Mitigating Risks of Oil Sands Process Affected Water Using Hybrid Constructed Wetland Treatment Systems

Andrew David McQueen
Clemson University, andrew.d.mcqueen@gmail.com

Follow this and additional works at: https://tigerprints.clemson.edu/all_dissertations

Recommended Citation
https://tigerprints.clemson.edu/all_dissertations/1827
MITIGATING RISKS OF OIL SANDS PROCESS AFFECTED WATER USING HYBRID CONSTRUCTED WETLAND TREATMENT SYSTEMS

A Dissertation
Presented to
the Graduate School of
Forestry and Environmental Conservation
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Wildlife and Fisheries Biology

by
Andrew David McQueen
December, 2016

Accepted by:
Dr. John H. Rodgers Jr., Committee Chair
Dr. James W. Castle
Dr. Burton C. Suedel
Dr. George M. Huddleston
Dr. William C. Bridges
ABSTRACT

Mining leases in the Athabasca Oil Sands (AOS) region produce extensive volumes of oil sands process-affected water (OSPW) containing constituents that limit beneficial uses, including discharge into receiving aquatic systems. The aim of this research is to provide a scalable approach using hybrid constructed wetland treatment systems for the mitigation of problematic constituents in OSPW. In the first experiment in this dissertation, OSPW was characterized to identify constituents of concern (COCs) using chemical, physical, and toxicological analyses. Following identification of COCs, bench-scale manipulations (termed process-based manipulations [PBMs]) were used to remove or alter “classes” of COCs in an effort to eliminate toxicity to a sentinel aquatic invertebrate and discern treatment processes. COCs identified in OSPW included organics (naphthenic acids [NAs], oil and grease [O/G]), metals/metalloids, and suspended solids. Results from PBMs indicated that the organic fraction of OSPW was the primary source of toxicity, with oxidation (i.e. $\text{H}_2\text{O}_2 + \text{UV}_{254}$) and granular activated charcoal treatments eliminating toxicity to Ceriodaphnia dubia (7-8 d), in terms of mortality and reproduction. In the second experiment, photocatalytic degradation of commercial (Fluka) NAs was evaluated using fixed-film titanium dioxide (TiO$_2$) irradiated with sunlight for 8 hours. Changes in NA concentrations by photocatalytic degradation were confirmed analytically and with toxicity tests using sentinel fish and aquatic invertebrate species. The half-life for Fluka NAs achieved by photocatalytic degradation was approximately 2 hours, with toxicity eliminated for vertebrate and invertebrate sentinel organisms (Pimephales promelas and Daphnia magna) by the 5th
hour of the sunlight exposure. In the third experiment, toxicity of the NA fraction to microbial populations was evaluated to discern adverse impacts to microbially driven processes within wetlands. Following exposures to a commercial NA, potential effects on sulfate-reducing bacteria (SRB), production of sulfides (as acid-volatile sulfides [AVS]), and precipitation of divalent metals (i.e. Cu, Ni, Zn [as aqueous and simultaneously extracted metals; SEM]) were evaluated. Extent of AVS production was sufficient in all NA exposure concentrations tested to achieve ∑SEM:AVS <1, indicating conditions were conducive for treatment of divalent metals. In addition, no adverse effects to SRB (in terms of density, relative abundance, and diversity) were observed. The lines of evidence indicated that dissimilatory sulfate reduction and subsequent metal precipitation in wetlands will not be vulnerable to NA exposures. In the final experiment, a hybrid pilot-scale CWTS was designed to promote treatment processes to alter (transfer and transform) COCs using sequential reducing and oxidizing wetland reactors and a solar photocatalytic reactor using fixed film titanium dioxide (TiO₂). Performance criteria were achieved as the CWTS decreased concentrations of NAs, O/G, suspended solids, and metals to an extent that eliminated toxicity to the aquatic invertebrate C. dubia. Results from this study provide proof-of-concept data to inform hybrid passive or semi-passive treatment approaches (i.e. constructed wetlands) that could mitigate COCs contained in OSPWs. Data presented in this dissertation provide approaches to identify problematic constituents contained in complex energy derived waters (e.g. OSPW) and strategies for mitigating risks by altering exposures using passive (low-energy) treatment systems.
DEDICATION

I dedicate this dissertation to my wife Cindy, who continuously encourages me and to my sons Ethan and Samuel for helping me keep life in perspective.
ACKNOWLEDGMENTS

I would like to thank my major advisor Dr. John H. Rodgers Jr. for his devotion to students and for providing me the tools needed to achieve my goals. I would also like to thank my all my committee members, Dr. James W. Castle, Dr. Burton C. Suedel, Dr. George M. Huddleston, and Dr. William C. Bridges for their support, wisdom, technical reviews, and research guidance. I would also like to thank my fellow graduate students who have provided an exceptional research team; Alyssa Calomeni, Ciera Kinley, Kyla Iwinski, Maas Hendrikse, Tyler Geer, Daniel Gaspari, and Kayla Wardlaw. I also am grateful to the sponsors of this research for providing funding support. Finally, I thank Dr. Wayne Chao for providing analytical support and always a smiling face. I offer my extreme gratitude to all who were apart of this process.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>....................................................................................................................</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>.....................................................................................................................</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>..................................................................................................................</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>........................................................................................................</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>...............................................................................................................</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>...............................................................................................................</td>
<td>x</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>..............................................................................................................</td>
<td>1</td>
</tr>
<tr>
<td>II. A RISK-BASED APPROACH FOR IDENTIFYING CONSTITUENTS OF CONCERN IN OILS SANDS PROCESS AFFECTED WATER FROM THE ATHABASCA OIL SANDS MINING AREA</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>III. PHOTOCATALYSIS OF A COMMERCIAL NAPHTHENIC ACID IN WATER USING FIXED-FILM TIO₂</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>11</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>16</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>24</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>28</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>34</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>44</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>46</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>65</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>66</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>69</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>73</td>
</tr>
<tr>
<td>References</td>
<td>........................................................................................................</td>
<td>77</td>
</tr>
</tbody>
</table>
Table of Contents (Continued)

References................................................................................................. 79

IV. INFLUENCE OF COMMERCIAL (FLUKA) NAPHTHENIC ACIDS ON .
ACID VOLATILE SULFIDE PRODUCTION AND DIVALENT METAL PRECIPITATION ................. 93

Abstract................................................................................................. 94
Introduction.............................................................................................. 95
Materials and Methods........................................................................... 98
Results and Discussion .......................................................................... 104
Conclusion.............................................................................................. 111
References.............................................................................................. 113

V. PERFORMANCE OF HYBRID PILOT-SCALE CONSTRUCTED 
WETLAND SYSTEMS FOR TREATING OIL SANDS PROCESS ... AFFECTED WATER FROM THE ATHABASCA OIL SANDS AREA
............................................................................................................ 129

Abstract................................................................................................. 130
Introduction.............................................................................................. 131
Materials and Methods........................................................................... 135
Results and Discussion .......................................................................... 141
Conclusion.............................................................................................. 154
References.............................................................................................. 155
Supplemental Material............................................................................ 177

VI. CONCLUSIONS.................................................................................... 185

Objectives............................................................................................... 185
References.............................................................................................. 190

APPENDICES ............................................................................................ 177

A: Chapter V: Supplemental Material .................................................... 177
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Analytical methods for site-specific OSPW.</td>
</tr>
<tr>
<td>2.2</td>
<td>Manipulations and treatment objectives of process based manipulations (PBMs) performed on OSPW.</td>
</tr>
<tr>
<td>2.3</td>
<td>Comparison of constituents in OSPW to water quality guidelines (CCME 1999, USEPA 1999; ESRD 2014) and toxicity values for <em>C. dubia</em> (<em>Cd</em>), rainbow trout (<em>Oncorhynchus mykiss</em> [<em>Om</em>]), and fathead minnow (<em>P. promelas</em> [<em>Pp</em>]).</td>
</tr>
<tr>
<td>2.4</td>
<td>Comparison of inorganic constituents in OSPW to water quality guidelines (CCME 1999; USEPA 1999; ESRD 2014) and toxicity values for <em>C. dubia</em> (<em>Cd</em>), <em>D. magna</em> (<em>Dm</em>), fathead minnow (<em>P. promelas</em> [<em>Pp</em>]), and rainbow trout (<em>Oncorhynchus mykiss</em> [<em>Om</em>])</td>
</tr>
<tr>
<td>2.5</td>
<td>Bioassay data for OSPW using fish (<em>P. promelas</em>), aquatic invertebrates (<em>C. dubia</em> and <em>D. magna</em>), and a macrophyte (<em>T. latifolia</em>).</td>
</tr>
<tr>
<td>3.1</td>
<td>Methods for water characteristics, light, and NA concentrations.</td>
</tr>
<tr>
<td>3.2</td>
<td>Physical and chemical characteristics of Fluka naphthenic acids (Sigma-Aldrich)</td>
</tr>
<tr>
<td>3.3</td>
<td>Summary of toxicity test conditions for <em>P. promelas</em> and <em>D. magna</em> (USEPA, 2002)</td>
</tr>
<tr>
<td>3.4</td>
<td>Measurements of water characteristics during photocatalysis and photolysis treatments (and dark controls) and toxicity testing.</td>
</tr>
<tr>
<td>3.5</td>
<td>Fluka naphthenic acid removal rate coefficients and extents for photocatalysis, photolysis, and dark control treatments</td>
</tr>
<tr>
<td>3.6</td>
<td>Comparative rates and extents of removal for photocatalysis and photolysis of Fluka NA</td>
</tr>
<tr>
<td>3.7</td>
<td>Summary of mean NA concentrations (mg/L) and 96-hr percent survival for microinvertebrates (<em>D. magna</em>) and fish (<em>P. promelas</em>) for photocatalysis, photolysis, and dark control treatments. NA concentrations with different letters are significantly different (p&lt; 0.05)</td>
</tr>
</tbody>
</table>
List of Tables (Continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Analytical methods for experimental parameters</td>
</tr>
<tr>
<td>4.2</td>
<td>Chemical sources and nominal concentrations of constituents used for aqueous exposures</td>
</tr>
<tr>
<td>4.3</td>
<td>Water and sediment characteristics measured during treatment periods 3-d to 21-d (n=21)</td>
</tr>
<tr>
<td>5.1</td>
<td>Hybrid pilot-scale CWTS design features</td>
</tr>
<tr>
<td>5.2</td>
<td>Comparison of water quality characteristics and organic constituents in OSPW to water quality guidelines (USEPA 2007; CCME 2011, ESRD 2014) and toxicity values for C. dubia (Cd), rainbow trout (O. mykiss [Om]), and fathead minnow (P. promelas [Pp])</td>
</tr>
<tr>
<td>5.3</td>
<td>Comparison of metals and metalloids in OSPW to water quality guidelines (USEPA 2007; CCME 2011, ESRD 2014) and toxicity values for C. dubia (Cd), D. magna (Dm), fathead minnow (P. promelas [Pp]), and rainbow trout (Oncorhynchus mykiss [Om])</td>
</tr>
<tr>
<td>5.4</td>
<td>Targeted treatment processes, operational metrics, and measured ranges in hybrid CWTS designed for OSPW</td>
</tr>
<tr>
<td>5.5</td>
<td>Inflow concentrations, outflow concentrations, removal efficiencies, and removal rate coefficients of COCs for each CWTS replicate series</td>
</tr>
<tr>
<td>5.1.A</td>
<td>Treatment dates and conditions for hybrid pilot-scale CWTS</td>
</tr>
<tr>
<td>5.2.A</td>
<td>Mean water characteristics (n=5) measured in hybrid pilot-scale CWTS</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Athabasca oil sands (AOS) region located in Alberta, Canada</td>
<td>2</td>
</tr>
<tr>
<td>1.2</td>
<td>Conceptual model for context of experiments</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>Conceptual model of treatment pathways for naphthenic acids</td>
<td>6</td>
</tr>
<tr>
<td>2.1</td>
<td>Approach for identifying constituents of concern (COCs) in OSPW and informing potential treatment processes to mitigate ecological risk</td>
<td>61</td>
</tr>
<tr>
<td>2.2</td>
<td>Hereroatom classes present in OSPW using high-resolution Orbitrap MS</td>
<td>62</td>
</tr>
<tr>
<td>2.3</td>
<td>Responses of <em>Ceriodaphnia dubia</em>, in terms of survival (top) and reproduction (bottom), in 7-8 d static/renewal tests following process based manipulations (PBM)</td>
<td>63</td>
</tr>
<tr>
<td>3.1</td>
<td>Schematic of photocatalytic reaction chamber</td>
<td>91</td>
</tr>
<tr>
<td>3.2</td>
<td>Concentrations of Fluka naphthenic acids (mg/L) with time in photocatalysis, photolysis, and dark control treatments</td>
<td>92</td>
</tr>
<tr>
<td>4.1</td>
<td>Acid volatile sulfide (AVS) concentrations (µmol/g) measured during 21-day testing period (<em>n</em>=3). Error bars indicate standard deviations. Asterisks indicate AVS concentrations significantly different (<em>p</em>&lt;0.05; <em>α</em>=0.05) from untreated controls</td>
<td>123</td>
</tr>
<tr>
<td>4.2</td>
<td>Comparison of rate of acid volatile sulfide (AVS) production (µmol/g) in sulfate-reducing bacteria (SRB) biocide treatments, untreated control, and 80 mg/L naphthenic acid (NA) treatments (<em>n</em>=3) on days 3 through 15. Error bars indicate standard deviations</td>
<td>124</td>
</tr>
<tr>
<td>4.3</td>
<td>Simultaneously extracted metals (ΣSEM; Cu, Ni, Zn; µmol/g) and acid volatile sulfide (AVS) ratios among controls and highest NA treatment (80 mg NA/L) during 21-day testing period (<em>n</em>=3). Errors bars indicate standard deviations</td>
<td>125</td>
</tr>
<tr>
<td>4.4</td>
<td>Copper (top), nickel (middle), and zinc (bottom) removal extent (mg/L) measured during 21-day treatment durations</td>
<td>126</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 MPN of sulfate-reducing bacteria per gram of sample. Error bars indicate ... standard error of the average results of samples ( n = 3 )</td>
<td>127</td>
</tr>
<tr>
<td>4.6 Percentage of known SRB in the bacterial community based on genetic ....... sequencing. Names of organisms are either genus (g) or family (f) level classification. Relative abundance (%) for each sample is the average ... across three replicates.</td>
<td>128</td>
</tr>
<tr>
<td>5.1 A schematic diagram of the pilot-scale experiment</td>
<td>171</td>
</tr>
<tr>
<td>5.2 O/G concentrations in inflow and reactor outflows for Series A (solid line). .. and Series B (dashed line)</td>
<td>172</td>
</tr>
<tr>
<td>5.3 Total NA concentrations in wetland inflow and outflows for Series A (solid lines) and Series B (dashed lines), and inflow and outflow from ....... photocatalysis treatments</td>
<td>173</td>
</tr>
<tr>
<td>5.4 Inorganic COC (Al, B, Cu, Ni, Se, Zn, and TSS) concentrations in inflow and reactor outflows for Series A (solid line) and Series B (dashed line) for . treatment periods 1-5</td>
<td>174</td>
</tr>
<tr>
<td>5.5 Survival (a) and reproduction (b) of Ceriodaphnia dubia exposed to inflow . (untreated) and wetland outflow samples of OSPW treated by pilot-scale CWTS. (n=20 per treatment)</td>
<td>176</td>
</tr>
<tr>
<td>5.1.A Mean plant shoot density measured in reducing reactors (top graph; n=4) and oxidizing reactors (bottom graph; n=10) during CWTS maturation and... treatment periods for replicate wetland Series A and B</td>
<td>180</td>
</tr>
<tr>
<td>5.2.A Mean plant shoot height measured in reducing (n=6) and oxidizing (n=36) .. reactors during CWTS maturation and treatment periods</td>
<td>181</td>
</tr>
<tr>
<td>5.3.A Mean oxidation-reduction potential (ORP; mV) measured in reducing (n=4) and oxidizing reactors (n=10) during CWTS maturation and treatment .. periods</td>
<td>181</td>
</tr>
<tr>
<td>5.4.A Hereroatom classes present in untreated (inflow) OSPW using high-resolution Orbitrap MS</td>
<td>182</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5.A</td>
<td>UV/visible transmittance (250 to 700 nm) at 1.0 cm water depth of untreated (inflow) OSPW and post-wetland treatment outflow...</td>
<td>183</td>
</tr>
<tr>
<td>5.6.A</td>
<td>Light extinction coefficient (Kd cm(^{-1})) of untreated OSPW</td>
<td>184</td>
</tr>
</tbody>
</table>
CHAPTER ONE

