprenorphine	1.6 (0.08)	1.9 (0.48)	--
Norbuprenorphine	1.8 (0.29)	2.5 (0.42)	0.005
Methamphetamine	124 (8.0)	171 (10.5)	<0.0001
Cocaine	7.0 (1.4)	8.8 (1.6)	--
Benzoylecgonine	15 (1.9)	17 (1.9)	--
3,4-methylenedioxy-methamphetamine (MDMA)	1.5 (1.1)	2.0 (1.3)	--
Amphetamine	33 (1,.1)	50 (5.4)	0.001
Methylphenidate	0.19 (0.11)	0.26 (0.14)	--
Alprazolam	0.78 (0.02)	0.95 (0.3)	--
Alpha-alprazolam	0.23 (0.03)	0.03 (0.01)	<0.0001
Ethyl sulfate	614 (137)	741 (153)	--
Nicotine	40 (5.6)	44 (7.8)	--
Cotinine	39 (2.6)	45 (7.2)	--
trans-3'-hydroxcotinine	19 (2.4)	30 (15)	--
Caffeine	2982 (127)	3482 (240)	0.001
Paraxanthine	473 (32)	624 (248)	--

Per-capita consumption of caffeine, nicotine, alcohol, and various licit and illicit narcotics was estimated using mass loads, combined with previous excretion rates, and population estimates provided by the WWTP. For per-capita consumption, the metabolite for each parent compound was used to estimate consumption. However, parent compounds were used for methamphetamine, 3,4-methylenedioxy-methamphetamine (MDMA), amphetamine (Adderall), methylphenidate (Ritalin) (no metabolites included in this study), morphine (>99% non-detect in metabolite), and codeine (selected non-detected in metabolite). Additionally, internal standard normalization was included in this calculation to control for compound losses. Average per-capita consumption estimates provided by the C-IS2 were higher than the per-capita estimates generated by the discrete sampler. Mass percent difference between the two samples ranged from 3% to as high as 100%. Note that the disparity in alprazolam between the two samplers was corrected when taking into account the internal standards, which corrected for losses in the C-IS2 while deployed in the field. The degradation vulnerability of this compound agrees with results from other studies [235-238].

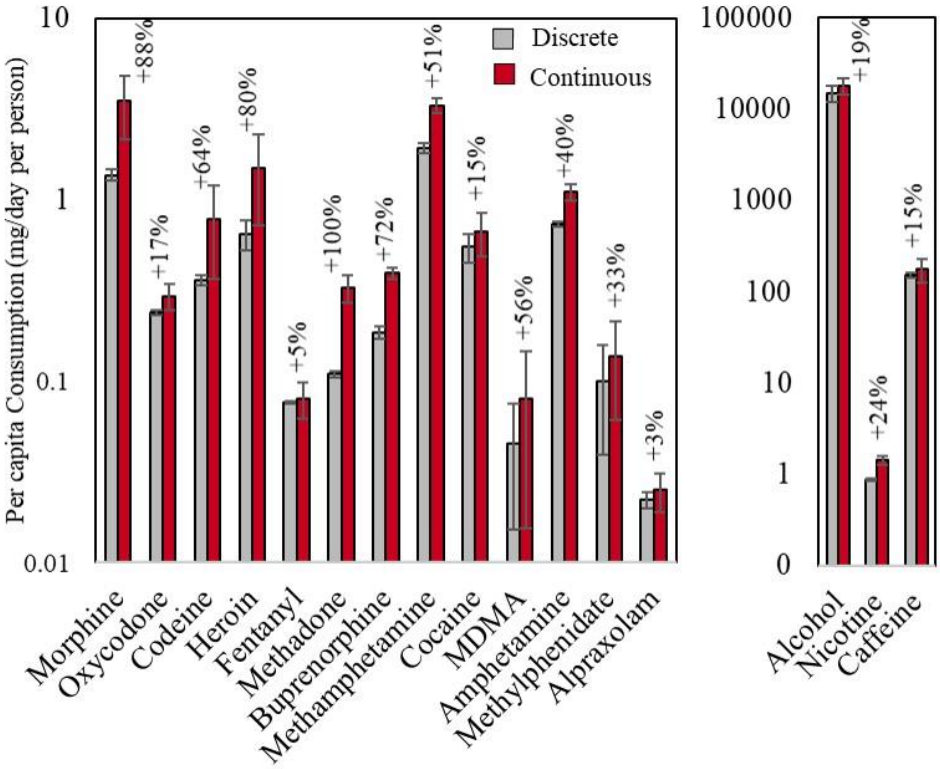


Fig. 22. Per-capita consumption rates of licit and illicit drugs of interest generated from mass loadings with the 15 min time-integrated sampler and the near- continuous *in situ* sampler (with and without internal standards being considered). Alcohol consumption is shown in units of g/day per person.

Results from the modeling and the field deployment show that continuous samplers offer the opportunity to capture analytes that are missed with less frequent sample collection. This was seen in the detections of heroin that were recorded as non-detects in the discrete sampler, and the singular events that increased the mass disparities between the two samplers on selected days (e.g., fentanyl, methadone). Additionally, with certain compounds, the continuous sampler captured more of the target compound, suggesting that the occurrence of events outside the 15-min interval, 30-s sample duration of the discrete sampler were enough to drive the overall concentration in the C-IS2 higher. In addition, the design of the C-IS2 also offers the option of less material transfer

to the laboratory (SPE cartridges only), and a shortened sample processing time than the liquid wastewater samplers if no additional SPE effluent analyses are necessary. Different SPE cartridges may be placed in tandem if all analytes are not amendable to capture by a single commercially available cartridge. Also, with the addition of internal standard to the cartridges prior to deployment, as was done here, *in situ* extraction by the sampler is able to quantify abiotic losses occurring in the field. Continuous samplers offer a viable alternative to conventional sampling methodologies in WBE studies.

CHAPTER 7

RESEARCH IMPLICATIONS AND RECOMMENDATIONS

This work has shown that the critical assessment of standard methodologies can highlight opportunities to improve the health of the natural environment and human populations. As highlighted in this dissertation, this can include assessments in well-defined fields such as environmental remediation, or those that are relatively new and adaptive as is the case with wastewater-based epidemiology. Assessments of this type can highlight inconsistencies or weakness in the methodology or create new opportunities to expand the field.

7.1 Groundwater remediation assessments

In Chapter 2, the differences between common bench-scale treatability studies were explored, first in a literature meta-analysis, with an emphasis on reaction kinetics in chlorinated solvent remediation, and second through experimental studies, which included three treatments technologies for *in situ* remediation of perchloroethylene. Results showed that there was a preference for reporting batch kinetic data, even when available continuous-flow column data were available, and that columns produced higher observed rate constants (faster rates) than batch bottles within comparable systems, by a factor of 6.1 ± 1.1. Experimentally, the same trend was found, with columns generating higher rate constants that were 8.0 ± 4.8 times faster than batch (1.23 ± 0.87 d^{-1} vs. 0.16 ± 0.05 d^{-1}). Additionally, kinetic data density in columns was also found to be higher than associated batch bottles, where column rates are a function of the number of samples collected after steady-state is achieved, while the number of batch bottle rates are

equivalent to the number of replicates. Progressing outside the traditional assessment of chemical speciation and kinetic analysis, a mass transformation assessment was included in this study, which found that columns were transforming 16.8 ± 8 times more mass than batch bottles of traditional design, and also had the potential to predict amendment longevity if column systems are conducted until rebound of the contaminant. The rate disparities between these bench-scale studies suggest that varying estimates of total cleanup time and associated costs vary significantly depending on which kinetic estimates are used in subsequent modeling of remediation at the site. Batch kinetics may underestimate *in situ* remediation, therefore overestimated time and costs, and potentially affecting downstream decision-making at the site. Additionally, increased kinetic data density with columns suggests these estimates are more relatable and indicative of both maximum and sustained rates in the system. This is not to say that continuous-flow columns are the superior option to groundwater remediation studies. Batch bottles in these cases may serve as a more conservative estimate for remediation in the system, and in the case fractured bedrock, the remediation occurring in vertical fractures may be more accurately assessed by a batch bottle system. In general, this study highlighted the need for a more unified framework for data use and reporting, to produce more representative outcomes at the field-scale.

Next steps in this research should focus on modeling kinetic data generated by batch and column treatability studies, to understand how differences in reaction kinetics translate into modeled cleanup times and costs. Additionally, one of the amendment technologies tested here, the controlled-release carbon source (CRCS), displayed very different patterns of contaminant speciation in each of the two treatability study types

(enhanced removal in the batch system). In batch bottles, release of electron donor is largely a function of the release rate of the materials, whereas in the column, donor availability is largely controlled by the flow rate of the system, which continually pushes the donor out of the column. Studying the differences of the CRCS in these variable conditions is important for understanding field-scale remediation activities using these materials.

In Chapter 3, a bench-scale continuous-flow column treatability study was performed and results compared to those of field-scale remedial activities, in which the test amendments were applied in the field as a method for source zone reduction of a DNAPL PCE plume. Results in the lab were qualitatively similar to those in the field, meaning that PCE reduction followed the similar trend towards formation of less chlorinated species, however bench-scale studies performed better. First-order degradation rates generated from column data were 0.71 ± 0.04 d^{-1}, while field rates were variable at <0.01 to 0.04 d^{-1}. Variably was largely driven by the challenges to creating fractured bedrock DNAPL remediation in the lab. Field conditions are spatial and temporal heterogeneous, the system is effectively open to outside perturbations, and there is no guarantee subsurface injections will reach DNAPL locations. Additionally, there is inherent variability in DNAPL dissolution, which drives downgradient measured concentrations. In lessons learned, valued quantitative parameters in bench-scale studies including chemical speciation, reaction kinetics, microbial numbers and gene copies do not all translate well into field-scale assessments. Compound speciation is the most useful of the three, microbial parameters are used only as secondary indicators of remediation to supplement chemical data, and kinetic estimates are insignificant. This suggests

alternative means of analyses are needed. Mass flux and mass discharge are two parameters that are more commonly being used in field-scale assessments, but have no counterpart in the lab. These analyses greatly improved the ability to interpret remedial successes at the site, as changes in DNAPL dissolution were collected in downgradient receptor wells, and showed changing slopes in flux diagrams. Assessments of mass flux into columns were two-orders of magnitude lower than those recorded in the field, suggesting maybe there is value to more closely aligning the flux between lab- and field-scale studies. It is important to note that in DNAPL source zone reduction studies, DNAPL is not included in the bench-scale assessment, either as an injected slug into the column or in the steady-stream of influent. No published studies could be found that tested either of these methods of DNAPL removal, and conversations with commercial laboratories have suggested this is too laborious and lacks reproducibility. However, there is merit is testing the feasibility of this type of treatability study in an effort to improve the understanding of source zone reduction in the lab.

7.2 Human population assessments

In Chapter 4, traditional wastewater-based epidemiology study methodologies were expanded to include the endogenous steroid hormones cortisol and cortisone, to assess human population stress on a university campus. The compounds were consistently detected, and followed a reproducible weekly trend of elevated concentrations during the week, correlating with class schedules, and lower measured concentrations during the weekend. The highest levels of per person total glucocorticoids occurred on Mondays (149 ± 62 μg d^{-1} per person) and lowest on Saturdays and Sundays (72 ± 18 μg d^{-1} per person and 71 ± 22 μg d^{-1} per person), and weekends were

significantly different than weekdays (p = 0.05). Higher stress levels were recorded during the first two months of the semester in August during the first week of school (161 ± 42 μg d^{-1} per person), and September (122 ± 54 μg d^{-1} per person), which were significantly different from the remaining months (p = 0.05, 0.01). Stress hormones were positively correlated with nicotine (r_s = 0.49) and caffeine (r_s = 0.63) consumption but not alcohol intake. This study showed promise in measuring glucocorticoids in wastewater as a metric of population stress, and has long-reaching implications for using WBE to assess stress in cities. Stress is known to degrade quality of life, and is linked to the six major causes of death in the U.S., so directly measuring primary physiological markers of stress in wastewater would allow for the identification of events and conditions putting populations at risk. For example, universities are notorious for representing high-stress environments, so consistent tracking the population would elucidate deviations from the baseline, such that high-stress situations could be identified and interventions implemented if necessary. Additionally, stress is often correlated with increases of substance abuse, so further studies into the relationship of concurrence with stressors is desirable. This study focused on a specific target population, and it is necessary to assess other non-student populations, and to scale up to treatment plant sampling, which generally has much longer travel times and travel distances than those in the current study. This is particularly important because cortisone and cortisol are relatively unstable, excreted at the μg/L concentrations and measured in WWTPs at ng/L [105, 120]. If degradation is an issue, tracking of the major metabolites tetrahydrocortisol and tetrahydrocoritsone, may add confidence and robustness to the data stream, as both compounds are excreted at mg/L concentrations in urine [239]. Additionally, other

compounds that occur during the stress response may also be options for fine-tuning the stress assessment, including: epinephrine and norepinephrine [240, 241].

In Chapter 5, WBE analysis was conducted to assess patterns of alcohol, caffeine and nicotine consumption in a target demographic (college students), and compared this to self-reported estimates generated from behavioral science peer-reviewed publications, as well as U.S. national statistics on consumption. Per capita wastewater-derived estimates for alcohol consumption were 11.3 ± 7.5 g d^{-1} per person (0.8 ± 0.5 drinks d^{-} per person), similar to estimates obtained from literature 10.1 ± 0.8 g d^{-1} per person (0.7± 0.006 drinks d^{-} per person). Caffeine and nicotine consumption were lower than the literature estimates, 114 ± 49 g d^{-1} per person vs. 178±19 g d^{-1} per person (caffeine), and 627 ± 219 g d^{-1} per person and 927 ± 243 g d^{-1} per person (nicotine). Strong correlations were found between alcohol and nicotine (r_s = 0.71), and nicotine and caffeine (r_s = 0.59), and weak with alcohol and caffeine (r_s = 0.17). In general, alcohol and nicotine consumption were higher on weekends than weekdays (p <0.0001, p = 0.01), and caffeine higher during the week (p = 0.05). Between semesters, alcohol consumption remained stable and caffeine and nicotine decreased. In this study, sampling occurred directly from the subsurface pipe infrastructure allowing for the isolation of a subpopulation for assessment, which was repeatedly and inexpensively monitoring for an entire academic year. Reported surveys often cite this target demographic as prone to abuse of these substances, however this alternative method of assessment showed this is not true for this specific university. This method of targeted sampling would greatly benefit subset locations containing at-risk populations, including schools, assisted living/retirement

communities, and socioeconomically disadvantaged neighborhoods. This is particularly true in the current climate of opioid abuse.

In the college-age demographic, caffeine consumption from energy drinks is a particular concern because: *(i)* caffeine concentrations in those beverages are high, increasing the likelihood of overconsumption; and *(ii)* energy drink consumption is often co-occurring with alcohol consumption. In the latter instance, the stimulant effects of caffeine mask the depressive effects of alcohol, leading to overconsumption of the latter. Future studies should try to elucidate consumption of energy drinks over other caffeine-containing products. This could be accomplished by monitoring common additives in this drinks, including compounds such as guarana, taurine, and ginseng, which themselves are natural stimulants and add to the health burden of the population [242]. Similarly, with the rise in e-cigarettes, there is a concern that these devices will increase nicotine consumption among youth, and will serve as a gateway to cigarette consumption. Tracking compounds in the nicotine liquids used by the e-cigarettes, would elucidate how these devices impact smoking patterns in this demographic.

In Chapter 5, a near-continuous *in situ* sampler was used to collected wastewater influent from a WWTP to assess compounds common to WBE, and to compare the associated mass loads to a traditional time-integrated sampler. Modeled data showed that the percentage of sample covered from each of the two samples varied, with 3% coverage by the 15-minute interval sampler, and 93% for the near-continuous sampler. Mass load results showed that the near-continuous sampler captured analytes when the discrete sampler did not (heroin), and captured pulse events that were not apparent in the discrete sampler (e.g., fentanyl, norbuprenorphine, and methadone). Additionally, the near-

continuous sampler consistently captured higher masses than the discrete sampler for the opioids morphine, 6-acetylmorphine (heroine metabolite), fentanyl, norfentanyl, the opioid antagonist norbuprenorphine, and the stimulants methamphetamine, amphetamine, and caffeine ($p \leq 0.01$). These results suggest that continuous capture may be an alternative sampling methodology to the current high frequency discrete sample design, and has the potential to improve our understanding of consumption estimates generated by WBE. More work is needed to fully explore this relationship. WBE practitioners tout flow-weighted samplers at the gold standard in sample collection. Similar to the time-averaged sampler this study, flow-weighted collection occurs at a brief interval after a certain volume of wastewater has moved through the system, which translates into high-flow periods being more heavily represented in the composite sample. Comparing the near-continuous sampler to the flow-weighted sampler will likely provide different results that the time-interval sampler. In terms of *in situ* sampler improvements, future instrument iterations should include: *(i)* integration of more sophisticated valve technology to allow for back-flushing; *(ii)* a mechanism to record channel flow so effluent need not be captured; *(iii)* incorporation of flow meter for near-continuous, flow-weighted studies. There is also a need to study abiotic degradation on SPE cartridges, and alternative mechanisms for internal standard addition.

This critical assessment of methodologies and devices provided here has shown that assessments of this type can highlight inconsistencies or weakness in the methodology, providing space for a discussion of avenues to improvement, and also can highlight previously unseen opportunities for advancement of the field.