INTRODUCTION

1.1 Background and Approach

Oil sands are comprised of sands and silts permeated with a highly biodegraded and viscous form of hydrocarbon known as bitumen (Carrigy, 1963). Due to the continued global demand for oil, “unconventional” sources of petroleum (e.g. oil sands) have developed, with many sources outpacing conventional oil recovery. The Athabasca Oil Sands (AOS) deposits in Alberta, Canada are the third largest proven oil reserve in the world, with ~168 billion barrels recoverable and an estimated oil production rate of 1.9 million barrels/day (Alberta Energy, 2014). Current bitumen extraction processes include open pit mining and in situ recovery (i.e. steam assisted gravity drainage and cyclic steam stimulation; Schramm, 2000). Due to the close surface proximity of bitumen deposits in the AOS region, open pit mining represents the majority (approximately 51%) of extraction (Alberta Energy, 2014). Surface mining in the AOS requires the removal of overburden (i.e. vegetation and sediments), which disturbs the naturally occurring landscape. The AOS landscape is predominantly wetlands (>50%), with approximately 90% of those wetlands existing as peatlands (Vitt et al., 1996; Raab and Bailey, 2012). In addition to peat-dominated wetlands, areas mined to date consist of boreal forests, bogs, and fens (Bayley and Mewhort, 2004). Legislation requires operators to reclaim the mined landscape to “equivalent land capabilities” that existed pre-disturbances (CEMA, 2014; Province of Alberta, 2014).
Spatial extent of the Athabasca deposits encompasses approximately 140,200 km², with 715 km² disturbed to date (Alberta Energy, 2014; Figure 1.1). Bitumen is commonly extracted from the oil sands by an alkaline warm water assisted extraction process, using water (50–80°C) and a conditioning agent (NaOH; Clark process; Schramm, 2000). Approximately 1.6 barrels of fresh water are required for every barrel of petroleum produced from surface mining operations (Shell Canada Ltd., 2016). The resulting water is referred to as oil sands process-affected water (OSPW).

Figure 1.1 Athabasca oil sands (AOS) region located in Alberta, Canada. (Source: Hein et al., 2013)
OSPW can contain complex mixtures of residual bitumen (petroleum hydrocarbons), suspended solids, organic acids, metals, metalloids, and salts (Mackinnon and Boerger, 1986; Allen, 2008a; Mahaffey and Dube, 2016). Ecological risks, in terms of toxicity to aquatic biota, have been attributed to the organic acid fraction of OSPW, referred to as naphthenic acids (NAs; e.g., Verbeek et al., 1994; Nero et al., 2006; Frank et al., 2008; Armstrong et al., 2008; Kavanagh et al., 2012; Leclair et al., 2013; Marentette et al., 2015). NAs are a natural component of bitumen and are transferred into the process-affected water during extraction. Warm water extraction with sodium hydroxide promotes retention of NA in the water column due to the alkaline pH (Rogers et al., 2002; Headley and McMartin, 2004). NAs present in OSPW are compositionally complex, comprised of over 3,000 individual acidic compounds (Ross et al., 2012), including carboxylic acids fitting classical definitions of NAs (C\(_n\)H\(_{2n+2}\)O\(_2\)) in addition to dibasic, heteroatomic, aromatic, and diamondoid adamantane acids (Headley et al., 2011, Rowland et al., 2011). Although the mechanisms of NA toxicity are not well known, it is thought that general narcosis, membrane disruption, and osmotic stress are possible factors in manifestation of adverse effects (Schramm, 2000). Recent research highlights that composition of NAs (and not concentration alone) is critical to understanding and predicting adverse effects (Morandi et al., 2015; Mahaffey and Dubé, 2016). Potency of NAs is typically greater in more readily biodegradable and lower-molecular weight NA fractions (i.e. <180 dalton range; Holowenko et al., 2002, Lo et al., 2006). OSPW sourced from tailings in the AOS typically contains a greater fraction of “weathered” NAs (higher molecular weight NAs [typically in the 180-300 dalton range]; Headley et al., 2007). In
addition to NAs, there are a number of other constituents in OSPW that may require treatment, including trace metals/metalloids (e.g., Al, As, Cr, Cu, Fe, Pb, Ni, Se, Zn), unrecovered bitumen (oil and grease), major anions (chloride), and suspended solids (Allen, 2008a; Mahaffey and Dubé, 2016).

To date, there has been a zero-discharge policy of OSPW in the AOS region (Province of Alberta, 2014). However, to achieve reclamation goals for mining leases in the AOS, OSPW will need to be returned to the environment (Mahaffey and Dubé, 2016). Due to the volume of OSPW currently stored in the AOS (~1 billion m³; Mahaffey and Dubé 2016), it is clear that economically viable mitigation approaches are required, offering passive (low-energy input) solutions. Constructed wetland treatment systems (CWTS) are plausible treatment options for renovating OSPW to achieve water return goals. However, for CWTS to be successful, a thorough design basis will need to be developed based on: 1) accurate characterization of problematic constituents contained in OSPW that impede water return goals, 2) identification and evaluation of treatment processes to mitigate risks of OSPW, and 3) evaluation of treatment performance using model CWTS (Figure 1.2).
1.1.1 OSPW Characterization

The composition of OSPW stored on the AOS landscape can vary spatially and temporally (Frank et al., 2016); therefore, to properly assess ecological risks, accurate characterization of constituents in OSPW is needed. This research focused on OSPW procured from Shell’s Muskeg River Mine External Tailings Facility (MRM-ETF). MRM-ETF OSPW is produced from a surface mining operation in the AOS region (near Fort McMurray, AB, Canada) and stored in a “fresh” tailings pond receiving ~94 million m³ of total fluid fine tailings annually (Shell Canada Ltd., 2016).
1.1.2 Naphthenic Acid Treatment Pathways

Due to the contribution of toxicity from NAs contained in OSPW (Verbeek et al., 1994, Morandi et al., 2015), a focus of this research is experimentally testing treatment processes for mitigating NAs as well as potential vulnerabilities of microbially mediated processes within wetland treatment systems to NA exposures. There are a number of NA treatment pathways that can be targeted or enhanced in wetland systems, including hydrolysis, oxidation, photolysis, volatilization, sorption, settling, and microbial degradation (Figure 1.2).

**Figure 1.3** Conceptual model of treatment pathways for naphthenic acids.

Note: “oxidation” includes advanced oxidation processes (e.g. photocatalysis).
Photocatalysis is a type of advanced oxidation that has shown promising results as a treatment option for degradation of NAs (Headley et al., 2009; Mishra et al., 2010; Leshuk et al., 2016). Photocatalytic oxidation occurs when a catalyst absorbs photons, and the corresponding energy increase causes electrons (i.e. energy) to be transferred. In the case of metal oxides such as titanium dioxide (TiO$_2$), the combination of excitation energy and surface moisture produces hydroxyl radicals (Linsebigler, 1995). Photocatalytic degradation has been used successfully in treating problematic organic constituents in the petroleum industry, offering an innovative alternative to conventional physical, chemical, and biological treatment strategies (Sain and Shahrezaei, 2011). To date, laboratory-scale photocatalytic degradation of both commercial and OSPW NA mixtures has been successful. Reported degradation rates for OSPW NA mixtures using photocatalytic treatment are orders of magnitude greater than conventional biodegradation techniques, with NA half-lives ranging between 1.55 and 4.50 h (Headley et al., 2009; Mishra et al., 2010). To date, this technology has been evaluated by adding the catalyst as a “slurry” (i.e. TiO$_2$ nanoparticles), potentially prohibiting larger scale application due to the cost of adding and recovering the catalyst. Part of this research focused on fixing catalyst to a film (i.e. fixed-film), coupled with natural solar irradiance, providing a passive treatment approach for NAs that can be translated to larger scale applications.

Many transfer and transformation processes that occur in wetlands are mediated by microbial activity (Lovley, 1997; Kadlec and Wallace, 2009). Microbial activity can include dissimilatory sulfate reduction, nitrification/denitrification, and biodegradation of
organic matter (Newman et al., 1997; Kadlec and Wallace, 2009). Microbial degradation pathways reported in the literature indicate some NAs fractions are biodegradable (MacKinnon and Boerger, 1986; Scott et al., 2005; Del Rio et al., 2006); however, higher molecular weight NAs (180-300 dalton range) are more recalcitrant (Quagraine et al., 2007). NA biodegradation rates are more favorable in aerobic than in anaerobic conditions (Del Rio et al., 2006). Under aerobic conditions, monocyclic NA concentrations were decreased by 30% in 14 days (Del Rio et al., 2006), with anaerobic degradation half-lives of years (Quagraine et al., 2007). Residual consumable organic matter (presumably residual hydrocarbons) in tailing ponds results in anaerobic conditions in portions of the water column, promoting conditions that minimize or eliminate degradation of NAs (Whitby, 2010). Half-lives for OSPW NAs in tailings ponds range from 12.8-13.6 years (Han et al., 2009). At this rate, it would take decades to decrease NA concentrations to non-toxic levels through in situ degradation.

In complex waters like OSPW, it is critical to understand the potential inhibitory effects that fractions (e.g. metals, salts, organics) may pose to treatment processes mediated by microbial activity. Due to potential toxicity of the organic acid fraction (NAs) to microbial assemblages (Microtox EC50 values ranging from 12 to 65 mg/L NAs; Rogers et al., 2002; Frank et al., 2008), efficiencies of targeted microbial treatment pathways may be minimized or eliminated. To date, limited data are available regarding effects of NAs on sulfate reducing bacteria (e.g. Desulfovibrio, Desulfotomaculum, and Desulfomonas). In order to design efficient and effective wetland treatment systems, it is
necessary to determine the effects of potentially toxic organic acid fractions of the water on biogeochemical pathways supporting treatment of constituents of concern.

1.1.3 Constructed Wetland Treatment Systems

To reclaim impacted waters, wet-landscape mitigation approaches (e.g. constructed wetland treatment systems) are among technologies considered for treatment of problematic constituents (e.g. metals, organics; Allen, 2008b; Foote 2012; Toor et al., 2013; Brown and Ulrich, 2015). Although CWTS have successfully treated many constituents in petroleum related waters (Kadlec and Wallace, 2009), a robust system is likely needed to treat the variety of COCs that may be found in OSPW with specific respect to mitigating risks to downstream aquatic biota. To date, information regarding the viability of CWTS for treatment of OSPW in the AOS has been limited (Quagraine et al., 2005; Allen, 2008b; Brown and Ulrich, 2015). Bench-scale constructed wetlands have been tested using OSPW with limited degradation of the total NA fraction (Toor et al., 2013); however, these systems were not explicitly designed using a focused process based approach. Assuming that NAs are a rate limiting constituent in OSPW, hybrid CWTS offer the flexibility to add or enhance pathways (e.g. photocatalysis) that may otherwise be a rate limiting step in a “traditional” design. To test the viability of hybrid CWTS, pilot-scale systems can be used to assess potential wetland performance for COC and toxicity reduction as they are easily manipulated to provide proof-of-concept data (Rodgers and Castle, 2008; Kadlec and Wallace, 2009). Constituent removal rates and percentages can be estimated from pilot-scale studies to decrease uncertainties and confirm design features for future, field-scale CWTS (Huddleston et al., 2000; Murray-
Gulde et al., 2005; Johnson et al., 2008; Huddleston and Rodgers 2008; Kadlec and Wallace, 2009). Carefully designed pilot-scale CWTS offer testable models that can be used to: 1) measure rates and extents of removal 2) measure bioavailability of COCs 3) measure effects of operational or environmental changes to performance 4) provide repetition and allowing greater confidence for meeting performance criteria, and 5) provide data for scaling.

To be sustainable and achieve performance goals (i.e. water return to receiving systems), treatment wetlands must be carefully designed and constructed with explicit consideration of OSPW composition and other materials such as hydrosoil and vegetation that are used to build them (Rodgers and Castle, 2008). Performance of these pilot-scale systems will be monitored not only analytically, but also using sentinel organisms. To accurately understand the rate and extent of treatment (i.e. removal of elements or compounds), changes in exposure of an element or compound can be monitored using aquatic organisms (e.g. aquatic invertebrates \textit{Ceriodaphnia dubia}). In the case of organic acids in OSPW, due to limitations in quantifying the more than 3000 acidic compounds that co-occur with NAs (Ross et al., 2012), it is not sufficient to rely on analytical summation of NAs alone to confirm successful mitigation. Therefore, use of sensitive sentinel organisms will be necessary endpoints for demonstration of treatment performance.
The rationale of this research is to provide a scalable approach using hybrid constructed wetland treatment systems for the mitigation of constituents in OSPW. This research has four main objectives:

1. Characterize the constituents of concern in oil sands process-affected water (OSPW) and discern potential treatment pathways in constructed wetland treatment systems (CWTS) (Chapter 2),

2. Determine the photocatalytic degradation rates and extents for a commercial naphthenic acid using fixed film TiO$_2$ (Chapter 3),

3. Measure the responses of sulfate reducing bacteria assemblages, acid volatile sulfide, simultaneously extracted and aqueous metal concentrations following exposures to a commercial naphthenic acid (Chapter 4), and

4. Measure the performance of a hybrid pilot-scale constructed wetland treatment systems using OSPW sourced from the Athabasca oil sands (Chapter 5)

1.2 Organization of Dissertation

This dissertation consists of six chapters: an Introduction (Chapter One), four independent manuscripts (Chapters Two, Three, Four, and Five), and Summary and Conclusions (Chapter Six). Chapters Three and Four have been accepted for publication in *Water, Air, and Soil Pollution* and *Ecotoxicology and Environmental Safety*, respectively. The manuscripts and their respective target journals are as follows:

- **Chapter 2:** A Risk-based Approach for Identifying Constituents of Concern in Oil Sands Process-affected Water from the Athabasca Oil Sands – *Chemosphere*
Collectively, these manuscripts provide information on problematic constituents contained in OSPW and treatment techniques for mitigating risks associated with those respective COCs using hybrid constructed wetland treatment systems. Brief overviews of the overall scope and objectives of each body manuscript (Chapters 2, 3, 4, and 5) are outlined below.

**Chapter 2: A Risk-based Approach for Identifying Constituents of Concern in Oil Sands Process-affected Water from the Athabasca Oil Sands Region**

The aim of this experiment was to identify COCs in OSPW sourced from an active settling basin with the goal of providing a sound rational for developing mitigation strategies using CWTS. COCs were identified through several lines of evidence with the following specific objectives:
1) compare concentrations of chemical and physical constituents in OSPW with numeric water quality guidelines (i.e. Alberta WQGs, CEQGs, and USEPA WQC) and toxicity threshold values for fish (*Pimephales promelas* and *Oncorhynchus mykiss*) and freshwater invertebrates (*Ceriodaphnia dubia* and *Daphnia magna*),

2) measure toxicity in OSPW using fish (*P. promelas*), aquatic invertebrates (*C. dubia* and *D. magna*), and seedlings from a rooted macrophyte (*Typha latifolia*),

3) conduct process-based manipulations (PBM) on OSPW to alter toxicity, in terms of mortality and reproduction, to the aquatic invertebrate *C. dubia*, and

4) discern potential treatment pathways to mitigate ecological risks of OSPW based on identification of COCs, toxicological analyses, and PBM results.

**Chapter 3: Photocatalysis of a Commercial Naphthenic Acid in Water using Fixed-film Titanium Dioxide (TiO₂)**

The overall objective of this study was to measure rates and extents of photolysis and photocatalytic degradation of a commercially available (Fluka) NA using bench-scale fixed-film TiO₂, and confirm changes in NA concentrations using sensitive vertebrate (fish = *Pimephales promelas*) and invertebrate (*Daphnia magna*) species. To achieve this overall objective, specific objectives were to:

1) measure the rates and extents of removal of commercial (Fluka) NAs throughout an 8 hour duration of natural sunlight (“photolysis”) and natural sunlight in the presence of fixed-film TiO₂ (“photocatalysis”), and
2) measure changes in toxicity after photolysis and photocatalysis treatments (in terms of mortality) with sentinel fish (*P. promelas*) and microinvertebrate (*D. magna*) species in 96-hr static tests.

**Chapter 4: Influence of Commercial (Fluka) Naphthenic Acids on Acid Volatile Sulfide (AVS) Production and Divalent Metal Precipitation**

The overall objective of this experiment was to measure responses of sulfate-reducing bacterial assemblages and microbially mediated treatment pathways (e.g. acid volatile sulfide concentrations) following a series of exposures to a commercial (Fluka) NA in bench-scale reactors. To achieve this overall objective, specific objectives were to:

1) measure relationships of acid-volatile sulfide (AVS), simultaneously extractable metal (SEM), and aqueous metal (copper, nickel, and zinc) concentrations following 21-d exposures to NAs, and

2) measure responses of sulfate-reducing bacterial (SRB) assemblages in terms of relative abundance, diversity, and density (most-probable number) in sediment to 21-d exposures of NAs.

**Chapter 5: Performance of a Hybrid Pilot-scale Constructed Wetland System for Treating Oil Sands Process-affected Water from the Athabasca Oil Sands**

The overall aim of this study was to evaluate the performance of a specifically designed hybrid pilot-scale CWTS for treating OSPW. In order to achieve this overall objective, specific objectives were to:
1) characterize OSPW in terms of chemical composition and targeted constituents of concern,

2) design and assemble a hybrid pilot-scale CWTS to treat target constituents in OSPW,

3) measure performance of the pilot-scale CWTS for OSPW based on rates and extents of constituent removal, and

4) measure the performance of the pilot-scale CWTS using toxicity testing with the aquatic invertebrate Ceriodaphnia dubia.
1.3 References


naphthenic acid mixtures, to fathead minnow (*Pimephales promelas*) embryos. *Aquatic Toxicology* 164:108-117.


CHAPTER TWO

A RISK-BASED APPROACH FOR IDENTIFYING CONSTITUENTS OF CONCERN IN OIL SANDS PROCESS AFFECTED WATER FROM THE ATHABASCA OIL SANDS MINING AREA

Andrew D. McQueen1,5; Ciera M. Kinley1; Maas Hendriks1; Daniel P. Gaspari2; Alyssa J. Calomeni1; Kyla J. Iwinski1; James W. Castle2; Monique C. Haakensen3; Kerry M. Peru4; John. V. Headley4; John H. Rodgers Jr.1

1 Department of Forestry and Environmental Conservation, 261 Lehotsky Hall, Clemson University, Clemson, SC 29634, USA

2Department of Environmental Engineering & Earth Sciences, 445 Brackett Hall, Clemson University, Clemson, SC 29634, USA

3Contango Strategies Limited, LFK Biotechnology Complex, 15-410 Downey Road, Saskatoon, SK S7N 4N1

4Water Science and Technology Directorate, Environment and Climate Change Canada, 11 Innovation Blvd, Saskatoon, SK, S7N 3H5

5Corresponding author: Andrew McQueen

Manuscript prepared for submission to: Chemosphere
2.1 Abstract

Mining leases in the Athabasca Oil Sands (AOS) region produce large volumes of oil sands process-affected water (OSPW) containing constituents that limit beneficial uses, including discharge into receiving systems. The aim of this research is to identify constituents of concern (COCs) in OSPW sourced from an active settling basin with the goal of providing a sound rational for developing mitigation strategies for using constructed treatment wetlands for COCs contained in OSPW. COCs were identified through several lines of evidence: 1) chemical and physical characterization of OSPW and comparisons with numeric water quality guidelines and toxicity endpoints, 2) measuring toxicity of OSPW using a taxonomic range of sentinel organisms (i.e. fish, aquatic invertebrates, and a macrophyte), 3) conducting process-based manipulations (PBM) of OSPW to alter toxicity and inform treatment processes, and 4) discern potential treatment pathways to mitigate ecological risks of OSPW based on identification of COCs, toxicological analyses, and PBM results. COCs identified in OSPW included organics (naphthenic acids [NAs], oil and grease [O/G]), metals/metalloids, phosphorus, and suspended solids. In terms of species sensitivities to undiluted OSPW, fish ≥ aquatic invertebrates > macrophytes. Bench-scale manipulations of the organic fractions of OSPW via PBMs (i.e. H2O2+UV254 and granular activated charcoal treatments) eliminated toxicity to Ceriodaphnia dubia (7-8 d), in terms of mortality and reproduction. Results from this study provide critical information to inform mitigation strategies using passive or semi-passive treatment processes (e.g., constructed treatment wetlands) to mitigate ecological risks of OSPW to aquatic organisms.
2.2 Introduction

Mining leases in the Athabasca Oil Sands (AOS) region, in Alberta Canada, produce large volumes of oil sands process affected water (OSPW) during the operational process of bitumen extraction, with approximately 1.6 barrels of fresh water required for every barrel of petroleum produced in surface mining operations (Shell Canada Ltd., 2016). OSPW often contains problematic constituents that need to be treated prior to beneficial uses (e.g., discharge into receiving aquatic systems; Allen, 2008a; Alberta Department of Energy, 2014). Ecological risks, in terms of toxicity to aquatic and terrestrial biota have been attributed to the organic acid fraction of OSPW, referred to as naphthenic acids (NAs; e.g., Verbeek et al., 1994; Nero et al., 2006; Frank et al., 2008; Armstrong et al., 2008; Kavanagh et al., 2011; Leclair et al., 2013; Marentette et al., 2015). In addition to NAs, other constituents in OSPW may require treatment including trace metals/ metalloids (e.g., Al, As, Cr, Cu, Fe, Pb, Ni, Se, Zn), unrecovered bitumen (e.g., oil and grease [O/G]), major cations and anions (e.g., Na⁺, Cl⁻, SO₄²⁻, HCO₃⁻), and suspended and dissolved solids (Mackinnon and Boerger, 1986; Allen, 2008a).

Identification of site-specific constituents of concern (COCs) is a crucial step in developing and implementing treatment systems for the goal of mitigating site-specific OSPW for discharge to aquatic receiving systems. For this purpose, COCs are defined as elements, compounds, or parameters measured in OSPW waters that can adversely affect receiving system biota. Wetlands designed to promote ecosystem services (e.g., habitat, flood protection, carbon storage, etc.) are part of a comprehensive reclamation plan for mined leases, restoring areas to “equivalent land capabilities” of pre-mined landscapes in
the AOS region (Allen, 2008b; CEMA, 2014; Province of Alberta 2014). Logically, constructed wetland treatment systems (CWTS) could be designed to actively treat problematic constituents in OSPW and transition to serve as passive (reclaimed) wetlands following their operational lifespan. To be successful, CWTS must be designed with explicit processes to alter (transfer or transform) COCs to concentrations or forms that mitigate ecological risks for aquatic organisms. Physical, chemical, and toxicological characterization of site-specific OSPW is necessary to provide a sound design basis for selection and prioritization of treatment processes. OSPW composition varies temporally and spatially (Frank et al., 2016), and with bitumen extraction processes (e.g., froth treatment, process aides, upgrading, etc.). Therefore, defining site-specific COCs will be necessary to confirm rates and extents of treatment required for process-specific OSPW. This study focuses on OSPW sourced from an active settling basin located in the AOS region. A risk-based approach was used to identify COCs, including: 1) chemical-specific approach comparing concentrations of parameters to relevant water quality criteria thresholds, 2) toxicological approach using sentinel aquatic species, and 3) manipulation of chemical and physical characteristics of OSPW samples using treatment processes relevant to wetland treatment systems, with the goal of decreasing toxicity to sentinel aquatic species.

The initial step for identifying COCs includes comparisons of concentrations of constituents in OSPW to surface water quality guideline limits (i.e. Canadian Environmental Quality Guidelines for the protection of freshwater aquatic life [CEQG], Alberta Environment Water Quality Guidelines [Alberta WQGs], and United States
Environmental Protection Agency [USEPA] aquatic life criteria; CCME, 2007; USEPA, 2007; ESRD, 2012, 2014). Further, comparisons of concentrations of constituents contained in OSPW to water quality thresholds was supported with acute and chronic toxicity endpoints for constituents reported in peer-reviewed literature for sentinel aquatic organisms (i.e. fish: *Pimephales promelas* Rafinesque [fathead minnow] and *Oncorhynchus mykiss* Walbaum [rainbow trout]; and aquatic invertebrates: *Ceriodaphnia dubia* Richard and *Daphnia magna* Straus). These species were selected based on availability of toxicological data for constituents present in OSPW (i.e. metals, organics, cations, and anions). In addition, these species are commonly used for evaluation of whole effluent for effluent discharge permits and for determination of water quality guidelines for protection of aquatic biota (CCME, 2007; USEPA, 2007; ESRD, 2014).

The second step for characterization of COCs included a toxicological evaluation of OSPW. Excursions from numeric criteria offer an initial step for identifying COCs; however, a chemical-specific approach is not sufficient to identify or confirm the presence of potential adverse effects to receiving system biota (USEPA, 1991). Toxicological characterization of OSPW was achieved using OSPW sourced from the AOS region (corresponding samples with analytical data). Toxicity testing using sentinel aquatic organisms provided an integrated response measure of ecological risk, accounting for interactions of constituents in complex mixtures of whole effluent (e.g., OSPW) and presence of unknown toxicants (USEPA, 1991). The toxicological analysis included a taxonomic range of aquatic animals and plants (i.e. fish, invertebrates, and a macrophyte)
to discern diagnostic responses of biota (e.g., relative sensivities among species and potencies within species), which may implicate sources of toxicity.

The third step in characterization of OSPW included manipulations of the water in an effort to decrease toxicity (if present) to sentinel aquatic species. Chemical and physical treatments can be evaluated in laboratory-scale experiments to simulate transfer or transformation processes that can be implemented in CWTS, with the goal of altering constituents into less bioavailable or non-toxic forms. These process-based manipulations (PBM)s are strategically selected based on the chemical-specific evaluation and toxicological analysis to inform potential wetland treatment processes required to alter exposures of COCs and mitigate risk. Fractions of OSPW (e.g., organics, metals, suspended solids) can be altered using bench-scale manipulations. Chemical and physical manipulations selected to alter toxicity included: filtration (removal of suspended solids), divalent metal chelation (removal of metals), activated charcoal (removal of non-polar organics), and coupled hydrogen peroxide (H₂O₂)/ UV₂₅₄ treatments (removal of organic compounds). Changes in toxicity (in terms of lethal and sub-lethal responses) were evaluated using the aquatic invertebrate C. dubia, based on their relative sensitivity to constituents contained in OSPW. PBM}s used in this study are not meant for direct translation as treatment strategies for altering ecological risk of OSPW (e.g., additions of chelators, oxidants, etc.), but to inform treatment processes that can be promoted in passive or semi-passive constructed wetland treatment systems (e.g., metal complexation and precipitation, microbial degradation of organic constituents). A synthesis of the
information gained from the OSPW characterization (steps 1-3) provide critical information to inform CWTS processes necessary to alter exposures and mitigate risk.

The aim of this research is to identify COCs in OSPW with the goal of providing a sound rational for developing mitigation strategies using CWTS. COCs were identified through several lines of evidence with the following specific objectives: 1) compare concentrations of chemical and physical constituents in OSPW with numeric water quality guidelines (i.e. Alberta WQGs, CEQGs, and USEPA WQC) and toxicity threshold values for fish (*P. promelas* and *O. mykiss*) and freshwater invertebrates (*C. dubia* and *D. magna*), 2) measure toxicity of OSPW using fish (*P. promelas*), aquatic invertebrates (*C. dubia* and *D. magna*), and seedlings from a rooted macrophyte (*T. latifolia*), 3) conduct process-based manipulations (PBM) on OSPW to alter toxicity, in terms of mortality and reproduction, to the aquatic invertebrate *C. dubia*, and 4) discern potential treatment pathways to mitigate ecological risks of OSPW based on identification of COCs, toxicological analyses, and PBM results.