REFERENCES

[1] United States. Environmental Protection Agency. Office of Solid Waste and Emergency, M. United States. Environmental Protection Agency. Office of Land and Emergency, Guide for conducting treatability studies under CERCLA: Final, in, Washington, D.C.: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, D.C., 1992.

[2] Risk Reduction Engineering. United States. Environmental Protection Agency. Office of Emergency and Remedial. Science Applications International, Science Applications, Leidos, National Risk Management Research Laboratory. Land Remediation and Pollution Control, Guide for conducting treatability studies under CERCLA : biodegradation remedy selection : interim guidance, Cincinnati, Ohio: U.S. Environmental Protection Agency, Risk Reduction Engineering Laboratory, Office of Research and Development; Washington, D.C.: Office of Emergency and Remedial Response, Office of Solid Waste and Emergency Response, Cincinnati, Ohio : Washington, D.C., 1993.

[3] S. Lee, D. Lalhmunsiama, D. Tiwari, Sericite in the remediation of Cd(II)- and Mn(II)-contaminated waters: batch and column studies, Environmental Science and Pollution Research, 21 (2014) 3686-3696.

[4] S.-W. Jeen, S. Lazar, L. Gui, R.W. Gillham, Degradation of chlorofluorocarbons using granular iron and bimetallic irons, Journal of Contaminant Hydrology, 158 (2014) 55-64.

[5] F. Suja, F. Rahim, M.R. Taha, N. Hambali, M. Rizal Razali, A. Khalid, A. Hamzah, Effects of local microbial bioaugmentation and biostimulation on the bioremediation of total petroleum hydrocarbons (TPH) in crude oil contaminated soil based on laboratory and field observations, International Biodeterioration & Biodegradation, 90 (2014) 115-122.

[6] S. Bilardi, R.T. Amos, D.W. Blowes, P.S. Calabrò, N. Moraci, Reactive Transport Modeling of ZVI Column Experiments for Nickel Remediation, Groundwater Monitoring & Remediation, 33 (2013) 97-104.

[7] P.M. Choi, B.J. Tscharke, E. Donner, Apos, J.W. Brien, S.C. Grant, S.L. Kaserzon, R. Mackie, E. Malley, N.D. Crosbie, K.V. Thomas, J.F. Mueller, Wastewater-based epidemiology biomarkers: Past, present and future, Trends in Analytical Chemistry, 105 (2018) 453-469.

[8] A. Venkatesan, H. Done, R. Halden, United States National Sewage Sludge Repository at Arizona State University—a new resource and research tool for environmental scientists, engineers, and epidemiologists, Environmental Science and Pollution Research, 22 (2015) 1577-1586.

[9] A.K. Venkatesan, R.U. Halden, Wastewater treatment plants as chemical observatories to forecast ecological and human health risks of manmade chemicals, Scientific reports, 4 (2014) 3731.

[10] B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring, Water Research, 72 (2015) 3-27.

[11] A.J. Gushgari, E.M. Driver, J.C. Steele, R.U. Halden, Tracking narcotics consumption at a Southwestern U.S. university campus by wastewater-based epidemiology, Journal of Hazardous Materials, 359 (2018) 437-444.

[12] M.J. Andrés-Costa, V. Andreu, Y. Picó, Liquid chromatography–mass spectrometry as a tool for wastewater-based epidemiology: Assessing new psychoactive substances and other human biomarkers, Trends in Analytical Chemistry, 94 (2017) 21-38.

[13] Z. Yang, G. Xu, J. Reboud, B. Kasprzyk-Hordern, J.M. Cooper, Monitoring Genetic Population Biomarkers for Wastewater-Based Epidemiology, Analytical chemistry, 89 (2017) 9941.

[14] Lincoln Park Zoo, Measuring Stress in Wild Species, in, 2018.

[15] L.K. Van Der Weyde, G.B. Martin, M.C.J. Paris, Monitoring stress in captive and free-ranging African wild dogs (Lycaon pictus) using faecal glucocorticoid metabolites, General and Comparative Endocrinology, 226 (2016) 50-55.

[16] M.H. Antoni, S. Cruess, D.G. Cruess, M. Kumar, S. Lutgendorf, G. Ironson, E. Dettmer, J. Williams, N. Klimas, M.A. Fletcher, N. Schneiderman, Cognitive-Behavioral Stress Managment Reduces Distress and 24-Hour Urinary Free Cortisol Output Among Symptomatic HIV-Infected Gay Men, Annals of Behavioral Medicine, 22 (2000) 29-37.

[17] APA, Stress in American: Missing the Health Care Connection, in, American Psychological Association, 2013.

[18] N.V. Heuett, C.E. Ramirez, A. Fernandez, P.R. Gardinali, Analysis of drugs of abuse by online SPE-LC high resolution mass spectrometry: communal assessment of consumption, Science of the Total Environment, 511 (2015) 319-330.

[19] D.A. Burgard, R. Fuller, B. Becker, R. Ferrell, M. Dinglasan-Panlilio, Potential trends in Attention Deficit Hyperactivity Disorder (ADHD) drug use on a college campus: wastewater analysis of amphetamine and ritalinic acid, Science of the Total Environment, 450 (2013) 242-249.

[20] D.R. Moore, D.A. Burgard, R.G. Larson, M. Ferm, Psychostimulant use among college students during periods of high and low stress: an interdisciplinary approach utilizing both self-report and unobtrusive chemical sample data, Addictive behaviors, 39 (2014) 987-993.

[21] J. Baz-Lomba, S. Salvatore, E. Gracia-Lor, R. Bade, S. Castiglioni, E. Castrignano, A. Causanilles, J. Kinyua, A.-K. McCall, C. Ort, B. Plosz, M. Reid, Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities, BMC Public Health, 16 (2016) n/a.

[22] I. González-Mariño, E. Gracia-Lor, N.I. Rousis, E. Castrignanò, K.V. Thomas, J.B. Quintana, B. Kasprzyk-Hordern, E. Zuccato, S. Castiglioni, Wastewater-Based Epidemiology To Monitor Synthetic Cathinones Use in Different European Countries, Environmental science & technology, 50 (2016) 10089.

[23] C. Ort, M. Lawrence, J. Rieckermann, A. Joss, Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid? A Critical Review, in, American Chemical Society, Easton, 2010, pp. 6024.

[24] C. Guo, T. Zhang, S. Hou, J. Lv, Y. Zhang, F. Wu, Z. Hua, W. Meng, H. Zhang, J. Xu, Investigation and Application of a New Passive Sampling Technique for in Situ Monitoring of Illicit Drugs in Waste Waters and Rivers, Environmental science & technology, 51 (2017) 9101.

[25] United States Environmental Protection Agency (USEPA), Cleaning up the nation's waste sites markets and technology trends, 2004 ed.. ed., Washington, D.C. : U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, D.C., 2004.

[26] National Research Council. Committee on Future Options for Management in the Nation's Subsurface Remediation Effort, Alternatives for managing the nation's complex contaminated groundwater sites, Washington, District of Columbia : National Academies Press, 2013.

[27] United States Environmental Protection Agency (USEPA), Remedial Investigation/Feasibiltiy in, 2016.

[28] G.D. McNelly, Guide for conducting treatability studies under CERCLA : chemical dehalogenation, Cincinnati, Ohio : Risk Reduction Engineering Laboratory, Office of Research and Development, U.S. Environmental Protection Agency ; Washington, DC : Office of Emergency and Remedial Response, Office of Solid Waste and Emergency Response, Cincinnati, Ohio : Washington, DC, 1992.

[29] L. Risk Reduction Engineering, R. United States. Environmental Protection Agency. Office of Emergency and Remedial, D. National Risk Management Research Laboratory . Land Remediation and Pollution Control, Guide for conducting treatability studies under CERCLA : aerobic biodegradation remedy screening : interim guidance, Cincinnati, Ohio : U.S. Environmental Protection Agency, Risk Reduction Engineering Laboratory, Office of Research and Development ; Washington, D.C. : Office of Emergency and Remedial Response, Office of Solid Waste and Emergency Response, Cincinnati, Ohio : Washington, D.C., 1991.

[30] M.R. Olson, J. Blotevogel, T. Borch, M.A. Petersen, R.A. Royer, T.C. Sale, Long-term potential of in situ chemical reduction for treatment of polychlorinated biphenyls in soils, Chemosphere, 114 (2014) 144-149.

[31] X. Hu, Z. Ding, A.R. Zimmerman, S. Wang, B. Gao, Batch and column sorption of arsenic onto iron-impregnated biochar synthesized through hydrolysis, Water Research, 68 (2015) 206-216.

[32] L.J. Matheson, P.G. Tratnyek, Reductive dehalogenation of chlorinated methanes by iron metal, Environmental science & technology, 28 (1994) 2045.

[33] H. Song, E.R. Carraway, Reduction of chlorinated ethanes by nanosized zero-valent iron: kinetics, pathways, and effects of reaction conditions, Environmental science & technology, 39 (2005) 6237.

[34] M. Lindsay, C. Ptacek, D. Blowes, W. Gould, Zero-Valent Iron and Organic Carbon Mixtures for Remediation of Acid Mine Drainage: Batch Experiments, Applied Geochemistry, 23 (2008).

[35] W. Orth, R. Gillham, Dechlorination of Trichloroethene in Aqueous Solution Using Fe super(0), Environmental Science & Technology, 30 (1996) 66-66.

[36] J. Klausen, P. Vikesland, T. Kohn, D. Burris, W. Ball, A. Roberts, Longevity of Granular Iron in Groundwater Treatment Processes: Solution Composition Effects on Reduction of Organohalides and Nitroaromatic Compounds, Environmental Science & Technology, 37 (2003) 1208-1208.

[37] B. Baumgarten, J. Jährig, T. Reemtsma, M. Jekel, Long term laboratory column experiments to simulate bank filtration: Factors controlling removal of sulfamethoxazole, Water Research, 45 (2011) 211-220.

[38] J. Conca, E. Strietelmeier, N. Lu, S.D. Ware, T.P. Taylor, J. Kaszuba, J. Wright, D.L. Naftz, S.J. Morrison, C.C. Fuller, J.A. Davis, Treatability study of reactive materials to remediate groundwater contaminated with radionuclides, metals, and nitrates in a four-component permeable reactive barrier, Amsterdam, Netherlands: Academic Press, Amsterdam, 2002.

[39] SiREM. labs, Treatability Testing, in, 2015.

[40] Y. Mamindy-Pajany, S. Sayen, E. Guillon, Impact of lime-stabilized biosolid application on Cu, Ni, Pb and Zn mobility in an acidic soil, Environmental Science and Pollution Research, 21 (2014) 4473-4481.

[41] H. Liu, Q. Wang, C. Wang, X.-Z. Li, Electron efficiency of zero-valent iron for groundwater remediation and wastewater treatment, Chemical Engineering Journal, 215-216 (2013) 90-95.

[42] S.D. Justicia-Leon, S. Higgins, E.E. Mack, D.R. Griffiths, S. Tang, E.A. Edwards, F.E. Löffler, Bioaugmentation with distinct Dehalobacter strains achieves chloroform detoxification in microcosms, Environmental science & technology, 48 (2014) 1851.

[43] P. Kumar, M. Nemati, G. Hill, Biodegradation kinetics of 1,4-benzoquinone in batch and continuous systems, Biodegradation, 22 (2011) 1087-1093.

[44] M.G. Mahmoodlu, S.M. Hassanizadeh, N. Hartog, Evaluation of the kinetic oxidation of aqueous volatile organic compounds by permanganate, Science of the Total Environment, 485-486 (2014) 755-763.

[45] Z. Li, Y. Inoue, S. Yang, N. Yoshida, A. Katayama, Mass balance and kinetic analysis of anaerobic microbial dechlorination of pentachlorophenol in a continuous flow column, Journal of Bioscience and Bioengineering, 110 (2010) 326-332.

[46] R.A. Brennan, R.A. Sanford, C.J. Werth, Biodegradation of Tetrachloroethene by Chitin Fermentation Products in a Continuous Flow Column System, Journal of Environmental Engineering, 132 (2006) 664-673.

[47] C. Kantar, C. Ari, S. Keskin, Z.G. Dogaroglu, A. Karadeniz, A. Alten, Cr(VI) removal from aqueous systems using pyrite as the reducing agent: Batch, spectroscopic and column experiments, Journal of Contaminant Hydrology, 174 (2015) 28-38.

[48] G. Lowry, M. Reinhard, Pd-Catalyzed TCE Dechlorination in Water: Effect of H(2)(aq) and H(2)-utilizing Competitive Solutes on the TCE Dechlorination Rate and Product Distribution, Environmental Science & Technology, 35 (2001) 696-696.

[49] United States. Environmental Protection Agency. Office of Solid Waste and Emergency. United States. Environmental Protection Agency. Office of Land and Emergency, Superfund NPL Characterization Project: National Results Highlights in, Washington, D.C.: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, D.C., 1991.

[50] D.M. Mackay, P.V. Roberts, J.A. Cherry, Transport of organic contaminants in groundwater, Transport of organic contaminants in groundwater, (1985) 384-392.

[51] C. Carr, J. Hughes, Enrichment of high-rate PCE dechlorination and comparative study of lactate, methanol, and hydrogen as electron donors to sustain activity, Environmental Science & Technology, 32 (1998) 1817-1824.

[52] G. Davis, B.R. Baldwin, A.D. Peacock, D. Ogles, G.M. White, S.L. Boyle, E. Raes, S.S. Koenigsberg, K.L. Sublette, Integrated approach to PCE-impacted site characterization, site management, and enhanced bioremediation, Remediation Journal, 18 (2008) 5-17.

[53] Interstate Technology and Regulatory Cooperation Work, Dense Non-aqueous Phase Liquids (DNAPLs): Review of Emerging Characterization and Remediation Technologies, in, 2000.

[54] D.W. Major, M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, L.W. Buonamici, Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene, Environmental Science and Technology, 36 (2002) 5106-5116.

[55] J.G. Arnason, M. Harkness, B. Butler-Veytia, Evaluating the subsurface distribution of zero-valent iron using magnetic susceptibility, Groundwater Monitoring and Remediation, 34 (2014).

[56] W. Zhang, Soy proteins and/or soy derivatives with zero-valent iron compositions and use for environmental remediation, in, United States, 2008.

[57] M. Ziv-El, T. Kalinowski, R. Krajmalnik-Brown, R.U. Halden, Simultaneous Determination of Chlorinated Ethenes and Ethene in Groundwater Using Headspace Solid-Phase Microextraction with Gas Chromatography, Journal of Chromatographic Science, 52 (2014) 137-142.

[58] G.D. Wardlaw, R.K. Nelson, C.M. Reddy, D.L. Valentine, Biodegradation preference for isomers of alkylated naphthalenes and benzothiophenes in marine sediment contaminated with crude oil, Organic Geochemistry, 42 (2011) 630-639.

[59] J.-H. Choi, Y.-H. Kim, S.J. Choi, Reductive dechlorination and biodegradation of 2,4,6-trichlorophenol using sequential permeable reactive barriers: Laboratory studies, Chemosphere, 67 (2007) 1551-1557.

[60] E. García-Peña, P. Zarate-Segura, P. Guerra-Blanco, T. Poznyak, I. Chairez, Enhanced Phenol and Chlorinated Phenols Removal by Combining Ozonation and Biodegradation, An International Journal of Environmental Pollution, 223 (2012) 4047-4064.

[61] P.J. Haest, J. Philips, D. Springael, E. Smolders, The reactive transport of trichloroethene is influenced by residence time and microbial numbers, Journal of Contaminant Hydrology, 119 (2011) 89-98.

[62] Y.P. Katsenovich, F.R. Miralles-Wilhelm, Evaluation of nanoscale zerovalent iron particles for trichloroethene degradation in clayey soils, Science of the Total Environment, 407 (2009) 4986-4993.

[63] T. Lee, C.H. Benson, G.R. Eykholt, Waste green sands as reactive media for groundwater contaminated with trichloroethylene (TCE), Journal of Hazardous Materials, 109 (2004) 25-36.

[64] K. Mackenzie, J. Battke, R. Koehler, F.-D. Kopinke, Catalytic effects of activated carbon on hydrolysis reactions of chlorinated organic compounds: Part 2. 1,1,2,2-Tetrachloroethane, Applied Catalysis B, Environmental, 59 (2005) 171-179.

[65] T.T. Tsai, C.M. Kao, T.Y. Yeh, S.H. Liang, H.Y. Chien, Application of surfactant enhanced permanganate oxidation and bidegradation of trichloroethylene in groundwater, Journal of Hazardous Materials, 161 (2009) 111-119.

[66] C.E. Schaefer, C.W. Condee, S. Vainberg, R.J. Steffan, Bioaugmentation for chlorinated ethenes using Dehalococcoides sp.: Comparison between batch and column experiments, Chemosphere, 75 (2009) 141-148.

[67] S. Yu, L. Semprini, Kinetics and modeling of reductive dechlorination at high PCE and TCE concentrations, Biotechnology and Bioengineering, 88 (2004) 451-464.

[68] C.M. Kao, S.C. Chen, J.Y. Wang, Y.L. Chen, S.Z. Lee, Remediation of PCE-contaminated aquifer by an in situ two-layer biobarrier: laboratory batch and column studies, Water Research, 37 (2003) 27-38.

[69] M.F. Azizian, S. Behrens, A. Sabalowsky, M.E. Dolan, A.M. Spormann, L. Semprini, Continuous-flow column study of reductive dehalogenation of PCE upon bioaugmentation with the Evanite enrichment culture, Journal of Contaminant Hydrology, 100 (2008) 11-21.