### 2.3 Materials and Methods

#### 2.3.1 Identification of Constituents of Concern

The OSPW used in this study was procured from the Muskeg River Mine External Tailings Facility (MRM-ETF) operated by Shell Canada Limited. MRM-ETF OSPW is produced from a surface mining operation in the AOS region (near Fort McMurray, AB, Canada) using a Clark caustic warm water extraction process (NaOH assisted at water temperatures of 50-80°C) to separate bitumen from ore. The approach
for identifying COCs in OSPW used chemical and toxicological data (Figure 1). In the initial step, chemical or physical parameters that exceeded water quality criteria/guidance limits (i.e. Alberta WQGs, CEQGs, and USEPA WQC) or toxicity endpoint values (point estimates were chosen when available; e.g., LC50s) were identified as COCs. The toxicity testing species *O. mykiss, P. promelas, C. dubia*, and *D. magna* were chosen for this comparison because of their sensitivity to many constituents found in OSPW, availability of data, and use in developing and enforcing water quality criteria (USEPA, 2002; CCME, 2007). COCs were parameters that exceeded the most conservative values from the selected criteria (Equation 1).

$$\text{COCs} = \text{OSPW Parameter} > \text{WQC or Toxicity Endpoint}$$

Equation 1

2.3.2 Analysis of OSPW

OSPW samples were transported from the MRM-ETF to Clemson University, SC (USA) for physical, chemical, and toxicological analyses. Samples for elemental analysis were collected in acid-cleaned 50-ml centrifuge tubes and preserved with concentrated trace metal-grade nitric acid (1% v/v; Fisher Scientific). Concentrations of Ag, Al, As, B, Ba, Cd, Cr, Cu, Co, Fe, Pb, Mn, Mo, Ni, Se, and Zn were measured with an Inductively Coupled Plasma Atomic Emission Spectrometer (Spectro Flame Modula; ICP-AES) following EPA method 200.7 (USEPA, 2001). Dissolved oxygen and pH were measured using YSI (model 85) and Orion® (model 410A+) instruments, respectively. Alkalinity, hardness, conductivity, and total suspended and dissolved solids were determined according to Standard Methods (APHA, 2012; Table 2.1).
Methods for NAs derivatization and analysis using High Performance Liquid Chromatography HPLC (Dionex, UltiMate-3000; Sunnyvale, CA) were based on Yen et al. (2004). Commercially available (Fluka) NAs (Sigma-Aldrich; St. Louis, Mo) were used to prepare stock solutions of NAs for developing standard curves. “Total” NA concentrations were quantified based on derivatization of carbonyl compounds amendable to derivatization and detected at 400 nm (Yen et al., 2004). The HPLC column was an Agilent LiChrospher 100 RP-18 (5 µm particle size, 125mm x 4 mm) with a guard column packed with 2 µm RP-18 solid phase material. Column temperature was maintained at 40° C with a sample injection volume of 60 µL mobilized with HPLC grade methanol (Fisher Scientific) at a flow rate of 60 µL/min. Results were reported with a range of 85-115% recovery. NA concentrations were reported as means of triplicate analyses.

The chemical composition and profile of NAs contained in OSPW were identified by use of direct injection linear ion trap-orbitrap mass spectrometer (Orbitrab MS; Orbitrap Elite, Thermo Fisher Scientific, San Jose, CA, USA) in negative (ESI−) electrospray according to the method described by Headley et al. (2016) and Leshuk et al. (2016). Solid phase extraction (SPE), as previously described by Headley et al. (2002) was used as a cleanup and concentration technique for test samples. Chemical species detected in each fraction were grouped according to heteroatom empirical formula classes in ESI− electrospray: Ox− (where x = 1–5), N−, NOx− (where x = 1–4), S−, SOx− (where x = 1–5), or NOxS− (where x = 2).
2.3.3 Toxicity Testing Procedures

Freshwater organisms (*P. promelas* and *C. dubia*) were cultured at Clemson University’s Aquatic Animal Research Laboratory according to USEPA (2002), under protocols in compliance with Clemson University’s Institutional Animal Care and Use Committee. Toxicity testing protocols for *P. promelas*, *C. dubia*, and *D. magna* were based on Environment Canada protocols (1996; 2007; 2011). Toxicity tests for *P. promelas* were conducted by exposing 30 organisms (< 24 h old larvae) per concentration (10 organisms per replicate for 3 replicates) in 250 ml borosilicate beakers. During exposures, fish were fed *Artemia sp.* once daily. Toxicity tests for *C. dubia* and *D. magna* were conducted by exposing 20 organisms (< 24 h old neonates) per concentration (1 organism per replicate for 20 replicates) in 15 ml borosilicate vials. During exposures, *C. dubia* and *D. magna* were fed 200 µL of a 1:1 mixture of *Pseudokirchneriella subcapitata* and YCT (yeast, cerophyll, trout chow) once daily.

For phytotoxicity testing, mature *T. latifolia* inflorescences were collected in August and September, 2015, from a wetland site at Clemson University, Clemson, SC (34°40'7.12"N, 82°50'53.98"W) and seeds were separated from bristle hairs by placing in a blender filled with NANOpure® water and blending. Seeds that sank to the bottom after blending were considered viable and used for testing (Kinley et al., 2016). Viable seeds were then added to a small volume (~1 ml) of moderately hard water and incubated for 2 days to induce germination. Toxicity experiments for *T. latifolia* were initiated by adding 10 germinated *T. latifolia* seedlings (2 d old) to each replicate 50mL beaker (three replicates/concentration) under fluorescent lighting (1,500-3,000 Lux) with a 16 h light/8
h dark photoperiod at 24 ± 1°C. Exposure concentrations were pipetted into treatment chambers and volumes were maintained as necessary. Control (untreated) exposures were moderately hard water. After 7 days, seedlings were removed from exposures and preserved in 70% ethanol until analysis. Root and shoot lengths (mm) of seedlings were measured using a Leica® M80 Stereoscope and software (Leica Microsystems®).

Reference toxicity tests were conducted for all test species using copper sulfate (CuSO₄·5H₂O; Fisher Scientific) for intra- and inter-laboratory comparisons (USEPA, 1991; USEPA, 2002). Acid soluble copper concentrations (exposures) were confirmed using flame atomic absorption spectroscopy and graphite atomic absorption spectroscopy (Agilent PSD 120 atomic absorption spectrometer; APHA, 2012).

Water characteristics of exposures were measured at test initiation and completion of toxicity tests, with the exception of T. latifolia exposures, which were measured at test initiation. Dissolved oxygen, pH, and conductivity of exposure waters were measured using a YSI® Model 52 dissolved oxygen meter, Orion® Model 250A pH meter, and Orion® Model 142 conductivity meter, respectively. Hardness and alkalinity of samples were measured according to Standard Methods for Examination of Water and Wastewater (APHA, 2012).

2.3.4 Process-based Manipulations (PBMs)

The purpose of the PBMs outlined below is to remove or alter “classes” of COCs (i.e. nonpolar organics, divalent metals, oxidizable compounds) which could be targeted using constructed wetlands. Treatment processes were conducted using 100% OSPW in an effort to reduce toxicity to the aquatic invertebrate C. dubia. PBMs consist of: 1)
filtration, 2) chelation with ethylenediaminetetraacidic acid (EDTA) addition, 3) granular activated charcoal (GAC) treatment, and 4) coupled hydrogen peroxide H$_2$O$_2$/UV$_{254}$ treatments (Table 2.2).

All manipulations were conducted at initial OSPW pH (~8.2). For fractionation experiments, OSPWs were stored in 1 liter HDPE Nalgene® bottles at 4 ± 1°C and warmed to 25°C prior to manipulations. To obtain a filterable fraction, OSPW was passed through a 0.45μm nitro cellulose membrane filter using a glass separatory funnel applying vacuum suction (-700 mm Hg). To chelate the metal fraction (e.g., cationic metals: Al$^{3+}$, Ba$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Pb$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$), 50 mL of 0.1 M EDTA was used per liter of OSPW (based on hardness of untreated OSPW). Non-polar organic fractions were treated using granular activated charcoal (GAC; coconut 6-14 mesh; Fisher Scientific) at concentrations of 5 g/L. GAC was added to OSPW and allowed a 1-2 h contact time, after which the residual GAC was removed prior to toxicity testing. To remove compounds susceptible to oxidation and photolysis, 100 ug/L hydrogen peroxide (H$_2$O$_2$; Fisher Scientific) was added to OSPW and exposed to UV$_{254}$ (8W fluorescent tube; Philips Ltd.) for a duration of 12 h.

No observable effect concentrations (NOECs) and lowest observable effect concentrations (LOECs) of OSPW (as % of undiluted OSPW) were determined by statistically significant differences relative to untreated controls using one way analysis of variance (ANOVA) and Dunnett’s multiple range test ($\alpha = 0.05$; JMP Pro V.11). Median lethal effect concentrations (LC50s) were estimated using the Probit model using JMP Pro V.11 (SAS Institute Inc., Cary, NC).
2.4 Results and Discussion

2.4.1 Chemical-specific Approach for Identifying COCs

2.4.1.1 Naphthenic acids

Using two analytical methods, HPLC and Orbitrap MS, OSPW NAs concentrations ranged from 80 to 128 mg/L (HPLC; n=10) and 93 to 103 mg/L (Orbitrap MS; n=2). In addition, speciation and quantification of NAs were conducted via pH-dependent extractions and quantification using Orbitrap MS analysis to investigate the abundance of “classical” NAs (NAs, \( \text{C}_n\text{H}_{2n+z}\text{O}_2 \)), oxidized NAs (Ox-NAs), and nitrogen- and sulfur-containing NAs. The sum of \( \text{O}_2^- \)-NA species accounted for ~50% of the total abundance of extracted organic matter (Figure 2.2). The abundance of \( \text{O}_2^- \)-NAs in OSPW is consistent with distribution of NAs reported for “fresh” OSPW, containing a greater abundance of monocarboxylic acids (\( \text{C}_n\text{H}_{2n+z}\text{O}_2 \); Marentette et al., 2015; Barrow et al., 2016). Morandi and coauthors (2015) implicated “classical” NAs as the primary contributor to toxicity to fathead minnows (\( \text{P. promelas} \)), with non-acidic organic species as minor contributors to toxicity. NAs concentration and species present in OSPW are generally consistent with OSPW-NAs quantified using similar extraction techniques and analysis (Grewer et al., 2010; Jones et al., 2015; Kavanagh et al., 2011; Marentette et al., 2015; Barrow et al., 2016; Frank et al., 2016). NAs were identified as COCs based on the concentration and species present as compared to published toxicity data (Table 2.3).

2.4.1.2 Residual Petroleum Hydrocarbons

The OSPW samples had a slight hydrocarbon sheen, with oil and grease (O/G) concentrations ranging from 8 to 13 mg/L (Table 2.3). Reported concentrations of O/G
(an aggregate measure of residual hydrocarbons) in OSPW exceed narrative criteria for discharge water (i.e. no visible film or sheen of oil present; Table 2.3). Interestingly, residual bitumen (O/G) concentrations measured in OSPW used in this study were lower than ranges in OSPW from other sources, with a range of 9 to 92 mg/L as O/G (Allen, 2008a). Organics present in OSPWs studied in the AOS region exceeding numeric criteria also include: benzene, toluene, ethylbenzene, xylene (BTEX), polycyclic aromatic hydrocarbons (PAHs), and phenols (Allen, 2008a; Rogers 2002; Galarneau et al., 2014); however, data on lower-molecular weight hydrocarbons in OSPW are generally limited in peer reviewed literature (Mahaffey and Dubé, 2016). In this study, petroleum hydrocarbons (as O/G) were identified as COCs.

2.4.1.3 Inorganics

OSPW was analyzed for 16 elements (Table 2.4). Elements not above the MDL included: cadmium (MDL = 0.0002 mg/L), chromium (MDL = 0.004), cobalt (MDL = 0.0002 mg/L), and silver (MDL = 0.0002 mg/L). Based on the chemical-specific characterization approach, COCs for metals/metalloids in OSPW included: Al, B, Cu, Fe, Pb, Ni, Se, and Zn (Table 2.4). These elements measured as “total” exceed conservative concentrations in water quality criteria or toxicity endpoints for sensitive sentinel aquatic biota measured in unconfounded laboratory studies. Although metal speciation and concentrations in tailings ponds differ due to geologic heterogeneities and recycling of tailing pond water, concentration ranges of elements contained in OSPW are generally consistent with other OSPWs from the AOS (MacKinnon and Boerger, 1986; Siwik et al., 2000; Allen, 2008a).
Maximum total ammonia (N) and phosphorus concentrations in OSPW samples (n=10) were 0.099 mg/L and 0.082 mg/L, respectively. Published concentrations of ammonia in other OSPWs have been reported as high as 18.4 mg/L (± 1.2; Lai et al., 1996), exceeding toxicity values or water quality criteria guidelines (CCME, 2007; ESRD, 2014). Ammonia toxicity is dependent upon pH of a solution (Thurston and Russo, 1981). At 14.1°C and pH of 8.29, the 96 h LC50 of ammonia to rainbow trout is 0.563 mg/L (Thurston and Russo, 1981), approximately 5.5x greater than ammonia concentrations observed in the source OSPW. In this study, total phosphorus maximum concentration (0.082 mg/L) was above CCME (2007) guidelines, and phosphorus was identified as a COC (Table 2.4). However, in the context of passive treatment, nutrient concentrations (e.g., TP) can influence microbially mediated wetland biogeochemical processes (e.g., biodegradation of NAs [Herman et al., 1994; Lai et al., 1996]; dissimilatory sulfate-reduction).

2.4.1.4 Cations and Anions

Dissolved salts are found in OSPW due to the connate water (water that forms part of the ore body), extraction processes, and recycling of process water (Allen, 2008a). Ions in OSPW were predominantly composed of bicarbonate (HCO₃⁻), sodium (Na⁺), and chloride (Cl⁻). These ions are associated with the ore body that forms the regional geology (Mikula, 2013) and become part of the OSPW during the warm water extraction process. Aggregate measures of major ions in OSPW (i.e. total dissolved solids [TDS]) indicate the ionic strength is greater than “background” receiving aquatic systems (Golder Associates, 2002), with TDS in OSPW ranging from 510 to 1100 mg/L. TDS and
conductivity (generic measures for salinity of water) are not reliable predictors of aquatic toxicity (Goodfellow et al., 2000), therefore, evaluation of the composition and strength of ions present in OSPW is necessary. The role of total dissolved ions in terms of the strength and balance (or imbalance) is important in determining potential risk to receiving aquatic biota (Goodfellow et al., 2000). The concentrations and distribution of ions can directly cause adverse effects or indirectly alter toxicity of co-occurring elements due to competition for ions at active sites (Di Toro et al., 2001). The ionic balance (ratio of the summation of cations to anions [as meq/L]) in OSPW is near neutral, ranging from 0.85 to 1.0. Relative toxicity for major ions is K\(^+\) > HCO\(_3^-\) ~Mg\(^{2+}\) > Cl\(^-\) > SO\(_4^{2-}\), and ion deficiencies can be as detrimental to aquatic organisms as excessive concentrations (Goodfellow et al., 2000). Sodium is introduced to OSPW by the use of NaOH in the caustic hot water extraction process, and is the predominant cation in OSPW (Allen, 2008a). Sodium ion (Na\(^+\)) concentration ranged from 150 to 364 mg/L; however, Na\(^+\) ions are generally not a major contributor to aquatic toxicity as compared to the associated Cl\(^-\) anion (Mount et al., 1997; Goodfellow et al., 2000). Chloride ion concentration are ~240 mg/L in OSPW, which at the maximum concentrations observed, exceed recommended water quality guidelines (CEQG guidance of 120 mg/L). Chloride ions in OSPW are elevated as compared to regional background (Athabasca River concentrations range from 2 to 50 mg/L). Based on OSPW concentrations of major ions present in OSPW, chloride ions are at concentrations exceeding ambient water quality criteria and are identified as a constituent of concern (CCME, 2007; ESRD, 2014). Verbeek et al. (1994) indicated that major cations and anions were not significantly
contributing to toxicity following bench-scale toxicity identification evaluations of OSPW.

2.4.1.5 General water characteristics

In terms of general water characteristics, OSPW is well buffered (alkalinity range from 320 to 340 mg/L as CaCO₃) due to the concentration of bicarbonates (HCO₃⁻; range from 300 to 320 mg/L; Table 2.3). Based on presence of bicarbonates, pH of OSPW is relatively stable, ranging from 7.91 to 8.45. OSPW hardness and conductivity range from 160 to 178 mg/L (as CaCO₃) and 1791 to 1800 µS/cm, respectively. Total suspended solids range from 130 to 400 mg/L in OSPW, therefore exceeding narrative WQC (e.g., based on the potential for increasing background receiver turbidity; CCME, 2007; ESRD, 2014).

2.4.2 Toxicity of OSPW

In this study, a fish (P. promelas), two aquatic invertebrates (C. dubia and D. magna), and seedlings from a rooted macrophyte (T. latifolia) were used to evaluate toxicity of OSPW (Table 2.5). In terms of species sensitivities, P. promelas and C. dubia were more sensitive to exposures of OSPW as compared to D. magna and T. latifolia. Lowest observed effect concentrations (LOECs), expressed as percentage of OSPW, were 50% and 25% for larval P. promelas (7 d; biomass) and C. dubia (7-8 d; reproduction), respectively. In undiluted OSPW, mortality was not observed for 48 h D. magna. The freshwater aquatic macrophyte seedlings were not as sensitive to OSPW as fish and aquatic invertebrates. T. latifolia seedling root growth was not adversely affected in 7 d exposures (LOEC >100% OSPW).
Availability of exposure-response data for an array of taxonomic groups provides a unique opportunity to integrate information, allowing general inferences regarding “diagnostic” effects implicating sources of toxicity. Direct comparisons should be made with caution, as differences in exposures (e.g., duration of tests, renewals, etc.) may complicate comparisons. Data from this study indicate that sensitivities of sentinel aquatic organisms to ABS-OSPW were: fish ≥ invertebrates > macrophytes. Relative sensitivities of organisms observed in this study imply that the toxicity is attributed to organic fractions (i.e. petroleum hydrocarbons, organic acids; Verbeek et al., 1994; Morandi et al., 2015). In general, Cladocera (i.e. C. dubia and D. magna) are more sensitive to cationic metals as compared to fish (P. promelas; USEPA, 2002; Suter and Tsao 1996). Interestingly, OSPW did not adversely affect D. magna (no mortality present in 100% OSPW), implying metals and metalloids are within the environmental tolerances of these aquatic invertebrates for chronic effects.

Although comparisons among other OSPWs from the AOS is challenging due to differences in OSPW composition (i.e. influenced by extraction processes, age, etc.), some general observations can be made from other studies. Relative species sensitivities to OSPW are generally consistent with observations among OSPWs produced in the AOS region. Other studies have indicated that fish are more sensitive to OSPW as compared to D. magna (Verbeek et al., 1994; Zubot et al., 2012). Zubot and coauthors measured toxicity of rainbow trout (96 h toxicity tests) exposed to OSPWs (produced using caustic soda extraction process stored in settling basins) and observed 100% mortality for fish exposed to OSPW, with a LC50 of 35% (vol% of “raw” water). In addition, Verbeek et
al. (1994) observed that rainbow trout bioassays (96 h) were seven times more sensitive (in terms of mortality endpoints) to OSPW as compared to *D. magna* (48 h exposures; Verbeek et al., 1994). In this study, *C. dubia* were slightly more sensitive to constituents contained in OSPW as compared to fish (*P. promelas*). Sensitivity differences among *Cladocera* (*C. dubia* and *D. magna*) to constituents in OSPWs is surprising. One possible explanation is differences in exposure durations (48 h [*D. magna*] as compared to 6-8 d [*C. dubia*]). Based on results from the OSPW toxicity testing data, *C. dubia* were chosen as the sensitive species for conducting PBMs. *C. dubia* were selected based on their sensitivity to OSPW in terms of lethal and sub-lethal (fecundity) endpoints (Table 2.5).

2.4.3 Process-based Manipulations (PBMs)

The purpose of PBMs was to use bench-scale treatments to remove fractions of compounds analogous to biogeochemical processes used for designing and implementing processes in CWTS (Rodgers and Castle, 2008). In this study, PBMs were evaluated using *C. dubia*. Initial (pre-treated) OSPW resulted in 76% *C. dubia* mortality, and a statistically significant (p<0.005; α = 0.05) decrease in reproduction. Following H2O2 + UV254 treatments (targeting oxidizable organics in OSPW), toxicity to *C. dubia* was eliminated (96% *C. dubia* survival, 7 d), with no statistically significant differences in reproduction as compared to a laboratory control (Figure 2.3). Additionally, GAC treatments eliminated toxicity to *C. dubia*, in terms of both mortality and reproduction (7 d survival was 100%; no statistically significant differences [p=0.68; α =0.05] in reproduction as compared to laboratory control). Filtered OSPW decreased mortality (7 d survival was 86%); however, reproduction was still impaired (<2 neonates per live adult
as compared to an average of 34 neonates per adult in untreated control). EDTA treated OSPW did not exhibit any measurable change in toxicity to *C. dubia* as compared to untreated water (Figure 2.3).

Results from PBMs implicate non-polar or slightly polar organics as contributing to toxicity of OSPW. Decreased *C. dubia* toxicity following H₂O₂ + UV₂₅₄ treatments in this study parallels decreases in toxicity from other studies using advanced oxidation treatments (i.e. ozonation and photocatalysis) to alter toxicity of OSPWs (Scott et al., 2008; El-Din et al., 2011; Anderson et al., 2012; He et al., 2012; Leshuk et al., 2016). GAC treatments used in this study support observations from the coupled H₂O₂ + UV₂₅₄ treatments targeting organic fractions of OSPW. Other studies have used adsorbents to target the non-polar fraction of OSPW in attempts to alter exposures of organic constituents to mitigate toxicity (McTernan et al., 1986; Marr et al., 1996; Zubot et al., 2012). Zubot et al. (2012) observed decreases in toxicity to rainbow trout and Microtox™ test (bioluminescent bacteria; *Vibrio fischeri*) following petroleum coke treatments of OSPW, attributing reduction in toxicity to the adsorption of NAs (removing 91%; 75 mg NA/L initial to 5.7 mg/L post-treatment).

Verbeek et al. (1994) identified non-polar compounds and surfactants (organic acids) contributed 100% of the observed toxicity in “fresh” tailings OSPW collected from the AOS region. Although these “fractions” of non-polar organic and surfactants contributing to toxicity contain a myriad of bitumen-derived constituents, more recent efforts have been made to identify species of these fractions using high resolution analytical techniques (Morandi et al., 2015). Morandi et al. (2015) identified NAs (i.e.
O2⁻ species) as among the most toxic fractions of OSPW contained in an end-pit lake in the AOS. Results from this study and others (Verbeek et al., 1994, Morandi et al., 2015) indicate minimal contributions from elemental fractions to the observed toxicity of OSPWs. Although numeric exceedances of elemental constituents (i.e. Al, B, Cu, Fe, Pb, Ni, Se, and Zn) were observed in this study, results from the PBMs indicate that elemental constituents are in concentrations or forms that limit their bioavailability. Data from PBMs in this study, in context with the identified COCs in OSPW, provide useful insight to processes needed in passive and semi-passive systems that could be used to alter exposures and mitigate ecological risks.

2.4.4 Implications to Constructed Wetland Treatment System Design

The goal of this risk-based approach was to characterize a site-specific OSPW to identify specific COCs needing treatment and informing CWTS processes for altering risks to aquatic biota. Clearly, a robust treatment strategy is needed for OSPW based on physical, chemical, and toxicological characteristics of these complex waters. To be economically viable, CWTS must be passive (i.e. low energy demand) due to the volume of OSPWs currently stored in the AOS (estimated 975 million m³; Alberta Department of Energy, 2013).

Based on the COCs and toxicity data for OSPW, clearly the mitigation strategy should focus on the organic fraction (e.g., non-polar organics and NAs) to achieve narrative discharge criteria (i.e. “no toxics in toxic amounts”). Treatment wetlands have been successfully designed to mitigate a variety of waters containing organic constituents (e.g., oil field produced water, natural gas storage produced water; Knight et al., 1999;
Pham et al., 2011; Horner et al., 2012). Organic constituents are microbially transformed in treatment wetlands due to the presence of organisms and enzymes capable of degrading organics (Knight et al., 1999; Pham et al., 2011; Horner et al., 2012). Treatment wetlands designed to mitigate oil field produced waters decreased O/G concentrations by > 98% with initial concentrations between 10 and 100 mg/L (Horner et al., 2012). In addition, microbial degradation processes could be coupled with advanced oxidation to increase biodegradability of recalcitrant organic fractions (i.e. NAs; Metcalf and Eddy 2003; Martin et al., 2010; El-Din et al., 2011; Shi et al., 2015; Vaiopoulou et al., 2015). Advanced oxidation is a promising pathway that has demonstrated environmentally relevant rates and extents of degradation of NAs in OSPW (Martin et al., 2010; Vaiopoulou et al., 2015) and has decreased toxicity of NAs extracted from OSPW (Scott et al., 2008; Anderson et al., 2012; He et al., 2012; Shi et al., 2015). Hybrid wetland treatment approaches such as coupling advanced oxidation and biodegradation may offer greater rates of removal for recalcitrant organic fractions (i.e. NAs) contained in OSPW and flexibility (in terms of footprint) in wetland design and placement.

PBM of OSPW did not indicate cationic metals were a source of toxicity; however, excursions in numeric criteria indicate potential for ecological risk. Therefore, wetland processes to reduce aqueous concentrations of inorganic COCs (i.e. Al, B, Cu, Fe, Pb, Ni, Se, and Zn) need to be promoted. Treatment wetlands can be designed to provide pathways that enable transformation and/or transfer of metals and metalloids to stable chemical forms, limiting their mobility, bioavailability, and re-distribution (solubility over time), thus enabling discharge of OSPW. Biogeochemical processes
targeted in wetland treatment systems for altering aqueous concentrations and bioavailability of elements include: sulfide sequestration (Huddleston et al., 2008; Murray-Gulde et al., 2008; Rodgers and Castle, 2008), formation of Fe- and Mn-oxyhydroxide co-precipitates (Rodgers and Castle, 2008; Schwindaman et al., 2014), microbial transformations (e.g., Se, As; Spacil et al., 2011; Lizma et al., 2011), and complexation with organic ligands (e.g., Al complexation with fulvic and humic acids; Driscol and Postek, 1995). These processes have been used successfully in treatment wetlands to achieve performance goals (i.e. numeric and narrative discharge criteria) for energy-derived waters (e.g., refinery effluents, oilfield produce waters, acid and neutral mine drainage; Mooney and Murray-Gulde, 2008; Haakensen et al., 2015).

Microbially-driven processes in wetlands (as described above) depend on the availability of macro- and micro-nutrients; therefore, concentrations of nitrogen (as ammonia; 0.13 to 0.99 mg/L) and phosphorus (as total phosphorus; 0.037 to 0.62 mg/L) in OSPW are necessary for facilitating treatment of some COCs (e.g., NAs, O/G). In addition, wetlands have been used extensively to treat excess nutrients (nitrogen [as ammonia, nitrate, and nitrite], total phosphorus; Gersberg et al., 1986; Braskerud et al., 2002; Kadlec and Wallace, 2008).

2.5 Conclusions

In this study, COCs were identified using a risk-based approach, including chemical, physical and toxicological characterization. COCs identified in OSPW include organics (NAs, O/G), metals/metalloids, and suspended solids. Toxicity testing confirmed that COCs were in sufficient forms and concentrations to have measurable
adverse effects on sentinel aquatic species. Sensitivities of aquatic organisms to OSPW indicated that fish ≥ aquatic invertebrates > macrophytes. The sensitivity distribution of organisms to OSPW in addition to strategic bench-scale manipulations indicate organic constituents are contributing to the observed toxicity. Alteration of the organic fraction of OSPW (i.e. H₂O₂ + UV₂⁵⁴ and GAC treatments) significantly increased survival and reproduction of *C. dubia* as compared to untreated OSPW. Based on multiple lines of evidence, these data indicate that organic fractions (i.e. O/G, NAs) of OSPW are sources of toxicity. In addition, metals and metalloids in OSPW exceeding numeric guidelines indicates the need to decrease concentrations to achieve WQC thresholds. Results from this study provide critical information to inform mitigation strategies using passive or semi-passive treatment processes (i.e. constructed treatment wetlands) to mitigate ecological risks of OSPW to aquatic organisms. The approach used in this study could be applied to other site-specific and compositionally complex process-affected waters (i.e. OSPWs and consolidated tailings) located in the AOS region.

### 2.6 Acknowledgements

Funding for this research was provided by Shell Canada Ltd. and Suncor Energy. We are grateful to our colleagues at Shell Canada Ltd. for procuring source water for this research. The authors are also grateful to Dr. Wayne Chao of Clemson University for providing analytical support.
2.7 References


and oil-sand petroleum systems in Alberta and beyond: AAPG Studies in Geology. 64:689-699.


Table 2.1 Analytical methods for site-specific OSPW.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
<th>Method Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Direct Instrumentation: Orion Model 420A (Standard Methods 4500-H+ B) (APHA, 2012)</td>
<td>0.01 SU</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Direct Instrumentation: YSI 30 (Standard Method 2510 B) (APHA, 2012)</td>
<td>0.1 μS/cm</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Standard Methods: 2320 B (APHA, 2012)</td>
<td>2 mg/L as CaCO3</td>
</tr>
<tr>
<td>Hardness</td>
<td>Standard Methods: 2340 B (APHA, 2012)</td>
<td>2 mg/L as CaCO3</td>
</tr>
<tr>
<td>TSS</td>
<td>Standard Methods: 2540 D (APHA, 2012)</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>TDS</td>
<td>Standard Methods: 2540 C (APHA, 2012)</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Oil and grease (O/G)</td>
<td>USEPA Method 1664 A (Environmental Express StepSaver Modification) (USEPA, 1999)</td>
<td>~2 mg/L</td>
</tr>
<tr>
<td>Cations/ Anions</td>
<td>Dionex ISC-2000 Ion Chromatograph (APHA, 2012)</td>
<td>2-7 mg/L</td>
</tr>
<tr>
<td>Total Ammonia</td>
<td>Standard Methods: 4500-NH3 (APHA, 2012)</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Total Phosphorous</td>
<td>Standard Methods: 4500-P (APHA, 2012)</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Metals and Metalloids (mg/L)</td>
<td>Inductively Coupled Plasma-Atomic Emissions Spectrometry (ICP-AES): 200.7 (USEPA, 2001);</td>
<td></td>
</tr>
<tr>
<td>Aluminum (Al)</td>
<td>0.045</td>
<td>Iron (Fe) 0.006</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>0.0006</td>
<td>Lead (Pb) 0.002</td>
</tr>
<tr>
<td>Barium (Ba)</td>
<td>0.003</td>
<td>Manganese (Mn) 0.002</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>0.006</td>
<td>Molybdenum (Mo) 0.012</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.0002</td>
<td>Nickel (Ni) 0.015</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.004</td>
<td>Selenium (Se) 0.002</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.002</td>
<td>Silver (Ag) 0.0002</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.003</td>
<td>Zinc (Zn) 0.002</td>
</tr>
<tr>
<td>Naphthenic Acids (NAs)</td>
<td>HPLC; Derivatization based on Yen et al., 2004</td>
<td>5 mg/L</td>
</tr>
<tr>
<td></td>
<td>Orbitrap MS; Headley et al., 2015; Leshuk et al., 2016</td>
<td>1 mg/L</td>
</tr>
</tbody>
</table>

aMDL represents samples which were concentrated using heat (60°C) assisted evaporative loss
Table 2.2 Manipulations and treatment objectives of process based manipulations (PBM) performed on OSPW.