[70] R.M. Powell, United States. Environmental Protection Agency. Technology Innovation, National Risk Management Research, Permeable reactive barrier technologies for contaminant remediation, Washington, DC: Technical Information Office, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency; Cincinnati, Ohio: National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC: Cincinnati, Ohio, 1998.

[71] K. McClellan, A New Approach to Groundwater Remediation Treatability Studies - Moving Flow-through Column Experiments from Laboratory to In Situ Operation, in: R.U. Halden, P. Johnson, R. Krajmalnik-Brown (Eds.), ProQuest Dissertations Publishing, 2013.

[72] K. Chandran, B.F. Smets, Biokinetic Characterization of the Acceleration Phase in Autotrophic Ammonia Oxidation, Water Environment Research, 80 (2008) 732-739.

[73] M.P. Shah, Microbial Removal of Ammonical Nitrogen by Nitrification and Denitrification, Journal of Bioremediation and Biodegradation, 9 (2015) 1-10.

[74] A.J. Saldanha, M.J. Brauer, D. Botstein, Nutritional homeostasis in batch and steady-state culture of yeast, Molecular biology of the cell, 15 (2004) 4089.

[75] D.P. Searson, M.J. Willis, S.J. Horne, A.R. Wright, Inference of Chemical Reaction Networks Using Hybrid S-system Models, Chemical Product and Process Modeling, 2 (2007).

[76] U. Jenal-Wanner, P.L. McCarty, Development and evaluation of semicontinuous slurry microcosms to simulate in situ biodegradation of trichloroethylene in contaminated aquifers, Environmental Science and Technology, 31 (1997) 2915-2922.

[77] K. Choi, W. Lee, Reductive dechlorination of carbon tetrachloride in acidic soil manipulated with iron(II) and bisulfide ion, Journal of Hazardous Materials, 172 (2009) 623-630.

[78] T. Kalinowski, Technical, Economical and Social Aspects of Moving Treatability Studies for In Situ Bioremediation of Contaminated Aquifers from the Laboratory to the Field, in: R.U. Halden, I. Bennett, P. Johnson, R. Krajmalnik-Brown (Eds.), ProQuest Dissertations Publishing, 2013.

[79] W.R. Kelly, G.M. Hornberger, J.S. Herman, A.L. Mills, Kinetics of BTX biodegradation and mineralization in batch and column systems, Journal of Contaminant Hydrology, 23 (1996) 113-132.

[80] United States. Environmental Protection Agency. Office of Solid Waste and Emergency. United States. Environmental Protection Agency. Office of Land and Emergency, Cleaning up the nation's waste sites markets and technology trends, 2004 ed.. ed., Washington, D.C.: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, D.C., 2004.

[81] M.C. Kavanaugh, United States Environmental Protection Agency (USEPA) National Risk Management Research, The DNAPL remediation challenge is there a case for source depletion?, Cincinnati, OH : U.S. Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH, 2003.

[82] C. Su, R.W. Puls, T.A. Krug, M.T. Watling, Amp, Apos, S.K. Hara, J.W. Quinn, N.E. Ruiz, A two and half-year-performance evaluation of a field test on treatment of source zone tetrachloroethene and its chlorinated daughter products using emulsified zero valent iron nanoparticles, Water Research, 46 (2012) 5071-5084.

[83] C.M. Schaefer, J.; Clement, P., DNAPL Dissolution in Bedrock Fractures And Fracture Networks, in, Strategic Environmental Research & Development Program, 2011.

[84] Government of Canada, Past Weather and Climate. Historical Data, in, 2016.

[85] B.P. Neff, Estimation of shallow ground-water recharge in the Great Lakes Basin, Reston, VA: U.S. Dept. of the Interior, U.S. Geological Survey, 2006.

[86] E. Cox, Elucidation of the Mechanisms and Environmental Relevance of cis-Dichloroethene and Vinyl Chloride Biodegradation, in, 2012.

[87] P.M. Bradley, Reinterpreting the Importance of Oxygen-Based Biodegradation in Chloroethene-Contaminated Groundwater, Ground Water Monitoring & Remediation, 31 (2011) 50-55.

[88] S. Rosansky, W. Condit, R. Sirabian, Best Practices for Injection and Distribution of Amendments, in, Naval Facilities Engineering Command, 2013.

[89] R. Yu, D.L. Freedman, R.G. Andrachek, Use of Novel, Intact Rock-Core Microcosms to Evaluate Natural and Enhanced Attenuation of TCE in Fractured Sandstone, in: Battelle Tenth International Conferences of Remediation of Chlorinated and Recalcitrant Compounds, Palm Springs, CA, 2016.

[90] United States Environmental Protection Agency (USEPA), Environmental Fracturing, Overview, in, CLU-IN, 2016.

[91] C.E. Schaefer, D.R. Lippincott, R.J. Steffan, Field-Scale Evaluation of Bioaugmentation Dosage for Treating Chlorinated Ethenes, Ground Water Monitoring & Remediation, 30 (2010) 113-124.

[92] D. Swift, J. Rothermel, L. Peterson, B. Orr, G.H. Bures, J. Weidhaas, Remediating TCE-contaminated groundwater in low-permeability media using hydraulic fracturing to emplace zero-valent iron/organic carbon amendment, Remediation Journal, 22 (2012) 49-67.

[93] Interstate Technology and Regulatory Countil (ITRC), Use of Measurements of Mass Flux and Mass Discharge, Technology Overview., in, 2010.

[94] H.F. Stroo, A. Leeson, J.A. Marqusee, P.C. Johnson, C.H. Ward, M.C. Kavanaugh, T.C. Sale, C.J. Newell, K.D. Pennell, C.A. Lebrón, M. Unger, Chlorinated ethene source remediation: lessons learned, Environmental science & technology, 46 (2012) 6438.

[95] M. Harkness, A. Fisher, Use of emulsified vegetable oil to support bioremediation of TCE DNAPL in soil columns, Journal of Contaminant Hydrology, 151 (2013) 16-33.

[96] N. Mastroianni, E. López-García, C. Postigo, D. Barceló, M. López de Alda, Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends, Science of the Total Environment, 609 (2017) 916-926.

[97] A. Kankaanpää, K. Ariniemi, M. Heinonen, K. Kuoppasalmi, T. Gunnar, Use of illicit stimulant drugs in Finland: a wastewater study in ten major cities, Science of the Total Environment, 487 (2014) 696-702.

[98] Y. Ryu, D. Barceló, L.P. Barron, L. Bijlsma, S. Castiglioni, P. de Voogt, E. Emke, F. Hernández, F.Y. Lai, A. Lopes, M.L. de Alda, N. Mastroianni, K. Munro, Apos, J. Brien, C. Ort, B.G. Plósz, M.J. Reid, V. Yargeau, K.V. Thomas, Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: An international study in 20 cities, Science of the Total Environment, 565 (2016) 977-983.

[99] T. Boogaerts, A. Covaci, J. Kinyua, H. Neels, A.L.N. van Nuijs, Spatial and temporal trends in alcohol consumption in Belgian cities: A wastewater-based approach, Drug and Alcohol Dependence, 160 (2016) 170-176.

[100] F.Y. Lai, F. Been, A. Covaci, A.L.N. van Nuijs, Novel Wastewater-Based Epidemiology Approach Based on Liquid Chromatography-Tandem Mass Spectrometry for Assessing Population Exposure to Tobacco-Specific Toxicants and Carcinogens, Analytical chemistry, 89 (2017) 9268.

[101] S. Castiglioni, I. Senta, A. Borsotti, E. Davoli, E. Zuccato, A novel approach for monitoring tobacco use in local communities by wastewater analysis, Tobacco Control, 24 (2015) 38.

[102] E. Gracia-Lor, N.I. Rousis, E. Zuccato, R. Bade, J.A. Baz-Lomba, E. Castrignanò, A. Causanilles, F. Hernández, B. Kasprzyk-Hordern, J. Kinyua, A.-K. McCall, A.L.N. van Nuijs, B.G. Plósz, P. Ramin, Y. Ryu, M.M. Santos, K. Thomas, P. de Voogt, Z. Yang, S. Castiglioni, Estimation of caffeine intake from analysis of caffeine metabolites in wastewater, Science of the Total Environment, 609 (2017) 1582-1588.

[103] R. Yeonsuk, G.-L. Emma, B. Richard, J.A. Baz-Lomba, G.B. Jørgen, C. Sara, C. Erika, C. Ana, C. Adrian, V. Pim De, H. Felix, K.-H. Barbara, K. Juliet, M. Ann-Kathrin, O. Christoph, G.P. Benedek, R. Pedram, I.R. Nikolaos, J.R. Malcolm, V.T. Kevin, Increased levels of the oxidative stress biomarker 8-iso-prostaglandin F2α in wastewater associated with tobacco use, Scientific Reports, 6 (2016).

[104] B.F.G. Pycke, I.B. Roll, B.J. Brownawell, C.A. Kinney, E.T. Furlong, D.W. Kolpin, R.U. Halden, Transformation products and human metabolites of triclocarban and triclosan in sewage sludge across the United States, Environmental science & technology, 48 (2014) 7881.

[105] Mayo Clinic, Test ID: COCOU Cortisol/Cortisone, Free, 24 Hour, Urine; Clinical and Interperative in, Mayo Medical Laboratories, 2018.

[106] P.J. Rosch, The Quandary of Job Stress Compensation, in: Health and Stress, The American Institute of Stress, Yonker, NY, 2001.

[107] Mayo Clinic, Test ID: CORT Cortisol, Serum; Clinical and Interperative, in, Mayo Medical Laboratories, 2018.

[108] N.E. Steen, P. Methlie, S. Lorentzen, I. Dieset, M. Aas, M. Nerhus, M. Haram, I. Agartz, I. Melle, J.P. Berg, O.A. Andreassen, Altered systemic cortisol metabolism in bipolar disorder and schizophrenia spectrum disorders, Journal of Psychiatric Research, 52 (2014) 57-62.

[109] C.E. Herbison, D. Henley, J. Marsh, H. Atkinson, J.P. Newnham, S.G. Matthews, S.J. Lye, C.E. Pennell, Characterization and novel analyses of acute stress response patterns in a population-based cohort of young adults: influence of gender, smoking, and BMI, Stress, 19 (2016) 139-150.

[110] B. Keselman, M. Vergara, S. Nyberg, F. Nystrom, A randomized cross-over study of the acute effects of running 5 km on glucose, insulin, metabolic rate, cortisol and Troponin T, PLoS One, 12 (2017) e0179401.

[111] D.H. Hellhammer, S. Wüst, B.M. Kudielka, Salivary cortisol as a biomarker in stress research, Psychoneuroendocrinology, 34 (2009) 163-171.

[112] D. Lee, E. Kim, M. Choi, Technical and clinical aspects of cortisol as a biochemical marker of chronic stress, in: BMB Rep., 2015, pp. 209-216.

[113] E. Russell, G. Koren, M. Rieder, S. Van Uum, Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions, Psychoneuroendocrinology, 37 (2012) 589-601.

[114] G.D. Cruess, H.M. Antoni, A.B. McGregor, M.K. Kilbourn, E.A. Boyers, M.S. Alferi, S.C. Carver, S.M. Kumar, Cognitive-Behavioral Stress Management Reduces Serum Cortisol By Enhancing Benefit Finding Among Women Being Treated for Early Stage Breast Cancer, Psychosomatic Medicine, 62 (2000) 304-308.

[115] D. Nesan, M.M. Vijayan, Embryo exposure to elevated cortisol level leads to cardiac performance dysfunction in zebrafish, Molecular and Cellular Endocrinology, 363 (2012) 85-91.

[116] T. Isobe, K. Sato, K. Joon-Woo, S. Tanabe, G. Suzuki, K. Nakayama, Determination of natural and synthetic glucocorticoids in effluent of sewage treatment plants using ultrahigh performance liquid chromatography-tandem mass spectrometry, Environmental Science and Pollution Research, 22 (2015) 14127-14135.

[117] Á. Tölgyesi, Z. Verebey, V.K. Sharma, L. Kovacsics, J. Fekete, Simultaneous determination of corticosteroids, androgens, and progesterone in river water by liquid chromatography–tandem mass spectrometry, Chemosphere, 78 (2010) 972-979.

[118] S. Liu, H. Chen, X.-R. Xu, S.-S. Liu, K.-F. Sun, J.-L. Zhao, G.-G. Ying, Steroids in marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioconcentration, and human dietary exposure, Science of the Total Environment, 502 (2015) 400-407.

[119] S. Liu, G.-g. Ying, J.-l. Zhao, L.-j. Zhou, B. Yang, Z.-f. Chen, H.-j. Lai, Occurrence and fate of androgens, estrogens, glucocorticoids and progestagens in two different types of municipal wastewater treatment plants, Journal of Environmental Monitoring, 14 (2012) 482-491.

[120] H. Chang, J. Hu, B. Shao, Occurrence of natural and synthetic glucocorticoids in sewage treatment plants and receiving river waters, Environmental science & technology, 41 (2007) 3462.

[121] M. Schriks, J. van Leerdam, S. van Der Linden, B. van Der Burg, A. van Wezel, P. de Voogt, High-Resolution Mass Spectrometric Identification and Quantification of Glucocorticoid Compounds in Various Wastewaters in The Netherlands, Environmental Science & Technology, 44 (2010) 4766.

[122] C. Chen, C. Kostakis, J.P. Gerber, B.J. Tscharke, R.J. Irvine, J.M. White, Towards finding a population biomarker for wastewater epidemiology studies, Science of the Total Environment, 487 (2014) 621-628.

[123] United States Environmental Protection Agency (USEPA), Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, in, Office of Water, CWA Methods Team, Washington, D.C., 2016.

[124] L.H. Keith, W. Crummett, J. Deegan, R.A. Libby, G. Wentler, J.K. Taylor, Principles of Environmental Analysis, Analytical Chemistry, 55 (1983) 2210-2218.

[125] USEPA, Guidance for Data Useability in Risk Assessment (Part A) Final, in, 1991.

[126] J. Hukkanen, P. Jacob, N.L. Benowitz, Metabolism and disposition kinetics of nicotine, Pharmacological reviews, 57 (2005) 79-115.

[127] J. Ahmed, E. Phillips, A.S. Gentzke, D.M. Homa, S.D. Babb, B.A. King, L.J. Neff, Current Cigarette Smoking Among Adults - United States, 2016, in, Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report (MMWR), 2018, pp. 53-59.

[128] ADHS, Annual Report FY2017 in, Arizona Department of Health Services, Phoenix, Arizona, 2017.

[129] L.P. Somogyi, Caffeine Intake by the U.S. Population, in, The Food and Drug Administration, Oakridge National Laboratory 2010, pp. 1-85.

[130] F.L. Miles, S.L. Navarro, Y. Schwarz, H. Gu, D. Djukovic, T.W. Randolph, A. Shojaie, M. Kratz, M.A.J. Hullar, P.D. Lampe, M.L. Neuhouser, D. Raftery, J.W. Lampe, Plasma metabolite abundances are associated with urinary enterolactone excretion in healthy participants on controlled diets, Food Funct., 8 (2017) 3209-3218.

[131] C.-L. Lin, T.-J. Wu, D.A. Machacek, N.-S. Jiang, P.C. Kao, Urinary Free Cortisol and Cortisone Determined by High Performance Liquid Chromatography in the Diagnosis of Cushing's Syndrome1, The Journal of Clinical Endocrinology & Metabolism, 82 (1997) 151-155.

[132] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, Determination of glucocorticoids in sewage and river waters by ultra-high performance liquid chromatography–tandem mass spectrometry, Determination of glucocorticoids in sewage and river waters by ultra-high performance liquid chromatography–tandem mass spectrometry, 1224 (2012) 19-26.

[133] S. Liu, G.-G. Ying, J.-L. Zhao, F. Chen, B. Yang, L.-J. Zhou, H.-J. Lai, Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry, Journal of Chromatography A, 1218 (2011) 1367-1378.

[134] G. Antonelli, C. Artusi, M. Marinova, L. Brugnolo, M. Zaninotto, C. Scaroni, R. Gatti, F. Mantero, M. Plebani, Cortisol and cortisone ratio in urine: LC-MS/MS method validation and preliminary clinical application, Clinical Chemistry and Laboratory Medicine 52 (2014) 213-220.

[135] N.I. Bahr, R. Palme, U. Möhle, J.K. Hodges, M. Heistermann, Comparative Aspects of the Metabolism and Excretion of Cortisol in Three Individual Nonhuman Primates, General and Comparative Endocrinology, 117 (2000) 427-438.

[136] A. Weltring, F.S. Schaebs, S.E. Perry, T. Deschner, Simultaneous measurement of endogenous steroid hormones and their metabolites with LC–MS/MS in faeces of a New World primate species, Cebus capucinus, Physiology & Behavior, 105 (2012) 510-521.

[137] M. Rico, M.J. Andrés-Costa, Y. Picó, Estimating population size in wastewater-based epidemiology. Valencia metropolitan area as a case study, Journal of Hazardous Materials, 323 (2017) 156-165.

[138] I. Senta, E. Gracia-Lor, A. Borsotti, E. Zuccato, S. Castiglioni, Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment, Water Research, 74 (2015) 23-33.

[139] J.W. O'Brien, A.P.W. Banks, A.J. Novic, J.F. Mueller, G. Jiang, C. Ort, G. Eaglesham, Z. Yuan, P.K. Thai, Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model, Environmental Science & Technology, 51 (2017) 3816-3823.

[140] J.W. O'Brien, P.K. Thai, G. Eaglesham, C. Ort, A. Scheidegger, S. Carter, F.Y. Lai, J.F. Mueller, A model to estimate the population contributing to the wastewater using samples collected on census day, Environmental science & technology, 48 (2014) 517.