<table>
<thead>
<tr>
<th>Process Based Manipulation (PBM)</th>
<th>Manipulation Objective</th>
<th>Analogous Passive or Semi-passive Treatment Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration^a</td>
<td>Remove suspended solids</td>
<td>Precipitation, settling, and sedimentation</td>
</tr>
<tr>
<td>EDTA chelation</td>
<td>Remove cationic metals</td>
<td>Dissimilatory sulfate reduction and sulfide-metal [MeS] precipitation</td>
</tr>
<tr>
<td>Granular activated charcoal (GAC)^b</td>
<td>Remove non-polar organic compounds</td>
<td>Sorption; microbial biotransformation and biodegradation</td>
</tr>
<tr>
<td>Hydrogen peroxide H₂O₂ + UV₂₅⁴</td>
<td>Remove compounds susceptible to oxidation</td>
<td>Advanced oxidation^c (e.g., solar photocatalysis)</td>
</tr>
</tbody>
</table>

^a0.45 µm nitrocellulose filter  
^bCharcoal, coconut (6-14 mesh; Fisher Scientific)  
^cAdvanced oxidation proposed for coupled "hybrid" semi-passive treatment options (Shi et al., 2015; Vaiopoulou et al., 2015; Leshuk et al., 2016; McQueen et al., 2016)
Table 2.3 Comparison of water quality characteristics and organic constituents in OSPW to water quality guidelines (CCME 2007, USEPA 2007; ESRD 2014) and toxicity values for *C. dubia* (Cd), rainbow trout (*O. mykiss* [Om]), and fathead minnow (*P. promelas* [Pp]). COCs (i.e. concentration > guideline) are bolded.

<table>
<thead>
<tr>
<th>Parameter mg/L (unless noted)</th>
<th>OSPW (n=10)</th>
<th>Water Quality Guidelines (mg/L)</th>
<th>Reported Toxicity Values (mg/L)</th>
<th>COC Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Alberta WQG</td>
<td>CEQG</td>
</tr>
<tr>
<td>pH (SU)</td>
<td>7.91</td>
<td>8.4</td>
<td>6.5-9.0</td>
<td>No</td>
</tr>
<tr>
<td>Alkalinity (CaCO&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>320</td>
<td>340</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Total Ammonia (N)</td>
<td>0.051</td>
<td>0.099</td>
<td>0.17-1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9</td>
</tr>
<tr>
<td>Total Phosphorus (TP)</td>
<td>0.037</td>
<td>0.082</td>
<td>0.01-0.02</td>
<td></td>
</tr>
<tr>
<td>Total Suspended Solids (TSS)</td>
<td>130</td>
<td>400</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Total Naphthenic Acids (NAs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAs (HPLC)</td>
<td>80</td>
<td>128</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>NAs (Orbitrap MS)</td>
<td>93</td>
<td>103</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Oil and grease (O/G)</td>
<td>8</td>
<td>13</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (HCO&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>300</td>
<td>320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (Ca&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>29</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride (Cl&lt;sup&gt;-&lt;/sup&gt;)</td>
<td>240</td>
<td>245</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Sodium (Na&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>360</td>
<td>364</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfate (SO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-2&lt;/sup&gt;)</td>
<td>150</td>
<td>175</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Minimum

<sup>b</sup>Temperature range from 5-20°C and pH of 8.0-8.5

<sup>c</sup>Thurston and Russon, 1981; @ 14.4°C and pH of 8.3

<sup>d</sup>Kavanagh et al., 2011; 5 d larvae; NAs quantified by ESI-MS

<sup>e</sup>Marentette et al., 2015; "fresh" OSPW; embryos; NA quantified by LC/QToF

<sup>f</sup>Narrative statement; no visible sheen or odor; or unreasonable turbidity or color

<sup>**</sup>"No toxics in toxic amounts"

Note: NAs quantified by different methods do not necessarily measure the same suite of compounds (Headley et al., 2015)
Table 2.4 Comparison of metals and metalloids in OSPW to water quality guidelines (CCME 2007; USEPA 2007; ESRD 2014) and toxicity values for *C. dubia* (Cd), *D. magna* (Dm), fathead minnow (*P. promelas* [Pp]), and rainbow trout (*O. mykiss* [Om]). Identified COCs (i.e. concentration > guideline) are bolded.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OSPW (n=10)</th>
<th>Water Quality Guidelines (mg/L)</th>
<th>Reported Toxicity Values (mg/L)</th>
<th>COC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Chronic</td>
<td>Chronic</td>
</tr>
<tr>
<td><strong>Aluminum (Al)</strong></td>
<td>0.36</td>
<td>10.9</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Arsenic (As)</strong></td>
<td>0.0006</td>
<td>0.0032</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Barium (Ba)</strong></td>
<td>0.21</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Boron (B)</strong></td>
<td>2.09</td>
<td>2.12</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Cadmium (Cd)</strong></td>
<td>&lt;0.0002</td>
<td>&lt;0.0002</td>
<td>0.00016&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00038&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Chromium (Cr)</strong></td>
<td>&lt;0.004</td>
<td>&lt;0.004</td>
<td>0.0089</td>
<td>0.0089</td>
</tr>
<tr>
<td><strong>Cobalt (Co)</strong></td>
<td>&lt;0.0002</td>
<td>&lt;0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Copper (Cu)</strong></td>
<td>0.003</td>
<td>0.12</td>
<td>0.007</td>
<td>0.0024&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Iron (Fe)</strong></td>
<td>1.2</td>
<td>1.5</td>
<td>0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Lead (Pb)</strong></td>
<td>&lt;0.0002</td>
<td>0.0014</td>
<td>0.0032&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00318&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Manganese (Mn)</strong></td>
<td>0.137</td>
<td>0.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Molybdenum (Mo)</strong></td>
<td>0.041</td>
<td>0.062</td>
<td>0.073</td>
<td>0.073</td>
</tr>
<tr>
<td><strong>Nickel (Ni)</strong></td>
<td>0.0056</td>
<td>0.01</td>
<td>0.052</td>
<td>0.0096</td>
</tr>
<tr>
<td><strong>Selenium (Se)</strong></td>
<td>0.002</td>
<td>0.004</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Silver (Ag)</strong></td>
<td>&lt;0.0002</td>
<td>&lt;0.0002</td>
<td>0.001</td>
<td>0.00025</td>
</tr>
<tr>
<td><strong>Zinc (Zn)</strong></td>
<td>0.014</td>
<td>0.113</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>pH: ≥6.5  
<sup>b</sup>Hardness: 100 mg/L as CaCO4  
<sup>c</sup>Dissolved fraction  
<sup>d</sup>Freeman and Everhart 1971; biomass  
<sup>e</sup>Biesinger and Christensen, 1972  
<sup>f</sup>OPP, 2000  
<sup>g</sup>Suedel et al. 1997  
<sup>h</sup>Kimball 1978  
<sup>i</sup>Mount, 1968  
<sup>j</sup>Pickering and Henderson, 1966  
<sup>k</sup>Boucher and Watzin, 1999  
<sup>l</sup>Birge 1978  
<sup>m</sup>Keithly et al. 2004
Table 2.5 Bioassay data for OSPW using fish (*P. promelas*), aquatic invertebrates (*C. dubia* and *D. magna*), and a macrophyte (*T. latifolia*).

<table>
<thead>
<tr>
<th>Species</th>
<th>Test Duration</th>
<th>Age</th>
<th>% Mortality (in 100% OSPW)</th>
<th>Endpoint Estimates (as % OSPW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td>LC50</td>
</tr>
<tr>
<td>Fathead minnow (<em>P. promelas</em>)</td>
<td>7 d</td>
<td>larval (&lt;24 h)</td>
<td>15 to 20</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Aquatic Invertebrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ceriodaphnia dubia</em></td>
<td>7-8 d</td>
<td>neonate (&lt;24 h)</td>
<td>10 to 80</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>48 h</td>
<td>neonate (&lt;24 h)</td>
<td>0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Macrophyte</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattail (<em>T. latifolia</em>)</td>
<td>7 d</td>
<td>2 d seedlings</td>
<td>-</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*a* Biomass (µg/live organism)

*b* Reproduction (average neonate/adult per day)

*c* No statistical difference (α = 0.05) observed in root growth
Figure 2.1 Approach for identifying constituents of concern (COCs) in OSPW and informing potential treatment processes to mitigate ecological risk.

Note: “numeric” criteria refer to constituent concentration comparisons to water quality guidelines/criteria and “narrative” criteria refer to “no toxics in toxic amounts” (USEPA, 1991).
Figure 2.2 Heteroatom classes present in OSPW using high-resolution Orbitrap MS.
Figure 2.3 Responses of *Ceriodaphnia dubia*, in terms of survival (top) and reproduction (bottom), in 7-8 d static/renewal tests following process based manipulations (PBM). Error bars represent standard error of replicates (n=3); Asterisks indicate significant differences (p<0.05; α=0.05) from controls.
CHAPTER THREE

PHOTOCATALYSIS OF A COMMERCIAL NAPHTENIC ACID IN WATER USING

FIXED-FILM TIO₂

Andrew D. McQueen¹, Ciera M. Kinley¹, Rebecca L. Kiekhaefer², Alyssa J. Calomeni¹;
John H. Rodgers Jr.¹ James W. Castle²

¹Department of Forestry and Environmental Conservation, 261 Lehotsky Hall, Clemson
University, Clemson, SC 29634, USA

²Department of Environmental Engineering & Earth Sciences, 445 Brackett Hall,
Clemson University, Clemson, SC 29634, USA

³Corresponding author: Andrew McQueen

Manuscript accepted in: Water, Air, and Soil Pollution
3.1 Abstract

Photolysis or photocatalysis may provide a process for mitigating ecological risks of naphthenic acids (NAs) contained in energy-derived waters such as refinery effluents and process waters. If effective, fixed-film TiO₂ photocatalysis of NAs could decrease operational expenses as well as capital costs for water treatment. The overall objective of this study was to measure rates and extents of photolysis and photocatalytic degradation of commercial NAs using bench-scale fixed-film TiO₂ and confirm changes in NA concentrations using sensitive vertebrate (fish = *Pimephales promelas*) and invertebrate (*Daphnia magna*) species. Specific objectives were to: 1) measure rates and extents of degradation of commercial (Fluka) NAs throughout an 8-hr duration of natural sunlight (“photolysis”) and natural sunlight in the presence of fixed-film TiO₂ (“photocatalysis”), and 2) measure changes in toxicity in terms of mortality with sentinel fish and microinvertebrate species. Bench-scale chambers using thin-film TiO₂ irradiated with natural sunlight were used to measure photocatalysis and HPLC was used to quantify NAs. After 4-hr in photocatalysis treatments, >92% decline was observed with an average removal rate of 15.5 mg/L hr⁻¹ and half-live of 2 hours. After 5-hrs of photocatalysis, there was no measurable NA toxicity for fish (*P. promelas*) or microinvertebrates (*D. magna*). Photocatalytic degradation achieved efficacious rates and extents of removal of Fluka NAs and eliminated acute toxicity to sentinel aquatic organisms, indicating the potential for application of this technology for mitigating ecological risks. Coupled with existing treatment processes (i.e. aerobic biodegradation), photocatalysis can augment rates and extents of NA removal from impacted waters.
3.2 Introduction

Naphthenic acids (NAs) are a complex group of organic acids associated with crude oils (Seifert and Teeter, 1969; Tomczyk et al., 2001) and energy derived process waters (Dorn, 1992; Allen, 2008). NAs are generally described by the formula \( \text{C}_n\text{H}_{2n+2}z\text{O}_2 \), where \( n \) is the number of carbons and \( Z \) is either zero or a negative even integer representing the hydrogen deficiency of the molecule due to rings or double bonds (Holowenko et al., 2002; Clemente and Fedorak, 2005). NAs are sources of toxicity in energy derived waters such as refinery effluents and oil sands process-affected waters (Dorn, 1992; Schramm, 2000), with adverse effects observed for fish, invertebrates, aquatic macrophytes, and microorganisms (Nero et al., 2006; Frank et al., 2008; Armstrong et al., 2008; Kavanagh et al., 2012; Leclair et al., 2013; Swigert et al., 2015). In addition, NAs are relatively persistent in water, with aerobic biodegradation half-lives for NAs in OSPW ranging from months to years (Scott et al., 2005; Han et al., 2009; Headley et al., 2010). To mitigate risks due to NAs, exposures must be sufficiently altered to eliminate toxicity to aquatic organisms. To effectively alter exposures of NAs, potential transformation pathways with reasonable rates and extents of removal should be thoroughly evaluated.

Naphthenic acids are susceptible to photolysis (USEPA, 2012; Headley et al., 2009), and the rate of this transformation process can be enhanced using catalysts (e.g. titanium dioxide \([\text{TiO}_2]\)). \( \text{TiO}_2 \) is widely used as a photocatalyst due to long term photostability, relative effectiveness, and stability in acidic and oxidative conditions (Bagheri et al., 2014). Degussa® and Aeroxide® P25 are commercial forms of \( \text{TiO}_2 \) previously
used in photocatalytic studies due to their relatively large surface area (~50 m²/g) and high ratio (4:1) of anatase to rutile (Wold, 1993). Photocatalytic degradation of NA has been accomplished using TiO₂ in aqueous suspensions (i.e. “slurries”) with both artificial ultraviolet (UV) irradiation (e.g. UV₂₅₄ lamps) and natural sunlight (McMartin et al., 2004; Headley et al., 2009; Mishra et al., 2010). Bench-scale studies have demonstrated that rates of NA degradation using photocatalysis are of practical significance (in terms of scalability), with half-lives achieved in hours (Headley et al., 2009; Mishra et al., 2010). However, some photocatalytic design features may limit full-scale application. Post-treatment recovery of aqueous suspensions of TiO₂ particles may be challenging (Kinsinger et al., 2015) and artificial UV is energy intensive (Parsons, 2004; Metcalf and Eddy, 2004). Immobilizing TiO₂ on a fixed-film eliminates the need to amend and recover catalyst offering greater flexibility in treatment design. In addition, natural sunlight could provide sufficient energy to accomplish photocatalytic degradation of NAs while decreasing operational costs of treatment.

To evaluate the feasibility of fixed-film photocatalysis irradiated by natural sunlight for achieving degradation of NAs, commercially available NAs were used for this study. To assess performance of photolysis and photocatalysis, rates and extents of degradation of Fluka NAs have been measured (McMartin et al., 2004; Headley et al., 2009; Mishra et al., 2010), providing an opportunity to compare results from this study. In addition, Fluka NAs have been well studied as a simplistic analogue to understand more compositionally complex mixtures of NAs present in energy-derived waters (Headley and McMartin 2004; Barrow et al., 2004; Scott et al., 2005; Rudzinski et al.,
Photolysis and photocatalytic oxidation can decrease concentrations and complexity of parent NA compounds (USEPA, 2012; McMartin et al., 2004; Headley et al., 2009); however, the question of post-treatment toxicity remains.

Decreased toxicity to sensitive species can confirm alteration of exposures of Fluka NAs achieved by fixed-film photocatalytic degradation. Sentinel species such as fathead minnow (*Pimephales promelas* Rafinesque) and microinvertebrate (*Daphnia magna* Straus) are relatively sensitive to NAs (Swigert et al., 2015; Kinley et al., 2016). Commercial NA 96-hr LC$_{50}$ for *P. promelas* is 5.6 mg NA/L and 48-hr EC$_{50}$ (immobilization) for *D. magna* is 20 mg NA/L (Swigert et al., 2015), demonstrating mortality is informative to assess changes in NA concentrations. Elimination of toxicity to these sensitive species in post-photocatalytic degradation samples can confirm mitigation of risks, supporting observations of NA degradation measured analytically.

The overall objective of this study was to measure rates and extents of photolysis and photocatalytic degradation of a commercially available (Fluka) NA using bench-scale fixed-film TiO$_2$ and confirm changes in NA concentrations using sensitive vertebrate (fish = *Pimephales promelas*) and invertebrate (*Daphnia magna*) species. To achieve this overall objective, specific objectives were to: 1) measure the rates and extents of removal of commercial (Fluka) NAs throughout an 8 hour duration of natural sunlight ("photolysis") and natural sunlight in the presence of fixed-film TiO$_2$ ("photocatalysis"), and 2) measure changes in toxicity after photolysis and photocatalysis treatments (in
terms of mortality) with sentinel fish (*P. promelas*) and microinvertebrate (*D. magna*) species in 96-hr static tests.

### 3.3 Materials and Methods

#### 3.3.1 Experimental Design

The experimental design included three treatments: 1) Fluka NAs irradiated by natural sunlight in the presence of TiO$_2$ film (“photocatalysis”), 2) Fluka NAs irradiated by natural sunlight without TiO$_2$ film (“photolysis”), and 3) Fluka NAs in the presence of TiO$_2$ film with no sunlight (“dark control”). Treatments were conducted outdoors near Clemson, SC USA (34°40'6.14"N, 82°50'52.02"W) in November with clear, sunny conditions. All treatments were conducted in 28x43 cm Sterilite® high-density polyethylene (HDPE) containers. For photocatalytic and dark control treatments, a thin-film of silicone caulk (DAP®; 100% silicone rubber sealant) was applied to a thickness of <0.2 cm to 28x43 cm on the bottom of the containers (Figure 3.1). Immediately after silicone application, TiO$_2$ (Aeroxide® P25; Fisher Scientific, Fairlawn NJ) particles were added to the surface of the film and air dried for 24-hr. The primary particle size of the TiO$_2$ was approximately > 45 μm and the specific surface area was 35 - 65 m$^2$/g with a crystalline composition of 10-20% rutile and 80-90% anatase. Water depths of <1.0 cm were used for sufficient light penetration based on preliminary experimentation. Dark control containers were shaded with opaque polypropylene covers. Aqueous samples were collected every hour through the 8-hr duration of the study in 60 mL amber glass vials for quantification of NAs and to conduct toxicity testing.
During the 8-hr treatments, *in situ* dissolved oxygen, pH, and conductivity of NA amended water were measured every hour using a YSI® Model 52 dissolved oxygen meter, Orion® Model 250A pH meter and Triode® electrode, and Orion Model 142 conductivity meter, respectively (Table 3.1). Alkalinity and hardness of aqueous samples were determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2012). Turbidity was measured using a Hach 2100AN Turbidimeter, (V2.2; APHA, 2012) and light intensity (LUX) was measured using a VWR® Traceable® dual-range light meter. Ultraviolet (UV) and visible irradiance were estimated using a methylene blue and peroxide actinometer (Alpert et al., 2010). A stock solution of 0.5 mg/L methylene blue with 15 µL of 30% hydrogen peroxide (Fisher Scientific) was used to estimate UV and visible light irradiance using sealed 1-cm quartz cuvettes at water depth intervals of 0, 0.5, and 1.0 cm. Measurements of methylene blue were performed using a SpectraMax®M2 spectrophotometer (Molecular Devices Corp. Sunnyvale, CA).

**3.3.2 Fluka Naphthenic Acid Exposure Preparation and Analysis**

Fluka NAs (Sigma-Aldrich; St. Louis, MO; Table 3.2) were used to prepare initial test concentrations and stock solutions in reconstituted moderately hard water (pH 8.2 ± 0.5 SU, alkalinity 65 ± 8 mg/L as CaCO₃, hardness 88 ± 10 mg/L as CaCO₃, conductivity 350 ± 20 µS/cm) which was prepared using reverse osmosis filtered water and reagent grade chemicals based on recommended culture methods (USEPA, 2002). The formulated water contained 5 mg/L CaCO₃, 102 mg/L NaHCO₃, 48 mg/L MgSO₄·7H₂O, 33 mg/L CaSO₄·2H₂O, 65 mg/L CaCl₂·2H₂O, 2 mg/L KCl, 0.8 mg/L KNO₃, 0.02 mg/L
K$_2$PO$_4$, and 0.002 mg/L of each Cu, Se, and Zn (from aqueous standards). All reagents were obtained from Fisher Scientific® (Pittsburgh, PA). Fluka NAs were added to moderately hard water in a 20 L HDPE Nalgene® container (initial nominal concentrations of 65 mg/L) and mixed to prepare a modified water accommodated fraction (WAF), where solutions were mixed with magnetic stir bars for 24 hours at a speed sufficient to create a vortex which extended 30-50% of the solution depth (OECD, 2000). Methods for NA derivatization and analysis were based on Yen et al. (2004) using high performance liquid chromatography (HPLC; Dionex, UltiMate-3000; Sunnyvale, CA). The HPLC analytical column was an Agilent LiChrospher 100 RP-18 (5 µm particle size, 125mm x 4 mm) with a guard column packed with 2 µm RP-18 solid phase material. Column temperature was maintained at 40°C with a sample injection volume of 60 µL mobilized with HPLC grade methanol (Fisher Scientific) at a flow rate of 60 µL per minute. The detection limit for this HPLC method is approximately 5 mg NA/L.

### 3.3.3 Removal Efficiency and Rate Calculations

Removal efficiencies (equation 1) were estimated using the following equation:

$$
\text{Removal efficiency (\%)} = \frac{[c_0] - [c]}{[c_0]} \times 100 \quad (1)
$$

Where, measured initial concentrations of NAs are designated as $[c_0]$ (mg/L) and $[C]$ (mg/L) is concentration of NAs at test completion. A linear relationship was observed between changes in NA concentration with time; therefore, removal rates (k=mg/L day$^{-1}$) were calculated using zero order kinetics, as the inverse slope of the line.
indicating change in concentration with change in time (hours). Correlation coefficients for the zero-order model are provided for each treatment. Half-lives were estimated using the following equation based on zero order kinetics:

\[ T_{1/2} = \frac{[C_0]}{2k} \quad (2) \]

Where, \( T_{1/2} \) is half-life (hours), \([C_0] \) (mg/L) is NA concentration at test initiation and \( k \) is degradation rate (mg/L day\(^{-1}\)).

### 3.3.4 Toxicity Testing

Photolysis and photocatalysis treatments were assessed for their ability to alter toxicity of Fluka NAs using sensitive sentinel species *P. promelas* and *D. magna*. Larval (<24h old) fish (*P. promelas*) and *D. magna* (<24h old) were obtained from cultures at Clemson University Aquatic Animal Research Laboratory (AARL). To conduct toxicity experiments, 50 mL aliquots of treatments were collected from reactors and organisms were exposed to treated waters in 30 mL HDPE chambers. Survival of *P. promelas* and *D. magna* were evaluated in 96-hr static/non-renewal toxicity tests conducted following a United States Environmental Protection Agency (USEPA) freshwater toxicity testing protocol with (n=30) organisms per exposure (USEPA, 2002; Table 3.3). Water characteristics (dissolved oxygen, pH, conductivity, alkalinity, and hardness) of test waters were measured at test initiation and completion using methods described in Table 3.1. Reference toxicity tests were conducted with copper sulfate (CuSO\(_4\)-5H\(_2\)O) to confirm the health of test organisms for quality assurance (USEPA, 2002) using the same toxicity testing procedures.

### 3.3.5 Statistical Analysis
Data were tested for normal distribution and homogeneity of variance using Chi-square and Bartlett’s tests, respectively. Normally distributed, homogeneous data were analyzed by one-way analysis of variance (ANOVA). Differences among treatments were determined using follow-up pairwise comparisons and contrasts using linear models. Differences were considered significant at $p \leq 0.05$ (JMP v11; SAS Institute Inc., Cary, NC, USA).

### 3.4 Results and Discussion

#### 3.4.1 Exposure conditions for photolysis and photocatalysis

Mean measured initial concentrations of NAs for aqueous samples were 63 ($\pm$9), 65 ($\pm$5), and 64 ($\pm$10) mg NA /L for photocatalysis, photolysis, and dark controls, respectively. Over the 8-hr treatment period in direct sunlight (excluding the dark control), measured light intensity ranged from 11,500 (at 8-hr) to 109,700 LUX (at 4-hr). There was no measurable incident light in dark controls. UV/Visible irradiance ranged from 12.6 W/m² at water surface to 6.27 W/m² at 1.0 cm water depth, respectively, indicating rapid light attenuation with water depth (attenuation coefficient $[K_d] = 0.69$ cm⁻¹). Ambient air temperatures during the 8-hr experiment ranged from 7.5 to 21.6°C and water temperatures in treatments and controls ranged from 13-19°C. Water containing NAs had no detectable turbidity ($<0.1$ NTU). pH in treatments and controls ranged from 8.02-8.26. *In situ* water characteristics measured during the 8-hr treatment durations (e.g. temperature, pH, DO, conductivity) did not differ among treatments (Table 3.4).
3.4.2 Photocatalysis

After 4-hr in photocatalysis treatments, NA concentrations declined to below detection limit (method detection limit = 5 mg/L), resulting in >92% removal (Figure 3.2). Data were fit to a zero-order model with a correlation coefficient ($R^2$) of 0.9792, based on 5 data points from Time 0 (test initiation) to Time 5 (hour 4). The calculated NA removal rate ($k$) and half-life for photocatalysis were 15.5 mg/L hr$^{-1}$ and 2.0-hr, respectively (Table 3.5). Results from this study are similar to results for Fluka NAs using TiO$_2$ “slurries” in photocatalysis studies irradiated with sunlight and UV$_{254}$ bulbs (Headley et al., 2009; Mishra et al., 2010; Table 3.6). For example, using sunlight, Headley et al. (2009) achieved 75% Fluka NA removal in 8-hr (initial concentration of 46 mg/L NA) using TiO$_2$ slurries with concentrations of 2 g TiO$_2$/L. Mishra et al. (2010) used aqueous suspensions of TiO$_2$ (at 0.3 g TiO$_2$/L concentrations) irradiated with UV$_{254}$ (8W lamps) and achieved first order rate coefficients ranging from 0.05 to 0.34 hr$^{-1}$ for Fluka NAs in South Saskatchewan River water and deionized water, respectively. Under the same laboratory conditions, Mishra et al. (2010) achieved relatively rapid degradation of NAs derived from oil sands process affected water (OSPW), with half-lives of 1.55 and 4.8-hr for deionized water and South Saskatchewan River water, respectively. Generally, results were similar among fixed-film and slurries of TiO$_2$, irradiance from sunlight and artificial UV$_{254}$, and Fluka and OSPW NAs (Headley et al., 2009; Mishra et al., 2010). Among these treatments, measured photocatalysis rates and extents of removal are of practical significance with NA half-lives achieved in hours.

3.4.3 Photolysis and Dark Control
In photolysis treatments, 51% removal of the initial NA concentration (65 mg/L ±5 SD) was achieved after 8-hr (final concentration 32 mg NA/L ±11 SD; Figure 3.2; Table 3.5). NA concentrations after 4-hr (α =0.05; p<0.001) were significantly different from test initiation, which corresponded with maximum measured light intensity during the experimental duration (109,700 LUX at 4-hr). Data were fit to a zero-order model with a correlation coefficient ($R^2$) of 0.9122, based on 9 data points from Time 0 (test initiation) to Time 9 (hour 8). The calculated NA removal rate (k) and half-life for photolysis were 4 mg/L hr$^{-1}$ and 8.1-hr, respectively (Table 3.5). Since one NA half-life was achieved with this treatment (rather than reaching the detection limit), this removal rate (4 mg/L hr$^{-1}$) applies to the first measured half-life only, and application of this estimated rate beyond the measured bounds in this study should be undertaken carefully. These photolysis results using Fluka NAs in sunlight are similar to modeled half-lives estimated for photo-degradation of commercial NAs (USEPA, 2012). Empirical models indicate photo-degradation half-lives for 1- to 4-ring structures with molecular weights of 254–325 amu (as compared to 210-250 amu of the NA used in this study) range from 3 to 6.8-hr (USEPA, 2012). Headley et al. (2009) estimated < 3% removal of Fluka NAs (initial concentration 46 mg/L) in 8-hr photolysis (no TiO$_2$) experiments irradiated with sunlight in Milli-Q water. A number of factors influence photolysis of NAs, including structure of the molecule (Headley et al., 2009), and exposure conditions (i.e. sunlight intensity, water depth, turbidity; Kirk, 1994). In this study, conditions that may confound photolysis and photocatalysis such as water depth, pH, and turbidity were managed to minimize their effects.
Dark controls containing fixed-film TiO₂ were used to account for sorption and volatilization of NAs, which may influence the measured concentration of NAs. Based on a one-way ANOVA, NA concentrations in dark controls did not differ from test initiation (64 mg/L ±10 SD) to completion at 8-hr (56 mg/L ±4 SD; α =0.05; p= 0.8487). In the absence of light, minimal decrease in NA concentration was anticipated due to the limited volatility (1.1 x 10⁻⁷ to 7.1 x 10⁻⁶ mm Hg at 25°C) and sorption (log Kd <0.5 [quartz sand]) of naphthenic acids at the experimental pH of 8.02-8.26 (Schramm, 2000; USEPA, 2012).

3.4.4 Toxicity testing to confirm changes in NA exposures

Toxicity tests using fish and microinvertebrates were used to confirm degradation of NAs within treatments. For all treatments at test initiation, complete mortality (0% survival) of both *D. magna* and *P. promelas* was observed in 96-hr exposures (NA concentrations 63-65 mg/L). In photocatalysis treatments, toxicity was completely eliminated (100% survival) for both *D. magna* and *P. promelas* after 5-hr (Table 3.7). Both photolysis and dark control treatments did not have any measurable change in toxicity after 8-hr durations (i.e. 100% mortality throughout treatments).

In this study, larval fish (*P. promelas*) were more sensitive to Fluka NA exposures than the microcrustacean *D. magna*, with 0% and 57% survival observed for *P. promelas* and *D. magna*, respectively, after 3-hr of photocatalytic treatment. Changes in percent mortalities with time observed for *D. magna* and *P. promelas* in this study provided an opportunity to compare with reported endpoint estimations (i.e. EC₅₀s and LC₅₀s) for commercial NAs (Swigert et al., 2015; Kinley et al. 2016). In photocatalysis treatments,
D. magna mortality was 43% in Fluka NA concentrations of 15 mg/L NA (±5), similar to the reported 48-hr EC₅₀ (immobilization) of 20 mg/L (17-23 mg/L C.I.) for D. magna exposed to Merichem NAs (Swigert et al., 2015). After photocatalysis treatment in this study, mortality of P. promelas declined from 100% to 40% after 4-hr and 0% mortality was observed after 5-hr with measured NA concentrations below the analytical detection limit of 5 mg/L NA. Swigert et al. (2015) measured a 96-hr LC₅₀ for juvenile P. promelas of 5.6 mg/L Merichem NAs. Comparatively, Kinley et al. (2016) observed a 7-day LC₅₀ for larval P. promelas of 1.9 mg/L as Fluka NAs. In this study, coupling bioassay response data with analytical quantification of NAs provided a robust approach for discerning changes in NA exposures and mitigation of ecological risks.