[141] L.V. Scott, T.G. Dinan, Urinary free cortisol excretion in chronic fatigue syndrome, major depression and in healthy volunteers, Journal of Affective Disorders, 47 (1998) 49-54.

[142] M. Maes, A. Lin, S. Bonaccorso, F. van Hunsel, A. Van Gastel, L. Delmeire, M. Biondi, E. Bosmans, G. Kenis, S. Scharpé, Increased 24-hour urinary cortisol excretion in patients with post-traumatic stress disorder and patients with major depression, but not in patients with fibromyalgia, Acta psychiatrica Scandinavica, 98 (1998) 328.

[143] Associated Regional and University Pathologists (ARUP) Laboratories, Cortisol Urine Free by LC-MS/MS, in, University of Utah, 2018.

[144] R.L. Taylor, D. Machacek, R.J. Singh, Validation of a high-throughput liquid chromatography-tandem mass spectrometry method for urinary cortisol and cortisone.(Endocrinology and Metabolism), Clinical Chemistry, 48 (2002) 1511.

[145] S.Y.L. Ching, E.M. Lim, J. Beilby, C. Bhagat, E. Rossi, J.P. Walsh, P. Pullan, Urine free cortisol analysis by automated immunoassay and high-performance liquid chromatography for the investigation of Cushing's syndrome, Annals of Clinical Biochemistry, 43 (2006) 402-407.

[146] S.B. Abraham, D. Rubino, N. Sinaii, S. Ramsey, L.K. Nieman, Cortisol, obesity, and the metabolic syndrome: A cross-sectional study of obese subjects and review of the literature, Obesity, 21 (2013) E105-E117.

[147] A.W. Schlotz, A.J. Hellhammer, A.P. Schulz, A.A. Stone, Perceived Work Overload and Chronic Worrying Predict Weekend–Weekday Differences in the Cortisol Awakening Response, Psychosomatic Medicine, 66 (2004) 207-214.

[148] G. Maina, A. Palmas, F. Filon, Relationship between self-reported mental stressors at the workplace and salivary cortisol, International Archives of Occupational and Environmental Health, 81 (2008) 391-400.

[149] K. Lacey, M.D. Zaharia, J. Griffiths, A.V. Ravindran, Z. Merali, H. Anisman, A prospective study of neuroendocrine and immune alterations associated with the stress of an oral academic examination among graduate students, Psychoneuroendocrinology, 25 (2000) 339-356.

[150] N. Weekes, R. Lewis, F. Patel, J. Garrison-Jakel, D.E. Berger, S.J. Lupien, Examination stress as an ecological inducer of cortisol and psychological responses to stress in undergraduate students, Stress: The International Journal on the Biology of Stress, 2006, Vol.9(4), p.199-206, 9 (2006) 199-206.

[151] V. Ng, D. Koh, S.-E. Chia, Examination Stress, Salivary Cortisol, and Academic Performance, Psychological Reports, 93 (2003) 1133-1134.

[152] G. McIlvain, M. Noland, R. Bickel, Caffeine Consumption Patterns and Beliefs of College Freshmen, American Journal of Health Education, 42 (2011) 235-244.

[153] T. Carpenter-Aeby, F. Overton Reginald, G. Aeby Victor, M. Malinauskas Brenda, K. Barber-Heidal, A survey of energy drink consumption patterns among college students, Nutrition Journal, 6 (2007) 35.

[154] W.R. Lovallo, L.T.L. Whitsett, H.M. Al'absi, B.S. Sung, A.F. Vincent, M.M. Wilson, Caffeine Stimulation of Cortisol Secretion Across the Waking Hours in Relation to Caffeine Intake Levels, Psychosomatic Medicine, 67 (2005) 734-739.

[155] A. Gavrieli, M. Yannakoulia, E. Fragopoulou, D. Margaritopoulos, J. Chamberland, P. Kaisari, S. Kavouras, C. Mantzoros, Caffeinated Coffee Does Not Acutely Affect Energy Intake, Appetite, or Inflammation but Prevents Serum Cortisol Concentrations from Falling in Healthy Men1-4, The Journal of Nutrition, 141 (2011) 703-707.

[156] J. Canals, M.T. Colomina, J. Domingo, E. Domènech, Influence of smoking and drinking habits on salivary cortisol levels, Personality and Individual Differences, 23 (1997) 593-599.

[157] S.R. Battista, S.H. Stewart, H.G. Fulton, D. Steeves, C. Darredeau, D. Gavric, A further investigation of the relations of anxiety sensitivity to smoking motives, Addictive Behaviors, 33 (2008) 1402-1408.

[158] R. Spencer, K. Hutchison, Alcohol, Aging, and the Stress Response, Alcohol Research and Health, 23 (1999) 272.

[159] A. Abbey, M.J. Smith, R.O. Scott, The relationship between reasons for drinking alcohol and alcohol consumption: An interactional approach, Addictive Behaviors, 18 (1993) 659-670.

[160] E. Gracia-Lor, N.I. Rousis, E. Zuccato, R. Bade, J.A. Baz-Lomba, E. Castrignanò, A. Causanilles, F. Hernández, B. Kasprzyk-Hordern, J. Kinyua, Estimation of caffeine intake from analysis of caffeine metabolites in wastewater, Science of the Total Environment, 609 (2017) 1582-1588.

[161] Mayo Clinic, Cortisol/Cortisone, Free, 24 Hour, Urine; Specimen in, Mayo Medical Laboratories, 2018.

[162] Center for Disease Control and Prevention (CDC), Fact Sheets - Alcohol Use and Your Health, in: Alcohol and Public Health, 2018.

[163] J.J. Sacks, K.R. Gonzales, E.E. Bouchery, L.E. Tomedi, R.D. Brewer, 2010 National and State Costs of Excessive Alcohol Consumption, American Journal of Preventive Medicine, 49 (2015) e73-e79.

[164] Center for Disease Control and Prevention (CDC), Tobacco-related Mortality in: Smoking and Tobacco Use, 2018.

[165] R. Courtney, The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General, 2014Us Department of Health and Human Services Atlanta, GA: Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 20141081 pp. online, in, 2015, pp. 694-695.

[166] S. Cappelletti, D. Piacentino, V. Fineschi, P. Frati, L. Cipolloni, M. Aromatario, Caffeine-related deaths: Manner of deaths and categories at risk, Nutrients, 10 (2018).

[167] N. Mastroianni, M. Lopez de Alda, D. Barcelo, Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona, Journal of Chromatography A, 1360 (2014) 93-99.

[168] M.J. Reid, K.H. Langford, J. Mørland, K.V. Thomas, Analysis and Interpretation of Specific Ethanol Metabolites, Ethyl Sulfate, and Ethyl Glucuronide in Sewage Effluent for the Quantitative Measurement of Regional Alcohol Consumption, Alcoholism: Clinical and Experimental Research, 35 (2011) 1593-1599.

[169] J.A. Baz-Lomba, S. Salvatore, E. Gracia-Lor, R. Bade, S. Castiglioni, E. Castrignanò, A. Causanilles, F. Hernandez, B. Kasprzyk-Hordern, J. Kinyua, Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities, BMC public health, 16 (2016) 1035.

[170] N.L. Benowitz, O.F. Pomerleau, C.S. Pomerleau, P. Jacob, Nicotine metabolite ratio as a predictor of cigarette consumption, Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco, 5 (2003) 621.

[171] Q.-D. Zheng, J.-G. Lin, W. Pei, M.-X. Guo, Z. Wang, D.-G. Wang, Estimating nicotine consumption in eight cities using sewage epidemiology based on ammonia nitrogen equivalent population, Science of the Total Environment, 590-591 (2017) 226-232.

[172] F.Y. Lai, C. Gartner, W. Hall, S. Carter, J. O' Brien, B.J. Tscharke, F. Been, C. Gerber, J. White, P. Thai, R. Bruno, J. Prichard, K.P. Kirkbride, J.F. Mueller, Measuring spatial and temporal trends of nicotine and alcohol consumption in Australia using wastewater-based epidemiology, Addiction, 113 (2018) 1127-1136.

[173] H.T. Nguyen, P.K. Thai, S.L. Kaserzon, J.W. O'Brien, G. Eaglesham, J.F. Mueller, Assessment of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam.(Report), The Science of the Total Environment, 631 (2018) 469.

[174] Substance Abuse and Mental Health Services Administration (SAMHSA), Results from the 2016 National Survey on Drug Use and Health: Detailed Tables, in: S.A.a.M.H.S. Administration (Ed.), U.S. Department of Health and Human Services, Rockville, MD, 2017.

[175] N. McKeganey, Vapers and vaping: E-cigarettes users views of vaping and smoking, Drugs: Education, Prevention & Policy, 25 (2018) 13-21.

[176] D.C. Mitchell, C.A. Knight, J. Hockenberry, R. Teplansky, T.J. Hartman, Beverage caffeine intakes in the U.S, Food and Chemical Toxicology, 63 (2014) 136-142.

[177] C.J. Reissig, E.C. Strain, R.R. Griffiths, Caffeinated energy drinks—A growing problem, Drug and Alcohol Dependence, 99 (2009) 1-10.

[178] B.M. Malinauskas, V.G. Aeby, R.F. Overton, T. Carpenter-Aeby, K. Barber-Heidal, A survey of energy drink consumption patterns among college students, Nutrition journal, 6 (2007) 35.

[179] C.A. Marczinski, M.T. Fillmore, A.L. Henges, M.A. Ramsey, C.R. Young, Mixing an Energy Drink with an Alcoholic Beverage Increases Motivation for More Alcohol in College Students, Alcoholism, 37 (2013) 276-283.

[180] R. McKetin, A. Coen, S. Kaye, A comprehensive review of the effects of mixing caffeinated energy drinks with alcohol, Drug and Alcohol Dependence, 151 (2015) 15-30.

[181] T. Rodríguez-Álvarez, I. Racamonde, I. González-Mariño, A. Borsotti, R. Rodil, I. Rodríguez, E. Zuccato, J.B. Quintana, S. Castiglioni, Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis, Science of the Total Environment, 536 (2015) 91-98.

[182] A.M. Lostia, J.L. Vicente, D.A. Cowan, Measurement of Ethyl Glucuronide, Ethyl Sulphate and Their Ratio in the Urine and Serum of Healthy Volunteers after Two Doses of Alcohol, Alcohol and Alcoholism, 48 (2013) 74-82.

[183] A. Lelo, D. Birkett, R. Robson, J. Miners, Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man, British Journal of Clinical Pharmacology, 22 (1986) 177-182.

[184] P. Jacob, III, L. Yu, A.T. Shulgin, N.L. Benowitz, Minor Tobacco Alkaloids as Biomarkers for Tobacco Use: Comparison of Users of Cigarettes, Smokeless Tobacco, Cigars, and Pipes, The American Journal of Public Health, 89 (1999) 731.

[185] S.G. Alsabri, W.O. Mari, S. Younes, M.A. Alsadawi, T.L. Oroszi, Kinetic and Dynamic Description of Caffeine, Journal of Caffeine Research, (2017).

[186] J.G. Teeguarden, C.J. Housand, J.N. Smith, P.M. Hinderliter, R. Gunawan, C.A. Timchalk, A multi-route model of nicotine–cotinine pharmacokinetics, pharmacodynamics and brain nicotinic acetylcholine receptor binding in humans, Regulatory Toxicology and Pharmacology, 65 (2013) 12-28.

[187] K. Uemura, T. Fujimiya, Y. Ohbora, M. Yasuhara, K.-I. Yoshida, Individual differences in the kinetics of alcohol absorption and elimination : A human study, Forensic science, medicine, and pathology, 1 (2005) 27.

[188] NIH, What is a standard drink?, in, National Institute on Alcohol Abuse and Alcoholism, 2018.

[189] B. Hoeppner, N. Barnett, K. Jackson, Daily college student drinking patterns across the first year of college, Alcoholism (NY), 33 (2009) 190A-190A.

[190] P.F. Tremblay, K. Graham, S. Wells, R. Harris, R. Pulford, S.E. Roberts, When Do First-Year College Students Drink Most during the Academic Year? An Internet-Based Study of Daily and Weekly Drinking, Journal of American College Health, 58 (2010) 401-411.

[191] M.E. Patrick, C.M. Lee, Comparing numbers of drinks: college students' reports from retrospective summary, followback, and prospective daily diary measures.(Report), Journal of Studies on Alcohol and Drugs, 71 (2010) 554.

[192] S.P. Haughwout, M.E. Slater, Apparent per capita alcohol consumption: National, state, and regional trends, 1977–2015, in, National Institute on Alcohol Abuse and Alcoholism Division of Epidemiology and Prevention Research Alcohol Epidemiologic Data System, 2017.

[193] F.K. Del Boca, J. Darkes, P.E. Greenbaum, M.S. Goldman, Up Close and Personal: Temporal Variability in the Drinking of Individual College Students During Their First Year, Journal of Consulting and Clinical Psychology, 72 (2004) 155-164.

[194] C.R. Mahoney, G.E. Giles, B.P. Marriott, D.A. Judelson, E.L. Glickman, P.J. Geiselman, H.R. Lieberman, Intake of caffeine from all sources and reasons for use by college students, Clinical Nutrition, (2018).

[195] T.R. Norton, A.B. Lazev, M.J. Sullivan, The “Buzz” on Caffeine: Patterns of Caffeine Use in a Convenience Sample of College Students, Journal of Caffeine Research, 1 (2011).

[196] J.-H. Bae, J.-H. Park, S.-S. Im, D.-K. Song, Coffee and health, Integrative Medicine Research, 3 (2014) 189-191.

[197] P. Nawrot, S. Jordan, J. Eastwood, J. Rotstein, A. Hugenholtz, M. Feeley, Effects of caffeine on human health, Food Additives and Contaminants, 20 (2003) 1-30.

[198] D.-C. Seo, J.T. Macy, M.R. Torabi, S.E. Middlestadt, The effect of a smoke-free campus policy on college students' smoking behaviors and attitudes, Preventive Medicine, 53 (2011) 347-352.

[199] K.M. Caldeira, K.E. O'Grady, L.M. Garnier-Dykstra, K.B. Vincent, W.B. Pickworth, A.M. Arria, Cigarette Smoking Among College Students: Longitudinal Trajectories and Health Outcomes, Nicotine & Tobacco Research, 14 (2012) 777-785.

[200] E.A. Pinsker, C.J. Berg, E.J. Nehl, A.V. Prokhorov, T.S. Buchanan, J.S. Ahluwalia, Intent to quit among daily and non-daily college student smokers, Health Education Research, 28 (2013) 313-325.

[201] Center for Behavioral Health Statistics and Quality (CBHSQ), 2016 National Survey on Drug Use and Health: Detailed Tables, in: C.f.B.H.S.a. Quality (Ed.), Substance Abuse and Mental Health Services Administration, Rockville, MD, 2017.

[202] T.W. Wang, B. Kenemer, M.A. Tynan, T. Singh, B. King, Consumption of Combustible and Smokeless Tobacco - United States, 2000-2015, MMWR. Morbidity and mortality weekly report, 65 (2016) 1357.

[203] S.A. McKee, R. Hinson, D. Rounsaville, P. Petrelli, Survey of subjective effects of smoking while drinking among college students, Nicotine & Tobacco Research, 6 (2004) 111-117.

[204] A.K. Littlefield, J.C. Gottlieb, L.M. Cohen, D.R.M. Trotter, Electronic Cigarette Use Among College Students: Links to Gender, Race/Ethnicity, Smoking, and Heavy Drinking, Journal of American College Health, 63 (2015) 00-00.

[205] M. Nichter, M. Nichter, A. Carkoglu, E. Lloyd-Richardson, Smoking and drinking among college students: "It's a package deal", Drug and Alcohol Dependence, 106 (2010) 16-20.

[206] T.M. Piasecki, S. Jahng, P.K. Wood, B.M. Robertson, A.J. Epler, N.J. Cronk, J.W. Rohrbaugh, A.C. Heath, S. Shiffman, K.J. Sher, The Subjective Effects of Alcohol–Tobacco Co-Use: An Ecological Momentary Assessment Investigation, Journal of Abnormal Psychology, 120 (2011) 557-571.

[207] B.A. Baiamonte, S.C. Stickley, S.J. Ford, Nicotine Deprivation Produces Deficits in Pain Perception that are Moderately Attenuated by Caffeine Consumption, Journal of Psychoactive Drugs, 48 (2016) 159-165.

[208] J.L. Treur, A.E. Taylor, J.J. Ware, G. McMahon, J.J. Hottenga, B.M.L. Baselmans, G. Willemsen, D.I. Boomsma, M.R. Munafò, J.M. Vink, Associations between smoking and caffeine consumption in two European cohorts, Addiction, 111 (2016) 1059-1068.

[209] A.M. Arria, K.M. Caldeira, S.J. Kasperski, K.B. Vincent, R.R. Griffiths, K.E. O'Grady, Energy Drink Consumption and Increased Risk for Alcohol Dependence, Alcoholism, 35 (2011) 365-375.

[210] C.O. Cobb, A. Nasim, K. Jentink, M.D. Blank, The role of caffeine in the alcohol consumption behaviors of college students, Substance Abuse, 36 (2013).

[211] C.E. Velazquez, N.S. Poulos, L.A. Latimer, K.E. Pasch, Associations between energy drink consumption and alcohol use behaviors among college students, Drug and Alcohol Dependence, 123 (2012) 167-172.

[212] A.P.W. Banks, F.Y. Lai, J.F. Mueller, G. Jiang, S. Carter, P.K. Thai, Potential impact of the sewer system on the applicability of alcohol and tobacco biomarkers in wastewater-based epidemiology, Drug Testing and Analysis, 10 (2018) 530-538.

[213] United States Bureau of Labor Statistics (USBLS), Consumer Expenditure Surveys, in, 2018.

[214] C. Uhlig, B. Seitz, N. Eter, J. Promesberger, H. Busse, Efficiencies of Internet-Based Digital and Paper-Based Scientific Surveys and the Estimated Costs and Time for Different-Sized Cohorts, PLoS One, 9 (2014) e108441.

[215] N.I. Rousis, E. Zuccato, S. Castiglioni, Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides, Environment international, 99 (2017) 213-220.

[216] I. González-Mariño, R. Rodil, I. Barrio, R. Cela, J.B. Quintana, Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers, Environmental Science & Technology, 51 (2017) 3902-3910.

[217] L. Feng, W. Zhang, X. Li, Monitoring of regional drug abuse through wastewater-based epidemiology—A critical review, Science China Earth Sciences, 61 (2018) 239-255.

[218] F. Been, L. Bijlsma, L. Benaglia, J.-D. Berset, A.M. Botero-Coy, S. Castiglioni, L. Kraus, F. Zobel, M.P. Schaub, A. Bücheli, F. Hernández, O. Delémont, P. Esseiva, C. Ort, Assessing geographical differences in illicit drug consumption—A comparison of results from epidemiological and wastewater data in Germany and Switzerland, Drug and Alcohol Dependence, 161 (2016) 189-199.

[219] J.H.P. van Wel, J. Kinyua, A.L.N. van Nuijs, S. Salvatore, J.G. Bramness, A. Covaci, G. Van Hal, A comparison between wastewater-based drug data and an illicit drug use survey in a selected community, International Journal of Drug Policy, 34 (2016) 20-26.

[220] D.A. Burgard, C. Banta-Green, J.A. Field, Working upstream: how far can you go with sewage-based drug epidemiology?, Environmental science & technology, 48 (2014) 1362.

[221] S. Castiglioni, L. Bijlsma, A. Covaci, E. Emke, F. Hernández, M. Reid, C. Ort, K.V. Thomas, A.L.N. van Nuijs, P. de Voogt, E. Zuccato, Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers, Environmental Science and Technology, 47 (2013) 1452-1460.

[222] E. Archer, E. Castrignanò, B. Kasprzyk-Hordern, G. Wolfaardt, Wastewater-based epidemiology and enantiomeric profiling for drugs of abuse in South African wastewaters, Science of The Total Environment, 625 (2018) 792-800.

[223] M.A. Humphries, R. Bruno, F.Y. Lai, P.K. Thai, B.R. Holland, J.W. O'Brien, C. Ort, J.F. Mueller, Evaluation of Monitoring Schemes for Wastewater-Based Epidemiology to Identify Drug Use Trends Using Cocaine, Methamphetamine, MDMA and Methadone, Environmental science & technology, 50 (2016) 4760.

[224] C. Ort, J.M. Eppler, A. Scheidegger, J. Rieckermann, M. Kinzig, F. Sörgel, Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design, Addiction, 109 (2014) 472-481.

[225] J.A. Baz-Lomba, S. Salvatore, E. Gracia-Lor, R. Bade, S. Castiglioni, E. Castrignano, A. Causanilles, F. Hernandez, B. Kasprzyk-Hordern, J. Kinyua, A.K. McCall, A. van Nuijs, C. Ort, B.G. Plósz, P. Ramin, M. Reid, R.I. Rousis, Y. Ryu, P. de Voogt, J. Bramness, K. Thomas, Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities, BMC Public Health, 16 (2016) urn:issn:1471-2458.

[226] C. Harman, M. Reid, K.V. Thomas, Concerning the viewpoint; "An anti-doping sampling strategy utilizing the sewerage systems of sport villages", Environmental science & technology, 45 (2011) 4191.

[227] United States Envrionmental Protection Agency (USEPA), Operating Procedure for Wastewater Sampling, in, 2017.

[228] I.B. Roll, R.U. Halden, Critical review of factors governing data quality of integrative samplers employed in environmental water monitoring, Water Research, 94 (2016) 200-207.

[229] A. Belles, N. Tapie, P. Pardon, H. Budzinski, Development of the performance reference compound approach for the calibration of "polar organic chemical integrative sampler" (POCIS), Analytical and bioanalytical chemistry, 406 (2014) 1131.

[230] I.B. Roll, E.M. Driver, R.U. Halden, Apparatus and method for time-integrated, active sampling of contaminants in fluids demonstrated by monitoring of hexavalent chromium in groundwater, Science of the Total Environment, 556 (2016) 45-52.

[231] M.P. Ensminger, M. Vasquez, H.-J. Tsai, S. Mohammed, A. Van Scoy, K. Goodell, G. Cho, K.S. Goh, Continuous low-level aquatic monitoring (CLAM) samplers for

pesticide contaminant screening in urban runoff: Analytical approach and a field test case, Chemosphere, 184 (2017) 1028-1035.

[232] J. Llorca, C. Gutiérrez, E. Capilla, R. Tortajada, L. Sanjuán, A. Fuentes, I. Valor, Constantly stirred sorbent and continuous flow integrative sampler: New integrative samplers for the time weighted average water monitoring, Journal of Chromatography A, 1216 (2009) 5783-5792.

[233] S. Supowit, I. Roll, V.D. Dang, K.J. Kroll, N.D. Denslow, R. Halden, I. Biodesign, S. Center for Environmental, I. Global Security, Active Sampling Device for Determining Pollutants in Surface and Pore Water – the In Situ Sampler for Biphasic Water Monitoring, in, 2016.

[234] N. Santiago Sánchez, S. Tejada Alarcón, R. Tortajada Santonja, J. Llorca-Pórcel, New device for time-averaged measurement of volatile organic compounds (VOCs), Science of the Total Environment, 485-486 (2014) 720-725.

[235] D.A. Devault, Y. Lévi, S. Karolak, Applying sewage epidemiology approach to estimate illicit drug consumption in a tropical context: Bias related to sewage temperature and pH, Science of the Total Environment, 584-585 (2017) 252-258.

[236] J. Li, Stability of Illicit Drugs as Biomarkers in Sewers: From Lab to Reality, Environmental Science & Technology, 52 (2018) 1561-1571.

[237] I. Senta, I. Krizman, M. Ahel, S. Terzic, Assessment of stability of drug biomarkers in municipal wastewater as a factor influencing the estimation of drug consumption using sewage epidemiology, Science of the Total Environment, 487 (2014) 659-665.

[238] A.-K. McCall, R. Bade, J. Kinyua, F.Y. Lai, P.K. Thai, A. Covaci, L. Bijlsma, A.L.N. van Nuijs, C. Ort, Critical review on the stability of illicit drugs in sewers and wastewater samples, Water Research, 88 (2016) 933-947.

[239] A. Raffaelli, A. Saba, E. Vignali, C. Marcocci, P. Salvadori, Direct determination of the ratio of tetrahydrocortisol + allo-tetrahydrocortisol to tetrahydrocortisone in urine by LC–MS–MS, Journal of Chromatography B, 830 (2006) 278-285.

[240] D.L. Delahanty, N.R. Nugent, N.C. Christopher, M. Walsh, Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims, Psychoneuroendocrinology, 30 (2005) 121-128.

[241] S.T. Yahyavi, M. Zarghami, F. Naghshvar, A. Danesh, Relationship of cortisol, norepinephrine, and epinephrine levels with war-induced posttraumatic stress disorder in fathers and their offspring, Revista Brasileira de Psiquiatria, 37 (2015) 93-98.

[242] J.P. Higgins, T.D. Tuttle, C.L. Higgins, Energy Beverages: Content and Safety, Mayo Clinic Proceedings, 85 (2010) 1033-1041.

[243] J. Hasselström, J. Säwe, Morphine pharmacokinetics and metabolism in humans, Clinical pharmacokinetics, 24 (1993) 344-354.

[244] B. Lalovic, E. Kharasch, C. Hoffer, L. Risler, L.Y. Liu-Chen, D.D. Shen, Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites, Clinical pharmacology & therapeutics, 79 (2006) 461-479.

[245] P.K. Thai, F.Y. Lai, R. Bruno, E. van Dyken, W. Hall, Apos, J. Brien, J. Prichard, J.F. Mueller, Refining the excretion factors of methadone and codeine for wastewater analysis — Combining data from pharmacokinetic and wastewater studies, Environment International, 94 (2016) 307-314.

[246] C. Postigo, M.L. de Alda, D. Barceló, Evaluation of drugs of abuse use and trends in a prison through wastewater analysis, Environment International, 37 (2011) 49-55.

[247] R.B. Labroo, M.F. Paine, K.E. Thummel, E.D. Kharasch, Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions, Drug Metabolism and Disposition, 25 (1997) 1072-1080.

[248] United States Food and Drug Administration (USFDA), Highlights of prescribing information: Suboxone, in, 2017.

[249] A.L. Van Nuijs, J.-F. Mougel, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Sewage epidemiology—a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium, Environment international, 37 (2011) 612-621.

[250] E. Gracia-Lor, E. Zuccato, S. Castiglioni, Refining correction factors for back-calculation of illicit drug use, Science of the Total Environment, 573 (2016) 1648-1659.

[251] E. Redalieu, M.F. Bartlett, L.M. Waldes, W.R. Darrow, H. Egger, W.E. Wagner, A study of methylphenidate in man with respect to its major metabolite, Drug Metabolism & Disposition, 10 (1982) 708-709.

[252] P.D. Garzone, P.D. Kroboth, Pharmacokinetics of the Newer Benzodiazepines, Clinical pharmacokinetics, 16 (1989) 337-364.

APPENDIX A

SUPPLEMENTAL MATERIAL FOR CHAPTER 2

Field Collection

Bedrock cores were collected following the guidelines outlined in the American Society for Testing and Materials (ASTM) D2113-Standard Practice for Rock Core Drilling and Sampling of Rock for Site Investigation. Rock cores were Ordovician limestone of the Ottawa formation collected from 9.5 to 10 m depth. Groundwater samples were collected in compliance with Ontario Regulation 154/03, as amended July 1, 2011, Record of the Site Condition Part XV.1 of the Environmental Protection Act, R.S.O. 1990, c. E.19, for preservation of volatile organic compounds (VOCs). Samples were collected in four L high-density polyethylene (HDPE) sample collection bottles and stored at 4°C.

Media Preparation

Site bedrock was prepared for BM construction by crushing with a construction grade hammer and steel plate. Bedrock fragments were further processed for CMs by passing the material through a steel plate rock crusher (Badger Crusher) and disc pulverizer (UA Disc Pulverizer) (Bico Braun International, Burbank, CA). Bedrock fragments were sieved with ASTM standard brass sieves to a desired size of 0.25 mm. Uniform grain size was necessary to sustain equivalent flow rates in continuous-flow columns.

Table 4 – Databases used in treatability study literature review.

Relevant Databases	
American Geophysical Union	National Academy of Sciences
ASCE Library	National Technical Information Service
ASTM Standards and Engineering Digital Library (SEDL)	ProQuest
Dissertations & Theses @ Arizona State University	ProQuest Dissertations & Theses Global
Earth and Environmental Science	PubMed
EDP Sciences	SAGE Premier
Environment Complete	Science Direct
GeoScienceWorld	Scientific American Archive Online
Google Scholar	SciFinder
JSTOR Health & General Science Collection	Scitation Publications
JSTOR Life Sciences Collection	SpringerLink
Knovel	Web of Science

Table 5 - Batch bottle and continuous-flow column construction details.

Control/Treatment Batch Bottle Microcosms									
Treatment/ Control	No.	Crushed Bedrock (g)	Ground-water (mL)	Proprietary (g)	nZVI (g)	Pd (mg)	Org. Carbon Source (mL)	EHC® (g)	KB-1®
Biotic Control	2	60	200	NA	NA	NA	NA	NA	NA
Treatment 1	3	60	200	2.3	NA	NA	NA	NA	Day 29
Treatment 2	3	60	170	NA	2.3	20	30	NA	Day 29
Treatment 3	3	60	200	NA	NA	NA	NA	1.2	Day 29
Control/Treatment Batch Bottle Microcosms									
Treatment/ Control	No.	Crushed Bedrock (g)	Ground-water (mL/Day)	Proprietary (g)	nZVI (g)	Pd (mg)	Org. Carbon Source (mL)	EHC® (g)	KB-1®
Biotic Control	2	38	3	NA	NA	NA	NA	NA	NA
Treatment 1	2	37	3	1.21	NA	NA	NA	NA	Day 30
Treatment 2	3	37	3	NA	1.21	12	11.6	NA	Day 30
Treatment 3	3	37	3	NA	NA	NA	NA	0.75	Day 30

Notes: NA- Not Applicable, Pd – palladium acetate, nZVI – nanoscale zero valent iron

Table 6 - Limits of detection and recoveries (n = 3) for target analytes.

Analyte	Batch Bottles	Continuous-Flow Columns	
	LOD (µg/L)	LOD (µg/L)	Recovery %
PCE	10	5	105 ± 4
TCE	10	1.3	95 ± 5
*c*DCE	10	2	104 ± 3
*t*DCE	10	2	97 ± 2
1,1-DCE	10	2.9	101 ± 3
VC	10	2.5	90 ± 5
Ethene	10	6	88 ± 7

Table 7 - Selected journal articles from treatability study literature review.

J.H. Choi, Y.H. Kim, S.J. Choi, Reductive dechlorination and biodegradation of 2,4,6-trichlorophenol using sequential permeable reactive barriers: Laboratory studies, *Chemosphere 67* (2007) 1151-1557.
E.I. Garcia-Pena, P. Zarate-Sequra, P. Guerra-Blanco, T. Poznyak, I. Chairez, Enhanced Phenol and Chlorinated Phenols Removal by Combining Ozonation and Biodegradation, *Water Air Soil Poll. 223* (2012) 2047-4064.
P.J. Haest, J. Philips, D. Springael, E. Smolders, The reactive transport of trichloroethene is influences by residence time and microbial numbers, *J. Contam. Hydrol. 119* (2011) 89-98.
Y.P. Katsenovich, F.R. Miralles-Wilhelm, Evaluation of nanoscale zerovalent iron particles for trichloroethene degradation in clayey soils, *Sci. Total Environ. 407* (2009) 4986–4993.
T. Lee, C.H. Benson, G.R. Eykholt, Waste green sands as reactive media for groundwater contaminated with trichloroethylene (TCE), *J. Hazard. Mater. 109* (2004) 25-36.
Z. Li, Y. Inoue, S. Yang, N. Yoshida, A. Katayama, Mass balance and kinetic analysis of anaerobic microbial dechlorination of pentachlorophenol in a continuous flow column, *J. Biosci. Bioeng. 110* (2010) 326-332.
K. Mackenzie, J. Battke, R. Koehler, F.D. Kopinke, Catalytic effects of activated carbon on hydrolysis reactions of chlorinated compounds Part 2. 1,1,2,2,-Tetrachloroethane, *Appl. Catal. B. 59* (2005) 171–179.
T.T. Tsai, C.M. Kao, T.Y. Yeh, S.H. Liang, H.Y. Chien, Application of surfactant enhanced permanganate oxidation and biodegradation of trichloroethylene in groundwater, *J. Hazard. Mater. 161* (2009) 111-119.
C.E. Schaefer, C.W. Condee, S. Vainbery, R.J. Steffan, Bioaugmentation for chlorinated ethenes using *Dehalococcoides sp.*: Comparison between batch and column experiments, *Chemosphere 75* (2009) 141-148.
G.V. Lowry, M. Reinhard, Pd-Catalyzed TCE Dechlorination in Groundwater: Solute Effects, Biological Control, and Oxidative Catalyst Regeneration. *Environ. Sci. Technol. 34* (2000) 3217-3223.
B. Yim, H. Okuno, Y. Negata, Y. Maeda, Sonochemical degradation of chlorinated hydrocarbons using batch and continuous flow system, *J. Hazard. Mater.* (2001) 253-263.
C.M. Kao, S.C. Chen, M.C. Su, Laboratory column studies for evaluating a barrier

system for providing oxygen and substrate for TCE biodegradation, *Chemosphere 44* (2001) 925-934.

J.E. Szecsody, J.S. Fruchter, M.D. Williams, V.D. Williams, V.R. Vermeul, D. Sklarew, In situ chemical reduction of aquifer sediments: enhancement of reactive iron phases and TCE dechlorination, *Environ. Sci. Technol. 38* (2004) 4656-4663.

S. Chen, S. Wu, Feasibility of using metals to remediate water containing TCE, *Chemosphere 43* (2001) 1023-1028.

G.F. Slater, B. Sherwood Lollar, R. Allen King, S. O'Hannesin, Isotopic fractionation during reductive dechlorination of trichloroethene by zero-valent iron: influence of surface treatment, *Chemosphere 49* (2002) 587-596.

C.K.J. Yeh, H.M. Wu, T.C. Chen, Chemical oxidation of chlorinated non-aqueous phase liquid by hydrogen peroxide in nation sand systems. *J Hazard. Mater*. (2003) 29-51.

C.M. Kao, S.C. Chen, J.Y. Wang, Y.L. Chen, S.Z. Lee, Remediation of PCE-contaminated aquifer by an in situ two layer biobarrier: laboratory batch and column studies, *Water Res. 37* (2003) 27-38.

G.R. Kassenga, J.H. Pardue, S. Blair, T. Ferraro, Treatment of chlorinated volatile organic compounds in upflow wetland mesocosms, *Ecol. Eng. 19* (2003) 305-323.

B.K. Taşeli, C.F. Gökçay, H. Taşeli, Upflow column reactor design for dechlorination of chlorinated pulping wastes by *Penicillium camemberti*, *J. Environ. Manage. 72* (2004) 175-179.

B.K. Taşeli, C.F. Gökçay, Degradation of chlorinated compounds by *Penicillium camemberti* in batch and up-flow column reactors, *Process Biochem. 40* (2005) 917-923.

J. Dries, L. Bastiaens, D. Springael, S.N. Agathos, L. Diels, Combined removal of chlorinated ethenes and heavy metals by zerovalent iron in batch and continuous flow column systems. *Environ. Sci. Technol. 39* (2005) 8460-8465.

L.A. Hellerich, N.P. Nikolaidis, Sorption studies of mixed chromium and chlorinated ethenes at the field and laboratory scales, *J. Environ. Manage. 75* (2005) 77-88.

R.A. Brennan, R.A. Sanford, C.J. Werth. Biodegradation of Tetrachloroethene by Chitin Fermentation Products in a Continuous Flow Column System, *J. Eng-ASCE 132* (2006) 664-673.

R.A. Brennan, R.A. Sanford, C.J. Werth, Chitin and corncobs as electron donor sources for the reductive dechlorination of tetrachloroethene, *Water Res. 40*(11) (2006) 2125–2134.

T.S. Kwon, J.S. Yang, K. Baek, J.Y. Lee, J.W. Yang, Silicone emulsion-enhanced recovery of chlorinated solvents: Batch and column studies, *J. Hazard. Mater. B136* (2006) 610-617.

F. He, D. Zhao, J.Liu, C.B. Roberts, Stabilization of Fe−Pd Nanoparticles with Sodium Carboxymethyl Cellulose for Enhanced Transport and Dechlorination of Trichloroethylene in Soil and Groundwater, *Ind. Eng. Chem. Res. 46*(1) (2007) 29–34.

Petersen MA, Sale TC, Reardon KF. Electrolytic trichloroethene degradation using mixed metal oxide coated titanium mesh electrodes. *Chemosphere 67 (*2007) 1573-1581.

L.H Chen, C.C. Huang, H.L. Lien, Bimetallic iron–aluminum particles for dechlorination of carbon tetrachloride *Chemosphere 73* (2008) 692–697.

M.F. Azizian, S. Behrens, A. Sabalowsky, M.E. Dolan, A.M. Spormann, L. Semprini, Continuous-flow column study of reductive dehalogenation of PCE upon bioaugmentation with Evanite enrichment culture, *J. Contam. Hydrol. 100* (2008) 11-21.

Y. Seol, I. Javandel, Citric acid-modified Renton's reactions for the oxidation of chlorinated ethylenes in soil solution systems, *Chemosphere 72* (2008) 537-542.

Z. Li, H. Hanlie, Combination of surfactant solubilization with permanganate oxidation for DNAPL remediation, *Water Res. 42* (2008) 605-614.

K. Choi, W. Lee, Reductive dechlorination of carbon tetrachloride in acidic soil manipulated with iron(II) and bisulfide ion, *J. Hazard. Mater. 172* (2009) 623-630.

M. Lee, R. Cord-Fuwisch, M. Manefield, A process for the purification of organochlorine contaminated activated carbon: Sequential solvent purging and reductive dechlorination, *Water Res. 44* (2010) 1580-1590.

M.F. Azizian, I.P.G. Marshall, S. Behrens, A.M. Spormann, L. Semprini, Comparison of lactate, formate, and propionate as hydrogen donors for the reductive dehalogenation of trichloroethene in a continuous-flow column, *J. Contam. Hydrol. 113* (2010) 77-97.

V. Nagpala, A.D. Bokarea, R.C. Chikateb, C.V. Rodec, K.M. Paknikara, Reductive dechlorination of γ-hexachlorocyclohexane using Fe–Pd bimetallic nanoparticles, *J. of Hazard. Mater. 175* (2010) 680-687.

X. Xu, H. Lin, L. Zhu, Y. Yang, J. Feng, Enhanced biodegradation of 2-chloronitrobenzene using a coupled zero-valent iron column and sequencing batch reactor system. *J. Chem. Technol. Biot. 86* (2010) 993-1000.

M.C. Shin, J.S. Yang, G.Y. Park, K. Baek, Influence of mixed-surfactant on

reductive dechlorination of trichloroethylene by zero-valent iron, *Korean J. Chem. Eng. 28*(4) (2011) 1047-1053.

A.J. Salter-Blanc, E.J. Suchomel, J.H. Fortuna, J.T. Nurmi, C. Walker, T. Krug, S. O'Hara, N. Ruiz, T. Morley, P.G. Tratnyek, Evaluation of zerovalent zinc for treatment of 1,2,3-trichloropropane-contamianted groundwater: laboratory and field assessment. *Groundwater Monit. R. 32*(4) (2012) 42-52.

Z. Öztürk, B. Tansel, Y. Katsenovich, M. Sukop, S. Laha, Highly organic natural media as permeable reactive barriers: TCE partitioning and anaerobic degradation profile in eucalyptus mulch and compost, *Chemosphere 89* (2012) 665-671.

S. H. Liang, K.F. Chen, C.S. Wu, Y.H. Lin, C.M. Kao, Development of KMnO4-releasing composites for in situ chemical oxidation of TCE contaminated groundwater, *Water Res. 54* (2014) 149-158.

Table 8 - Concentrations for batch bottle and continuous-flow microcosms (steady-state only).

Microcosm	Analyte	Concentrations (mg/L)
BM Control A	PCE	57, 24, 9, 37, BDL
BM Control B		57, 49, 16, 2.7, BDL
BM Control A	TCE	43, 61, 17, BDL
BM Control B		43, 71, 58, BDL
BM Control A	*c*DCE	31, 45, 110, 130, 130, 120
BM Control B		31, 43, 56, 78, 120, 120
BM Control A	VC	0.36, 0.34, 0.35, 0.34, 0.41
BM Control B		0.40, 0.94, 0.35, 0.37, 2.5
CM Control A	PCE	46, 47, 46, 47, 46, 47, 47, 46, 48, 50
CM Control B		45, 46, 44, 47, 45, 47, 48, 47, 48, 49
CM Control A	TCE	3.0, 0.9, 1,1. 1.5, 1.7, 1.0, 1.0, 0.8, 0.8
CM Control B		3.2, 1.7, 1.8, 1.7, 2.2, 1.6, 1.5, 1.4, 1.2, 1.2
CM Control A	*c*DCE	5.0, 5.8, 6.5, 4.8, 5.9, 5.3, 4.7, 5.1, 4.6, 4.0
CM Control B		4.2, 4.6, 5.0, 4.9, 4.7, 4.6, 4.8, 5.4, 4.2, 3.3
BM Treatment 1A	PCE	57, 25, 3.2, BDL
BM Treatment 1B		57, 34, 3.1, BDL
BM Treatment 1C		57, 31, 0.84, BDL
BM Treatment 1A	TCE	43, 42, 17, BDL
BM Treatment 1B		43, 44, 18, BDL
BM Treatment 1C		43, 45, 17, BDL
BM Treatment 1A	cDCE	31, 39, 40, 76, 80, 70, 44, 31, 18
BM Treatment 1B		31, 39, 40, 72, 78, 64, 35, 16, 4
BM Treatment 1C		31, 39, 46, 76, 75, 64, 30, 10, 1.4
BM Treatment 1A	VC	0.44, 0.37, 0.39, 1.2, 9.7, 27, 38, 40

BM Treatment 1B		0.36, 0.29, 0.27, 1.2, 14, 27, 45, 35
BM Treatment 1C		0.34, 0.27, 0.29, 1.4, 14, 30, 48, 42
BM Treatment 1A	Ethene	BDL, 0.56, 1.7, 4.5
BM Treatment 1B		BDL, 0.16, 0.81, 2.5, 5.6
BM Treatment 1C		BDL, 0.24, 0.89, 2.7, 5.8
CM Treatment 1A	PCE	2.7, 0.7, 1.0
CM Treatment 1B		17, 10, 11
CM Treatment 1A	TCE	31, 31, 32
CM Treatment 1B		23, 29, 30
CM Treatment 1A	*c*DCE	9, 10, 9
CM Treatment 1B		6, 6, 5
BM Treatment 2A		57, 35, 0.41, 0.12, 0.17, BDL
BM Treatment 2B	PCE	57, 29, 0.13, BDL
BM Treatment 2C		57, 34, 0.1, 0.17, 0.14, BDL
BM Treatment 2A		43, 45, 69, 22, BDL
BM Treatment 2B	TCE	43, 48, 80, 78, 71, 22, BDL
BM Treatment 2C		43, 46, 26, BDL
BM Treatment 2A		31, 40, 57, 97, 97, 110, 71, 9.1, 1.7
BM Treatment 2B	*c*DCE	31, 43, 48, 54, 53, 90, 66, 13, BDL
BM Treatment 2C		31, 40, 85, 110,110, 80, 8.1, BDL
BM Treatment 2A		0.3, 0.27, 0.31, 0.7, 4,4, 24, 36, 14
BM Treatment 2B	VC	0.42, 0.39, 0.43, 0.47, 1.2, 25, 37, 0.37
BM Treatment 2C		0.3, 0.26, 0.35, 1.2, 21, 16, BDL
BM Treatment 2A		BDL, 1.1, 6.3, 8.6
BM Treatment 2B	Ethene	BDL, 0.14, 0.17, 0.7, 4.3, 13
BM Treatment 2C		BDL, 1.1, 9.1, 13, 14
CM Treatment 2A	PCE	0.1, 0.5, 0.3, BDL, BDL, 5.1, 0.7, BDL, 0.2

CM Treatment 2B		6.0, 5.3, 4.9, 3.0, 5.5, 7.3, 6.2, 4.7, 6.5
CM Treatment 2C		4.7, 3.8, 3.6, 4.9, 3.9, 0.2, 3.9, 4.5, 8.7
CM Treatment 2A		11, 17, 16, 17, 16, 12, 15, 9.0, 4.5
CM Treatment 2B	*c*DCE	11, 13, 12, 15, 13, 13, 12, 12, 9.4, 7.6
CM Treatment 2C		12, 13, 12, 15, 13, 16, 11, 8.0, 4.7
CM Treatment 2A	VC	6.3, 3.1, 3.0, 2.5, 4.5, 2.8, 6.0, 8.2, 9.7
CM Treatment 2B		1.7, 1.2, 1.3, 0.9, 2.1, 2.3, 3.5, 3.4, 3.8
CM Treatment 2C		2.1, 1.5, 1.6, 0.9, 2.4, 4.6, 3.4, 4.4, 4.4
CM Treatment 2A		3.4, 2.9, 3.5, 3.5, 2.8, 3.7, 2.3, 3.1, 3.7
CM Treatment 2B	Ethene	4.5, 4.2, 4.5, 4.2, 3.9, 3.5, 3.4, 4.3, 4.3
CM Treatment 2C		4.2, 4.4, 4.5, 4.1, 3.7, 2.7, 3.8, 4.2, 4.4
BM Treatment 3A		57, 29, 0.39, BDL
BM Treatment 3B	PCE	57, 33, BDL
BM Treatment 3C		57, 38, 0.15, 0.17, BDL
BM Treatment 3A		43, 43, 12, BDL
BM Treatment 3B	TCE	43, 43, 18, BDL
BM Treatment 3C		43, 43, 14, 2.6, BDL
BM Treatment 3A	*c*DCE	31, 37, 72, 83, 81, 22, BDL
BM Treatment 3B		31, 37, 67, 89, 80, 13, BDL
BM Treatment 3C		31, 36, 67, 78, 75, 2.5, BDL
BM Treatment 3A	VC	0.43, 0.62, 0.65, 4.7, 28, BDL
BM Treatment 3B		0.32, 0.58, 0.64, 6.5, 25, BDL
BM Treatment 3C		0.28, 0.49, 0.61, 4.6, 9.7, BDL
BM Treatment 3A	Ethene	0.11, 0.42, 0.35, 0.41, 6.6, 13, 13
BM Treatment 3B		BDL, 0.42, 0.35, 0.44, 6.6, 9.6, 10
BM Treatment 3C		BDL, 0.33, 0.49, 0.49, 10, 7.8, 9
CM Treatment 3A	VC	0.14, 0.01, 0.01, 0.02, 0.08, BDL, BDL, BDL, 0.03,

		BDL, BDL
CM Treatment 3B		0.02, 0.01, 0.02, 0.04, 0.02, BDL, 0.02, 0.02, BDL, 0.01, 0.03
CM Treatment 3C		0.05, 0.03, 0.33, 0.50, 0.23, 0.07, 1.9, 1.6, 0.18, 0.04, 0.11
CM Treatment 3A	Ethene	9.58, 9.58, 9.63, 9.63, 9.63, 9.60, 9.64, 9.64, 9.64, 9.63, 9.64, 9.64
CM Treatment 3B		9.62, 9.62, 9.62, 9.62, 9.59, 9.62, 9.62, 9.62, 9.62, 9.62, 9.61, 9.61
CM Treatment 3C		9.60, 9.61, 9.47, 9.94, 9.51. 9.59, 8.75, 8.90, 9.54, 9.61, 9.58

Note: BM – Batch Microcom, CM – Column Microcoms, BDL- Below Detection Limits. A/B/C denotes replicate. Analytes with non-dectects during the entire study period in batch or during steady-state in columns were not included here. Once removal was achieved in batch, additional BDLs for subsequent sampling periods were not included.

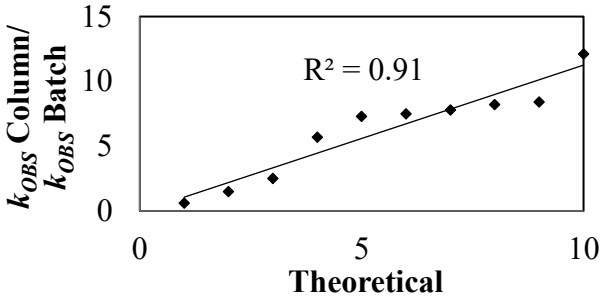


Fig. 23. Normal probability plot of column observed rate constants normalized to batch observed rate constants from combined studies captured in meta-analysis. Coefficient of determination of 0.91 illustrates normal data distribution, thus data extracted from captured results likely are representative of true values.

APPENDIX B

SUPPLEMENTAL MATERIAL FOR CHAPTER 4

Table 9 – Glucocorticoid mass spectrometry parameters.

Compound	Q1	Q3	DP	EP	CE	CXP
Cortisone	361	163/91	91/91	12/6	35/93	16/6
Cortisol	363	121/91	81/81	6/10	47/93	10/6
Cortisol-d_4	367.2	121/96.9	81/81	10/10	37/55	10/16
Cortisone- d_8	369.3	169/337	71/71	10/10	37/19	8/18

Notes:
Q1 = precursor ion, Q3 = product ions, DP = declustering potential, EP = entrance potential, CE = collision energy, CPX = collision cell exit potential

Table 10 – Glucocorticoid analytical method parameters.

Analyte	Absolute Recovery (%)	Relative Recovery (%)	MDL (ng/L)	LOD (ng/L)	LOQ (ng/L)
Cortisol	66 ± 4	88 ± 5	78	84	281
Cortisone	65 ± 3	94 ± 5	60	65	217
Cortisol- d_4	69 ± 4	NA	ND	ND	ND
Cortisone- d_8	70 ± 4	NA	ND	ND	ND

Notes:
NA = not applicable, ND = not determined, MDL = method detection limit
LOD = limit of detection, LOQ = limit of quantitation

Table 11 - Calculated total mass load, and per person cortisol and cortisone in selected studies. Estimated total mass load based on average urinary excretion of cortisol (23 μg d^{-1}) and cortisone (72 μg d^{-1}) [131].

Reference	Compound	Measured Concentration (ng/L)	Total Flow (L)	Population	g/day (actual)	μg/day person
Herrerro et al., 2012	Cortisol	190	3.00E+07	140000	5.7	40.7
		136	3.00E+07	140000	4.1	29.1
		270	1.60E+07	107000	4.3	40.4
		136	1.60E+07	107000	2.2	20.3
	Cortisone	285	1.60E+07	107000	4.6	42.6
		122	1.60E+07	107000	2.0	18.2
		135	3.00E+07	140000	4.1	28.9
		122	3.00E+07	140000	3.7	26.1
Liu et al., 2011	Cortisol	28.8	2.00E+08	425,000	5.8	13.6
		12.7	7.00E+07	230,000	0.9	3.9
	Cortisone	45.8	2.00E+08	425,001	9.2	21.6
		14.5	7.00E+07	230,001	1.0	4.4
Liu et al., 2012	Cortisol	9.67	7.44E+07	425,000	0.7	1.7
		123	9.60E+07	380,000	11.8	31.1
	Cortisone	4.58	7.44E+07	425,000	0.3	0.8
		48.2	9.60E+07	380,000	4.6	12.2
Chang et al., 2007	Cortisol	15	6.00E+07	400,000	0.9	2.3
		85	4.00E+07	100,000	3.4	33.9
		57	7.92E+08	2,400,000	45.3	18.9
		14	2.00E+08	480,000	2.9	6.0
		41	4.74E+08	814,000	19.3	23.7
		26	1.50E+07	180,000	0.4	2.2
		37	6.00E+08	1,925,000	21.9	11.4
	Cortisone	10	6.00E+07	400,000	0.6	1.6
		70	4.00E+07	100,000	2.8	28.1
		38	7.92E+08	2,400,000	29.9	12.4
		14	2.00E+08	480,000	2.7	5.7
		31	4.74E+08	814,000	14.7	18.1
		25	1.50E+07	180,000	0.4	2.0
		26	6.00E+08	1,925,000	15.8	8.2

Table 12 - Daily wastewater flow (L d^{-1}) from North and South campus sampling locations.

Month	Day	North Campus (L/day)	South Campus (L/day)
Aug	Mon	6,090,685	193,260
	Tue	5,896,581	184,272
	Wed	6,061,116	182,472
	Thu	6,121,856	271,625
	Fri	5,951,132	191,359
	Sat	5,586,937	125,876
	Sun	5,759,957	112,247
Sept	Mon	7,325,565	283,586
	Tue	7,325,565	312,928
	Wed	7,734,011	252,644
	Thu	7,261,903	253,164
	Fri	6,738,106	292,729
	Sat	6,203,102	119,123
	Sun	6,290,142	128,017
Oct	Mon	6,736,704	235,242
	Tue	6,586,472	251,998
	Wed	6,659,124	230,506
	Thu	6,650,750	271,294
	Fri	6,025,289	198,753
	Sat	5,833,181	101,395
	Sun	5,674,872	99,660
Nov	Mon	6,215,578	299,279
	Tue	5,961,781	251,830
	Wed	6,065,051	242,515
	Thu	5,928,467	256,215
	Fri	5,624,905	237,873
	Sat	5,132,319	114,527
	Sun	5,221,398	105,766
Dec	Mon	6,019,810	225,177
	Tue	5,993,890	210,178
	Wed	5,845,066	190,682
	Thu	5,743,360	198,269
	Fri	5,070,626	181,962
	Sat	4,349,397	75,931
	Sun	4,226,058	55,365

Table 13 - Calculated campus population with biomarkers caffeine (CAF), paraxanthine (PAR), nicotine (NIC), cotinine (COT), trans-3'-hydroxycotinine (3-OH-COT), enterolactone (ENT) and total wastewater flow normalized to 50 gallons per day (GPD) per person estimates.

Month	Day	CAF	PAR	Water (50 GPD)	NIC	COT	3-OH-COT	ENT
	Mon	224,027	9,530	33,196	17,362	15,258	6,090	15,072
	Tue	228,976	10,011	32,123	17,432	16,461	6,350	15,560
	Wed	187,350	9,483	32,983	16,399	16,384	6,679	17,594
Aug	Thu	213,555	11,861	33,774	17,463	16,925	7,890	17,957
	Fri	168,500	7,397	32,448	16,924	18,311	6,620	14,498
	Sat	108,101	6,992	30,179	16,589	17,672	7,317	10,266
	Sun	104,247	5,412	31,021	16,556	15,933	5,787	9,394
	Mon	133,409	7,785	27,896	17,152	16,105	3,054	27,024
	Tue	192,306	11,205	28,051	24,121	19,822	4,067	38,149
	Wed	269,874	14,390	42,190	30,417	23,844	4,368	41,705
Sept	Thu	247,227	18,086	39,699	27,924	23,641	4,479	39,320
	Fri	230,048	14,509	37,141	30,875	26,943	5,858	35,471
	Sat	137,108	9,328	33,398	27,537	24,714	4,907	19,267
	Sun	123,955	6,249	33,905	29,706	25,670	4,067	22,035
	Mon	229,660	24,215	34,416	39,202	33,686	4,709	33,295
	Tue	213,814	23,117	32,824	36,054	33,094	4,534	30,027
	Wed	201,184	19,304	33,320	34,099	30,134	3,899	20,023
Oct	Thu	161,503	15,036	32,671	29,209	28,049	3,362	17,981
	Fri	143,473	12,450	30,971	29,820	26,086	2,956	14,616
	Sat	95,730	7,415	27,717	30,167	27,286	3,008	15,037
	Sun	87,544	5,747	28,141	34,831	26,199	2,821	12,848
	Mon	220,251	9,696	34,416	44,986	20,657	4,221	34,519
	Tue	202,984	9,813	32,824	56,819	20,100	3,829	30,219
	Wed	284,918	10,127	33,320	90,773	24,135	4,618	50,147
Nov	Thu	190,453	9,652	32,671	40,767	20,472	3,914	38,835
	Fri	179,767	9,509	30,971	36,588	23,062	4,683	34,264
	Sat	114,334	6,169	27,717	35,824	18,790	3,555	22,804
	Sun	107,780	5,206	28,141	34,257	17,920	3,119	20,900
	Mon	204,909	10,227	32,990	49,140	21,669	4,609	62,525
	Tue	219,397	10,257	32,774	65,001	23,821	4,923	48,458
	Wed	169,936	7,753	31,885	43,496	20,145	3,836	29,013
Dec	Thu	185,438	8,191	31,387	46,736	25,407	4,980	31,510
	Fri	139,744	5,245	27,747	50,992	19,546	3,014	21,676
	Sat	120,106	3,380	23,377	36,346	17,955	2,798	14,817
	Sun	75,275	3,245	22,617	32,277	15,333	2,985	12,871

Notes:

GPD = gallons per day

Table 14 - Statistical comparisons between days, months, and weekdays/weekends. Mann-Whitney U values, associated Z-score and p-values showed in table. P-values ranked for each set of comparisons and combined with number of comparisons (m) and false discovery rate (FDR) of 0.1 to establish Benjamini-Hochberg correction. BH corrections not completed for weekday versus weekend because only a single comparison.

Pair 1	Pair 2	Mann-Whitney U	Z-score	*p*-value	p-value rank	BH
Mon	Tues	6	1.36	0.175	8	0.038
Mon	Wed	3	1.98	0.047	1	0.005
Mon	Thu	6	1.36	0.175	8	0.038
Mon	Fri	3	1.98	0.047	1	0.005
Mon	Sat	3	1.98	0.047	1	0.005
Mon	Sun	3	1.98	0.047	1	0.005
Tues	Wed	9	0.73	0.465	15	0.071
Tues	Thu	7	1.15	0.251	13	0.062
Tues	Fri	5	1.57	0.117	7	0.033
Tues	Sat	4	1.78	0.076	6	0.029
Tues	Sun	3	1.98	0.047	1	0.005
Wed	Thu	11	0.31	0.754	18	0.086
Wed	Fri	9	0.73	0.465	15	0.071
Wed	Sat	7	1.15	0.251	13	0.062
Wed	Sun	6	1.36	0.175	8	0.038
Thu	Fri	9	0.73	0.465	15	0.071
Thu	Sat	6	1.36	0.175	8	0.038
Thu	Sun	6	1.36	0.175	8	0.038
Fri	Sat	13	-0.10	0.917	21	0.100
Fri	Sun	11	0.31	0.754	18	0.086
Sat	Sun	11	0.31	0.754	18	0.086
Aug	Sep	12	1.60	0.110	7	0.070
Aug	Oct	0	3.13	0.002	1	0.010
Aug	Nov	0	3.13	0.002	1	0.010
Aug	Dec	0	3.13	0.002	1	0.010
Sep	Oct	8	2.11	0.035	5	0.050
Sep	Nov	8	2.11	0.035	5	0.050
Sep	Dec	6	2.36	0.018	4	0.040
Oct	Nov	27	-0.32	0.750	9	0.090
Oct	Dec	23	0.19	0.848	10	0.100
Nov	Dec	17	0.96	0.338	8	0.080
Day	End	63	2.59	0.010	NA	NA

Notes
BH = Benjamini-Hochberg correction
NA = Not Applicable

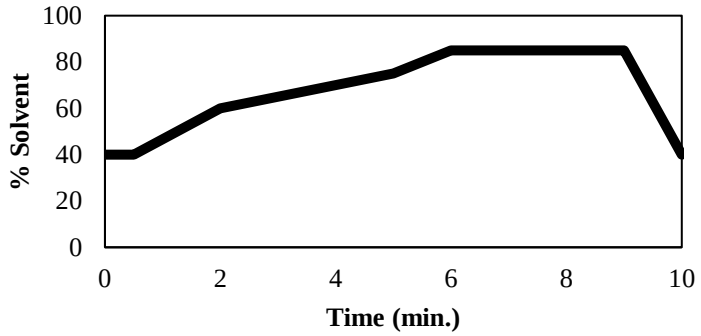


Fig. 24. Liquid chromatography gradient program.

APPENDIX C

SUPPLEMENTAL MATERIAL FOR CHAPTER 5

Table 15 - Measured analyte transitions and method detection limits (MDLs) for alcohol, caffeine, and nicotine compounds.

Compound	**Q1**	**Q3**	**MDL**
Ethyl Sulfate	124.773	96.8/79.8	73
Caffeine	195.195	137.9/42.2	12
Paraxanthine	181.032	124.0/162.9	24
Nicotine	162.996	84.1/79.8	21
Cotinine	177.113	79.6/98.0	2
trans-3'-hydroxycotinine	193.122	79.9/133.8	6
Ethyl Sulfate-d_5	129.713	98.0/79.9	ND
$^{13}C_3$-Caffeine	198.179	139.8/112.0	ND
Cotininie-d_3	180.000	79.8/101.0	ND

Notes: ND = not determined

Table 16 - Daily wastewater flow (L d^{-1}) from North and South Campus sampling locations, Fall 2017 – Spring 2017.

Month	Day	North Campus (L/d)	South Campus (L/day)
Aug	Mon	6,090,685	193,260
	Tue	5,896,581	184,272
	Wed	6,061,116	182,472
	Thu	6,121,856	271,625
	Fri	5,951,132	191,359
	Sat	5,586,937	125,876
	Sun	5,759,957	112,247
Sept	Mon	7,325,565	283,586
	Tue	7,325,565	312,928
	Wed	7,734,011	252,644
	Thu	7,261,903	253,164
	Fri	6,738,106	292,729
	Sat	6,203,102	119,123
	Sun	6,290,142	128,017
Oct	Mon	6,736,704	235,242
	Tue	6,586,472	251,998
	Wed	6,659,124	230,506
	Thu	6,650,750	271,294
	Fri	6,025,289	198,753
	Sat	5,833,181	101,395
	Sun	5,674,872	99,660
Nov	Mon	6,215,578	299,279
	Tue	5,961,781	251,830
	Wed	6,065,051	242,515
	Thu	5,928,467	256,215
	Fri	5,624,905	237,873
	Sat	5,132,319	114,527
	Sun	5,221,398	105,766
Dec	Mon	6,019,810	225,177
	Tue	5,993,890	210,178
	Wed	5,845,066	190,682
	Thu	5,743,360	198,269
	Fri	5,070,626	181,962

	Sat	4,349,397	75,931
	Sun	4,226,058	55,365
	Mon	6,036,375	243,782
	Tue	6,010,379	248,313
	Wed	5,961,489	226,156
Jan	Thu	6,052,731	236,367
	Fri	5,758,942	192,831
	Sat	5,267,119	109,662
	Sun	5,397,830	105,985
	Mon	6,391,610	232,124
	Tue	6,211,325	286,484
	Wed	6,352,415	241,714
Feb	Thu	6,052,006	298,174
	Fri	5,819,215	270,251
	Sat	5,351,702	184,081
	Sun	5,518,886	166,734
	Mon	6,330,403	249,771
	Tue	6,456,077	277,580
	Wed	6,361,471	212,558
Mar	Thu	6,330,250	235,590
	Fri	5,920,964	171,663
	Sat	5,457,231	74,934
	Sun	5,471,597	73,968
	Mon	6,309,493	213,524
	Tue	6,515,371	220,865
	Wed	6,299,732	215,553
Apr	Thu	6,302,010	204,520
	Fri	6,065,704	144,171
	Sat	6,011,489	82,040
	Sun	5,860,513	82,589
	Mon	6,243,979	149,102
	Tue	6,154,980	157,589
	Wed	5,948,889	125,754
May	Thu	6,132,745	142,308
	Fri	5,854,486	85,647
	Sat	5,303,718	14,008
	Sun	4,778,672	12,310

Table 17 - Estimated campus population from biomarkers Fall 2017- Spring 2018.

Month	Day	ENT	5-HIAA	Water (50 GPD)	Avg.
	Mon	15,072	7,536	33,196	18,601
	Tue	15,560	7,780	32,123	18,488
	Wed	17,594	8,797	32,983	19,791
Aug	Thu	17,957	8,979	33,774	20,237
	Fri	14,498	7,249	32,448	18,065
	Sat	10,266	5,133	30,179	15,193
	Sun	9,394	4,697	31,021	15,037
	Mon	27,024	13,512	27,896	22,811
	Tue	38,149	19,074	28,051	28,425
	Wed	41,705	20,853	42,190	34,916
Sept	Thu	39,320	19,660	39,699	32,893
	Fri	35,471	17,735	37,141	30,116
	Sat	19,267	9,634	33,398	20,766
	Sun	22,035	11,017	33,905	22,319
	Mon	33,295	16,262	34,416	27,991
	Tue	30,027	16,975	32,824	26,609
	Wed	20,023	12,785	33,320	22,043
Oct	Thu	17,981	10,658	32,671	20,437
	Fri	14,616	7,373	30,971	17,653
	Sat	15,037	9,204	27,717	17,320
	Sun	12,848	7,453	28,141	16,148
	Mon	34,519	14,496	34,416	27,810
	Tue	30,219	14,522	32,824	25,855
	Wed	50,147	26,905	33,320	36,791
Nov	Thu	38,835	15,078	32,671	28,861
	Fri	34,264	11,365	30,971	25,533
	Sat	22,804	8,645	27,717	19,722
	Sun	20,900	8,461	28,141	19,167
	Mon	62,525	24,059	32,990	39,858
	Tue	48,458	23,029	32,774	34,754
	Wed	29,013	19,436	31,885	26,778
Dec	Thu	31,510	18,890	31,387	27,262
	Fri	21,676	14,764	27,747	21,396
	Sat	14,817	11,404	23,377	16,533
	Sun	12,871	10,674	22,617	15,387

	Mon	45,513	15,171	33,176	31,287
	Tue	41,082	13,694	33,062	29,279
	Wed	53,700	17,900	32,687	34,762
Jan	Thu	52,674	17,558	33,223	34,485
	Fri	47,797	15,932	31,441	31,723
	Sat	31,491	10,497	28,403	23,464
	Sun	34,429	11,476	29,075	24,993
	Mon	55,388	17,560	34,991	35,980
	Tue	145,723	26,721	34,325	68,923
	Wed	66,113	19,610	34,834	40,186
Feb	Thu	65,410	17,703	33,546	38,886
	Fri	53,641	12,638	32,168	32,816
	Sat	43,480	6,747	29,243	26,490
	Sun	39,228	5,040	30,035	24,768
	Mon	50,959	11,870	34,761	32,530
	Tue	40,119	14,773	35,571	30,154
	Wed	34,986	11,610	34,728	27,108
Mar	Thu	25,561	12,377	34,685	24,208
	Fri	33,998	9,041	32,185	25,075
	Sat	38,043	14,288	29,224	27,185
	Sun	25,421	8,144	29,295	20,953
	Mon	45,202	37,033	34,761	38,999
	Tue	39,898	35,593	35,571	37,021
	Wed	36,278	31,141	34,728	34,049
Apr	Thu	30,830	31,584	34,685	32,366
	Fri	1,941	1,215	32,185	11,780
	Sat	18,136	22,576	29,224	23,312
	Sun	16,957	21,984	29,295	22,746
	Mon	37,637	19,752	33,772	30,387
	Tue	40,470	20,416	33,347	31,411
	Wed	21,102	12,873	32,090	22,022
May	Thu	14,438	11,204	33,149	19,597
	Fri	13,336	8,027	31,379	17,581
	Sat	9,400	6,155	28,092	14,549
	Sun	8,601	5,089	25,309	13,000

Notes: GPD = gallons per day

Table 18 – Published survey data of alcohol, caffeine, and nicotine consumption estimates in college students.

	Per Capita Consumption (SD)	Citation
Alcohol ($g\ d^{-1}$ per capita)	11.3 (7.5)	This Study
	10.5 (8.1)	Hoeppner et al., 2009
	9.0 (10.9)	Tremblay et al., 2009
	10.9 (29.9)	Patrick & Lee, 2009
Caffeine ($mg\ d^{-1}$ per capita)	114 (49)	This Study
	159	Mahoney et al., 2018
	196	Norton et al., 2011
Nicotine ($mg\ d^{-1}$ per capita)	0.63 (0.22)	This Study
	1361, 944, 618, 859	Seo et al., 2011
	853.5 (486)	Caldeira et al., 2012
	927 (243)	Caldeira et al., 2012

Notes: SD = Standard deviation

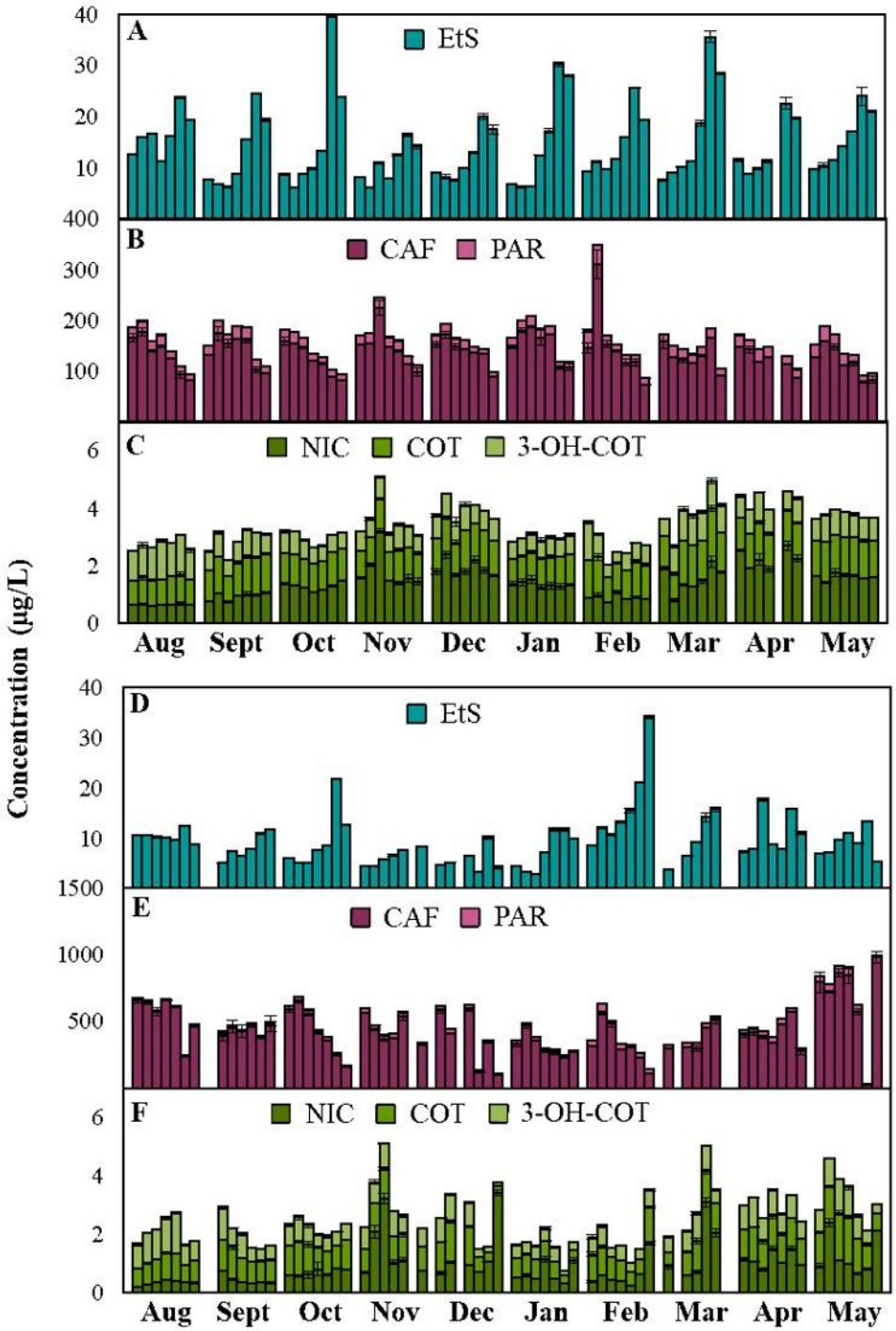


Fig. 25. Concentrations (ug L^{-1}) of EtS, caffeine (CAF), paraxanthine (PAR), nicotine (NIC), cotinine (COT), *trans*-3'-hydroxycotinine (3-OH-COT) in wastewater at the north (A-C) and south (D-F) sample locations, on a university campus.

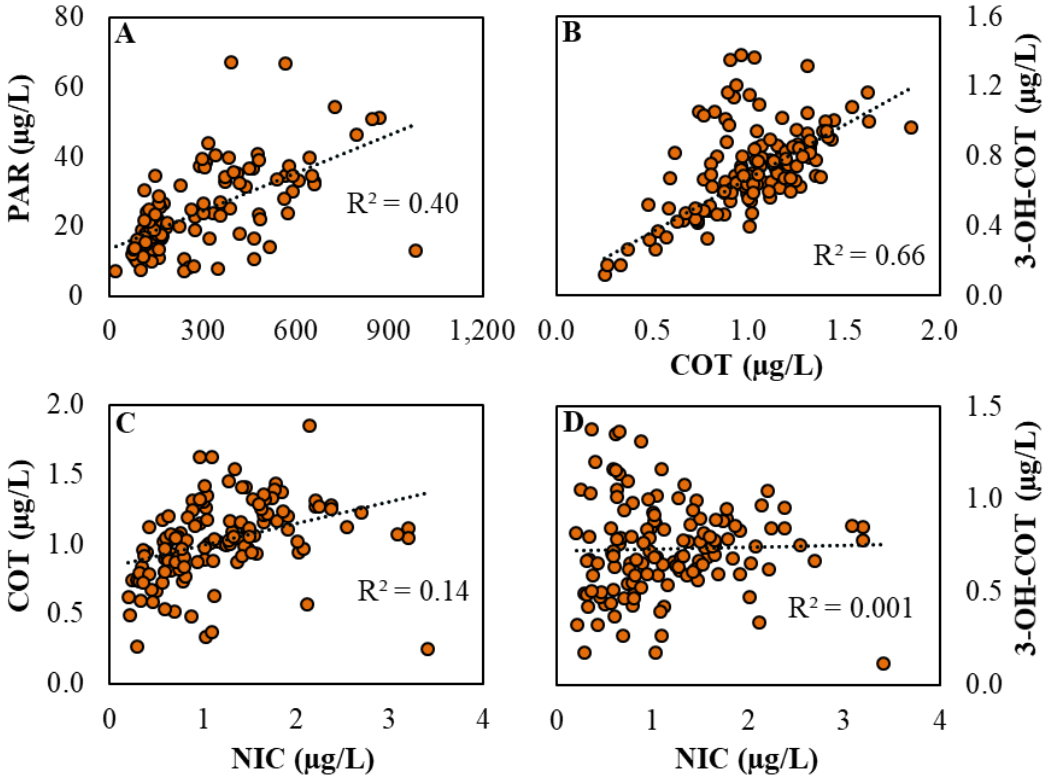


Fig. 26. Correlation plot of caffeine (CAF), paraxanthine (PAR) (A), *trans*-3'-hydroxycotinine (3-OH-COT), cotinine (COT) (B), nicotine (NIC), COT (C) and NIC, 3-OH-COT (D) in wastewater on a university campus.

APPENDIX D

SUPPLEMENTAL MATERIAL FOR CHAPTER 6

Table 19 – List of narcotic labeled standards.

Narcotic	Labeled Standard
Morphine	morphine-d_6
Morphine-3-Glucuronide	morphine-3-glucuronide-d_3
Oxycodone	oxycodone-d_3
Noroxycodone	noroxycodone-d_3
Codeine	codeine-d_6
Norcodeine	norcodeine-d_3
Heroin	heroin-d_9
6-Acetylmorphine	6-acetylmorphine-d_3
Fentanyl	fentanyl-d_5
Norfentanyl	nofentanyl-d_5
Methadone	methadone-d_3
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine-d_3	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine-d_3
Buprenorphine	buprenorphine-d_4
Norbuprenorphine	norbuprenorphine-d_3
3,4-methylenedioxy-methamphetamine	3,4-methylenedioxy-methamphetamine-d_5
Cocaine	cocaine-d_3
Benzoylecgonine	benzoylecgonine-d_8
Amphetamine	amphetamine-d_6
Methylphenidate	methylphenidate-d_9
Alprazolam	alprazolam-d_5
α-hydroxyalprazolam	α-hydroxyalprazolam-d_5

Table 20 – Licit and illicit compound method detection limits and recoveries.

Analyte	Method Detection Limit (ng/L)	Average Recovery (%)
Ethyl Sulfate	73	104
Caffeine	12	89
Paraxanthine	24	86
Nicotine	21	91
Cotinine	2	102
trans-3'-hydroxycotinine	6	84
Morphine	0.9	91
Morphine-3-Glucuronide	0.2	121
Codeine	1.4	99
Norcodeine	0.8	128
Oxycodone	0.2	104
Noroxycodone	0.3	110
Heroin	0.3	139
6-Acetylmorphine	0.3	116
Fentanyl	0.3	124
Norfentanyl	0.2	112
Methadone	1.4	125
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	1.7	109
Buprenorphine	140	99
Norbuprenorphine	120	138
Methamphetamine	71	88
3,4-Methylenedioxy-methamphetmaine (MDMA)	0.5	126
Amphetamine	0.9	135
Methylphenidate	0.3	83
Cocaine	0.6	141
Benzoylecgonine	0.7	161
Alprazolam	0.5	98
alpha-hydroxyalprazolam	0.2	144

Table 21 – Licit and illicit compound excretion values.

Indicator	Excretion Rate (%)	Source
Morphine	10	[243]
Noroxycodone	22.1	[244]
Codeine	30	[245]
6-Acetylmorphine	1.3	[246]
Norfentanyl	91.08	[247]
EDDP	27.5	[245]
Norbuprenorphine	15.3	[248]
Methamphetamine	43	[249]
Benzoylecgonine	39.1	[221]
MDMA	22.5	[250]
Amphetamine	36.3	[250]
Methylphenidate	1.5	[251]
alpha-hydroxyalprazolam	15	[252]

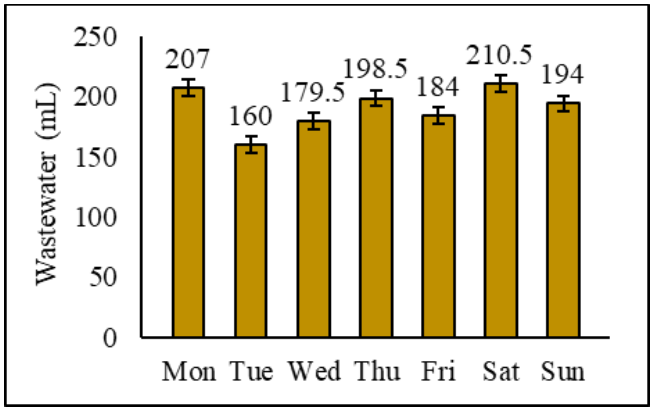


Fig. 27. Flow rates generated by *in situ* sampler. Error bars represent minimum/ maximum of duplicate samples.

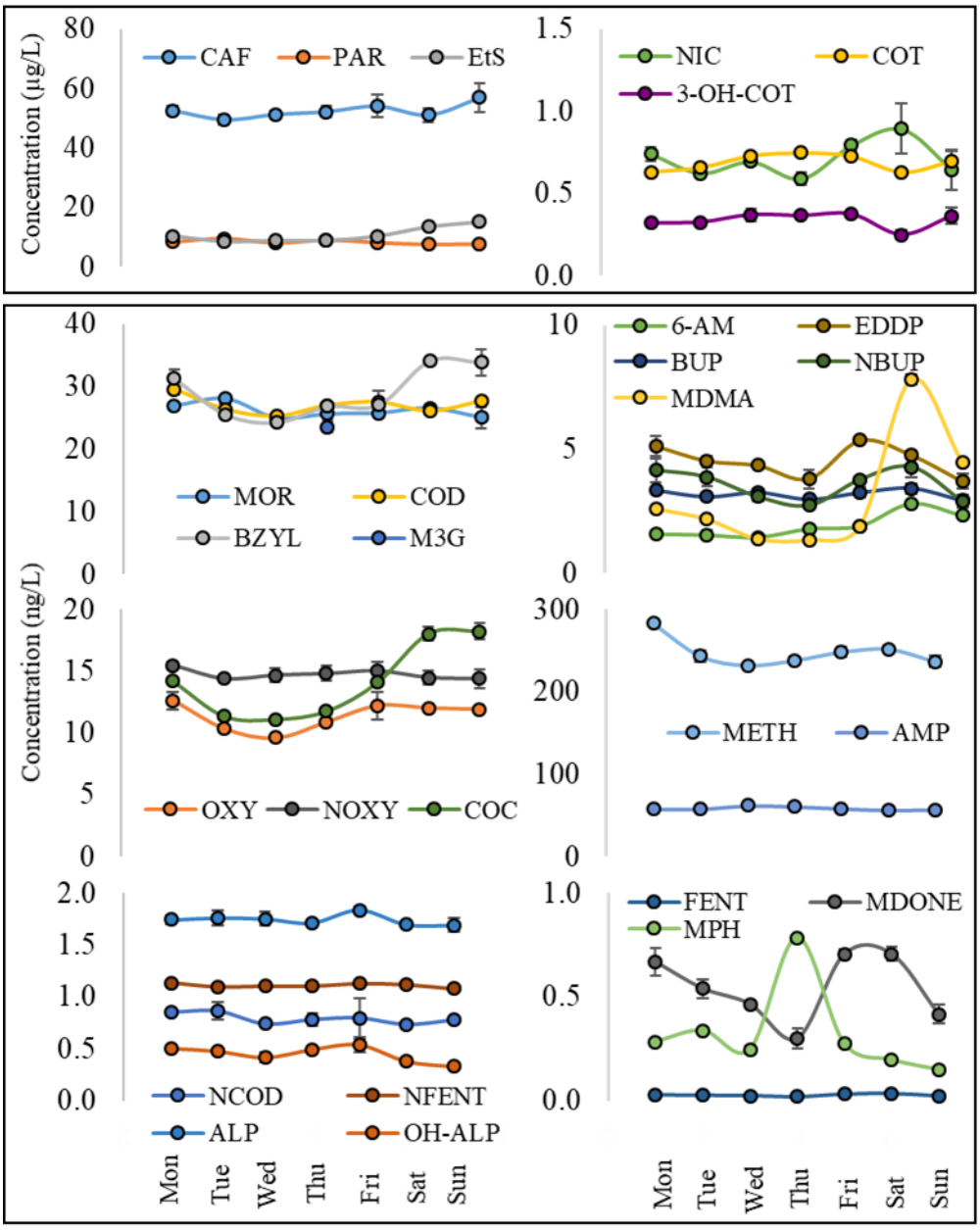


Fig. 28. Analyte concentrations measured by the discrete auto-sampler.

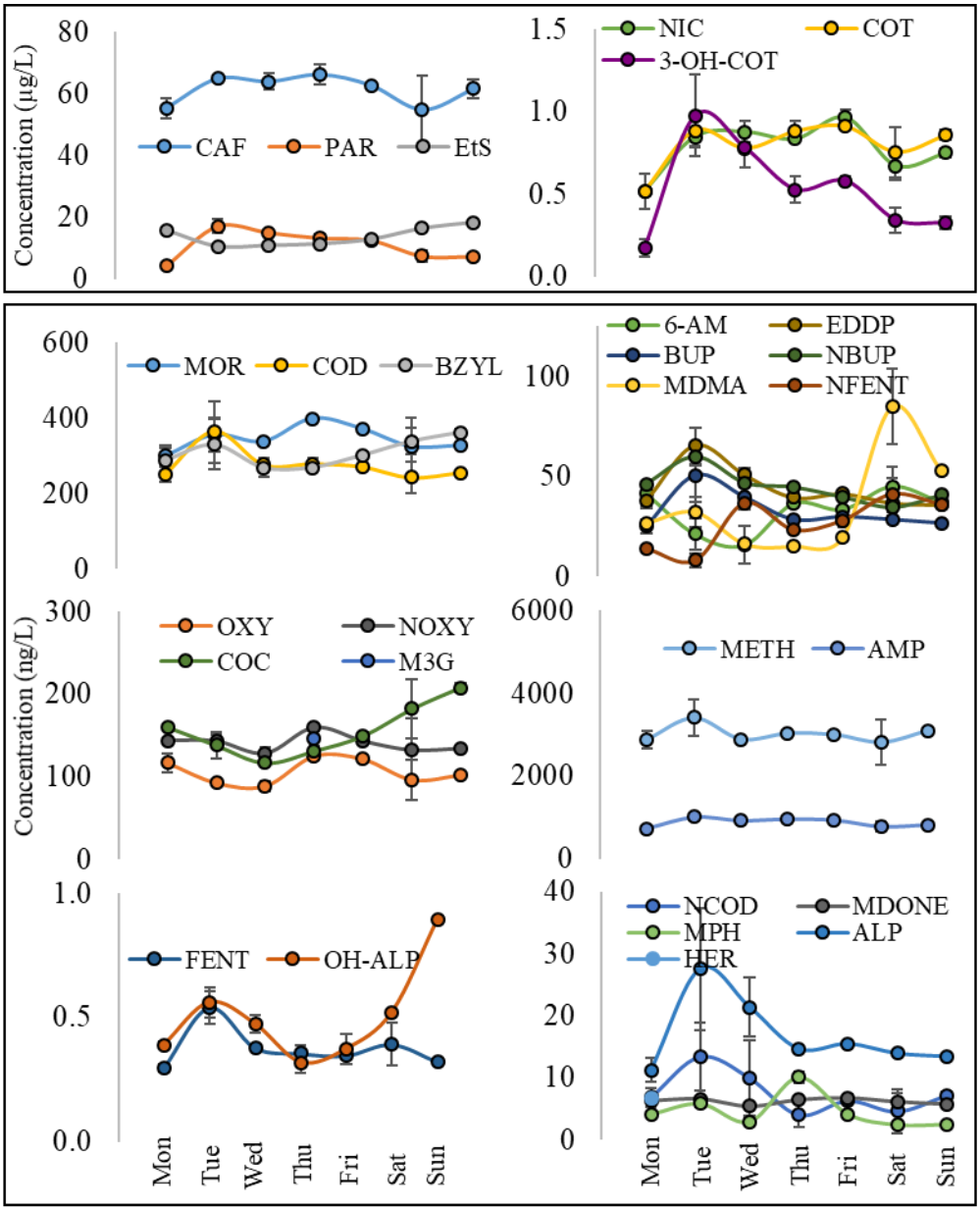


Fig. 29. Analyte concentrations measured by the continuous auto-sampler.