3.5 Conclusions

Greater than 90% removal of Fluka NAs (initial concentration 63 mg/L) was achieved in 4-hr with photocatalysis in fixed-film (TiO₂) reactors in direct sunlight. Photocatalysis also eliminated acute toxicity to sentinel species, with mortality decreasing from 100% to 0% after 5-hr of photocatalytic treatment for fathead minnow (Pimephales promelas) and after 4-hr for the freshwater invertebrate (Daphnia magna). In this experiment, measuring responses of aquatic organisms concomitantly with analytical quantification of Fluka NAs over time confirmed alteration of exposures as well as mitigation of risk. Fixed-film TiO₂ application may provide an alternative solution for scaling the technology for larger applications. Photocatalytic degradation using fixed-film TiO₂ irradiated with sunlight achieved efficacious rates and extents of
removal of Fluka NAs, indicating the potential for application of this technology for mitigating ecological risks associated with NAs.

3.6 Acknowledgements

Funding support for this research was provided by Shell Canada Ltd. and Suncor Energy. The authors are also grateful to Dr. Wayne Chao of Clemson University for providing analytical support.
3.7 References


Table 3.1 Methods for water characteristics, light, and NA concentrations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Method Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Electrometric method 4500-H⁺ B: Orion model 420A (APHA, 2007)</td>
<td>0.01 SU</td>
</tr>
<tr>
<td>Temperature</td>
<td>Laboratory method 2550 B: Orion Model 420A (APHA, 2007)</td>
<td>0.01 °C</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>Membrane electrode method 4500-O G: YSI Model 52 (APHA, 2007)</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Laboratory method 2510 B: YSI 30 (APHA, 2007)</td>
<td>0.1 μS/cm</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Titration method 2320 B (APHA, 2007)</td>
<td>2 mg/L as CaCO3</td>
</tr>
<tr>
<td>Hardness</td>
<td>EDTA Titrimetric Method 2340 C (APHA, 2007)</td>
<td>2 mg/L as CaCO3</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Nephelometric Method 2130 B (APHA, 2007)</td>
<td>0.1 NTU</td>
</tr>
<tr>
<td>Light Intensity</td>
<td>VWR® Traceable® Light Meter</td>
<td>0.1 LUX</td>
</tr>
<tr>
<td>UV/ Visible Irradiance</td>
<td>Methylene blue and peroxide actinometer (Alpert et al., 2010)</td>
<td>1 W/m²</td>
</tr>
<tr>
<td>Naphthenic acid</td>
<td>HPLC; Derivatization based on Yen et al. (2004)</td>
<td>5 mg/L</td>
</tr>
</tbody>
</table>
Table 3.2 Physical and chemical characteristics of Fluka naphthenic acids (Sigma-Aldrich)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>General Characteristic</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>1338-24-5 (CAS No)</td>
<td>Sigma-Aldrich, 2015</td>
</tr>
<tr>
<td>Color</td>
<td>Pale yellow, dark amber</td>
<td>Sigma-Aldrich, 2015</td>
</tr>
<tr>
<td>Physical State</td>
<td>Viscous liquid</td>
<td>Sigma-Aldrich, 2015</td>
</tr>
<tr>
<td>Molecular weight\textsuperscript{b}</td>
<td>210-250 amu</td>
<td>Brient et al., 1995</td>
</tr>
<tr>
<td>Water solubility</td>
<td>88.1 mg/L at pH 7.5</td>
<td>API, 2012</td>
</tr>
<tr>
<td></td>
<td>1.1 x 10\textsuperscript{-7} to 7.1 x 10\textsuperscript{-6} mm Hg at 25°C</td>
<td>API, 2012</td>
</tr>
<tr>
<td>Vapor pressure</td>
<td>25°C</td>
<td>USEPA, 2012</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>~4 at pH 1</td>
<td>Schramm, 2000</td>
</tr>
<tr>
<td>octanol/ water</td>
<td>~2.4 at pH 7</td>
<td>Schramm, 2000</td>
</tr>
<tr>
<td>(Log Kow)\textsuperscript{c}; pH dependent</td>
<td>&lt; 0.1 at pH 10</td>
<td>Schramm, 2000</td>
</tr>
<tr>
<td>Density</td>
<td>0.92 g/mL</td>
<td>Sigma-Aldrich, 2015</td>
</tr>
<tr>
<td>Viscosity</td>
<td>22 mm\textsuperscript{2}/s</td>
<td>Sigma-Aldrich, 2015</td>
</tr>
<tr>
<td>pKa</td>
<td>5 to 6</td>
<td>Brient et al., 1995</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Alkylated cyclopentane carboxylic acids (mixture)
\textsuperscript{b}Average molecular weight for refined naphthenic acids
\textsuperscript{c}Weathered naphthenic acid mixture; for OSPW NAs
Table 3.3 Summary of toxicity test conditions for *P. promelas* and *D. magna* (USEPA, 2002).

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test type</td>
<td>static non-renewal</td>
</tr>
<tr>
<td>Endpoint measured&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mortality</td>
</tr>
<tr>
<td>Test Duration (hours)</td>
<td>96</td>
</tr>
<tr>
<td>Test temperature (°C)</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Test chamber</td>
<td>30 mL HDPE chamber</td>
</tr>
<tr>
<td>Test water</td>
<td>Formulated moderately hard water&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of organisms/exposure</td>
<td>30</td>
</tr>
<tr>
<td>Number of organisms/chamber&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>Age of organisms</td>
<td>&lt; 24 h&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>16 h light, 8 h dark</td>
</tr>
</tbody>
</table>

<sup>a</sup>Immobilization  
<sup>b</sup>USEPA, 2002  
<sup>c</sup>3 replicates  
<sup>d</sup>Post-hatch for *P. promelas*; post-brood for *D. magna*
Table 3.4 Measurements of water characteristics during photocatalysis and photolysis treatments (and dark controls) and toxicity testing.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temp. (°C)</th>
<th>pH (S.U.)</th>
<th>DO (mg O₂/L)</th>
<th>Conductivity (µS/cm)</th>
<th>Alkalinity (mg CaCO₃/L)</th>
<th>Hardness (mg CaCO₃/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In Situ</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photocatalysis</td>
<td>13-19</td>
<td>8.02-8.22</td>
<td>8 ± 1</td>
<td>340-355</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Photolysis</td>
<td>13-19</td>
<td>8.06-8.26</td>
<td>8 ± 1</td>
<td>345-355</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dark Control</td>
<td>13-19</td>
<td>8.08-8.23</td>
<td>8 ± 1</td>
<td>345-352</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Toxicity Testing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photocatalysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. promelas</em></td>
<td>25 ± 1</td>
<td>8.08-8.26</td>
<td>8 ± 1</td>
<td>335-362</td>
<td>76-80</td>
<td>152-160</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>25 ± 1</td>
<td>8.05-8.26</td>
<td>8 ± 1</td>
<td>340-366</td>
<td>70-78</td>
<td>155-164</td>
</tr>
<tr>
<td>Photolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. promelas</em></td>
<td>25 ± 1</td>
<td>8.05-8.22</td>
<td>8 ± 1</td>
<td>346-365</td>
<td>70-80</td>
<td>152-160</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>25 ± 1</td>
<td>8.11-8.21</td>
<td>8 ± 1</td>
<td>344-353</td>
<td>76-84</td>
<td>155-160</td>
</tr>
<tr>
<td>Dark Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. promelas</em></td>
<td>25 ± 1</td>
<td>8.12-8.28</td>
<td>8 ± 1</td>
<td>345-360</td>
<td>68-82</td>
<td>152-160</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>25 ± 1</td>
<td>8.10-8.22</td>
<td>8 ± 1</td>
<td>344-364</td>
<td>70-85</td>
<td>155-160</td>
</tr>
</tbody>
</table>

*a measured at 1 hour sampling intervals (n=8)

*b measured at test completion (n=3)
Table 3.5 Fluka naphthenic acid removal rate coefficients and extents for photocatalysis, photolysis, and dark control treatments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Photocatalysis</td>
</tr>
<tr>
<td>Initial [NA] mg/L (±SD)</td>
<td>63 (± 9)</td>
</tr>
<tr>
<td>Ending [NA] mg/L (±SD)</td>
<td>BDL&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Removal Efficiency, %</td>
<td>&gt;92</td>
</tr>
<tr>
<td>Rate Equation&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Y = -15.502x +</td>
</tr>
<tr>
<td></td>
<td>64.727</td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.9792</td>
</tr>
<tr>
<td>k average (mg/L hour&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>15.5</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (hour)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

NA = Not applicable
<sup>a</sup>BDL = Below Detection Limit, detection limit = 5 mg/L NA
<sup>b</sup>Zero Order Rate Equation; Linear plot [C] versus time (hours), slope = -k
<sup>c</sup>Ending [NA] not statistically different from initial [NA] in dark control (<i>α</i> =0.05; <i>p</i> = 0.8487)
<sup>d</sup>Estimation based on 1 observed half-life

Note: Removal rate coefficient calculated from best fit using five data points for photocatalysis (Time 0-4 on Figure 3.2) and nine data points for photolysis (Time 0-8 on Figure 3.2)

---

87
<table>
<thead>
<tr>
<th>Treatment (Condition)</th>
<th>Initial NA (mg/L)</th>
<th>Ending NA (mg/L), Duration</th>
<th>Removal Efficiency (%)</th>
<th>Removal Rate* (mg/L hr⁻¹)</th>
<th>Estimated Half Life Values (hours)</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td>8-hr</td>
<td>&gt; 92</td>
<td>15.5</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Photocatalysis (sunlight; TiO₂ film)</td>
<td>63 (± 9)</td>
<td>BDL, 8-hr</td>
<td>&gt; 92</td>
<td>15.5</td>
<td>2.0</td>
<td>Current study</td>
</tr>
<tr>
<td>Photocatalysis (sunlight; TiO₂ slurryᵃ)</td>
<td>46</td>
<td>9, 8-hr</td>
<td>~80</td>
<td>-</td>
<td>-</td>
<td>Headley et al., 2009</td>
</tr>
<tr>
<td>Photocatalysis (UVᵇ; TiO₂ slurryᶜ)</td>
<td>40-100</td>
<td>NA; 5-hr</td>
<td>-</td>
<td>0.34</td>
<td>2.04</td>
<td>Mishra et al., 2010</td>
</tr>
<tr>
<td>Photocatalysis (UVᵇ; TiO₂ slurryᶜ; river waterᵈ)</td>
<td>40-100</td>
<td>NA; 5-hr</td>
<td>-</td>
<td>0.05</td>
<td>13.86</td>
<td>Mishra et al., 2010</td>
</tr>
<tr>
<td>Photolysis (sunlight)</td>
<td>65 (± 5)</td>
<td>32 (± 11), 8-hr</td>
<td>51</td>
<td>4.0</td>
<td>8.0</td>
<td>Current study</td>
</tr>
<tr>
<td>Photolysis (sunlight)</td>
<td>46</td>
<td>45, 8-hr</td>
<td>&lt; 3</td>
<td>-</td>
<td>-</td>
<td>Headley et al., 2009</td>
</tr>
<tr>
<td>Photolysis (simulated solar radiationᵉ; river waterᶠ)</td>
<td>50</td>
<td>NA, 168-hr</td>
<td>-</td>
<td>-</td>
<td>8120 ± 100</td>
<td>McMartin et al., 2004</td>
</tr>
<tr>
<td>Photolysis (UVᵇ)</td>
<td>40-100</td>
<td>NA, 5-hr</td>
<td>-</td>
<td>0.22</td>
<td>3.15</td>
<td>Mishra et al., 2010</td>
</tr>
<tr>
<td>Photolysis (UVᵇ; river waterᶠ)</td>
<td>40-100</td>
<td>NA, 5-hr</td>
<td>-</td>
<td>0.04</td>
<td>17.33</td>
<td>Mishra et al., 2010</td>
</tr>
<tr>
<td>Photolysis (UVᶜ; river waterᶠ)</td>
<td>10</td>
<td>NA, 8-hr</td>
<td>-</td>
<td>-</td>
<td>1050 ± 20</td>
<td>McMartin et al., 2004</td>
</tr>
<tr>
<td>Photolysis (UVᶜ; river waterᶠ)</td>
<td>50</td>
<td>NA, 8-hr</td>
<td>-</td>
<td>-</td>
<td>1200 ± 40</td>
<td>McMartin et al., 2004</td>
</tr>
<tr>
<td>Photolysis (UVᶜ; river waterᶠ)</td>
<td>10</td>
<td>NA, 8-hr</td>
<td>-</td>
<td>-</td>
<td>50 ± 1</td>
<td>McMartin et al., 2004</td>
</tr>
<tr>
<td>Photolysis (UVᶜ; river waterᶠ)</td>
<td>50</td>
<td>NA, 8-hr</td>
<td>-</td>
<td>-</td>
<td>52 ± 1</td>
<td>McMartin et al., 2004</td>
</tr>
<tr>
<td>Dark Control (TiO₂ film)</td>
<td>64 (± 10)</td>
<td>56 (± 4), 8-hr</td>
<td>~2</td>
<td>-</td>
<td>-</td>
<td>Current study</td>
</tr>
<tr>
<td>Dark Control (TiO₂ slurryᵃ)</td>
<td>46</td>
<td>45, 8-hr</td>
<td>&lt; 3</td>
<td>-</td>
<td>-</td>
<td>Headley et al., 2009</td>
</tr>
</tbody>
</table>

BDL = below detection limit (<5 mg/L)
NA = data not available
ᵃTiO₂ (Degussa P25) slurry concentration = 2 g/L
ᵇFluka NA concentrations prepared in unaltered Athabasca River Water
ᶜPhilips medium pressure black light bulb; 300-400 nm (15W)
ᵈLow pressure Phillips® UV<sub>254</sub> tube
ᵉSimulated full spectrum solar radiation with incandescent and fluorescent lamps
ᶠFluka NA concentrations prepared in unaltered Athabasca River Water
Note: Comparison studies quantified Fluka NAs using electrospray ionization mass spectrometry (ESI-MS)
NA mixtures prepared in South Saskatchewan River water (Saskatoon, SK).
Note: pH not reported in McMartin et al. (2004), Headley et al. (2009); Mishra et al. (2010)

*Zero and first order rates/coefficients not directly comparable
Table 3.7 Summary of mean NA concentrations (mg/L) and 96-hr percent survival for microinvertebrates (*D. magna*) and fish (*P. promelas*) for photocatalysis, photolysis, and dark control treatments. NA concentrations with different letters are significantly different (p< 0.05).

| Treatment Duration (hours) | Photocatalysis | | Photolysis | | Dark Control |
|----------------------------|----------------|----------------|----------------|----------------|
|                            | NAs (±SD) | % Survival | NAs (±SD) | % Survival | NAs (±SD) | % Survival |
|                            | D. Magna | P. Promelas | D. Magna | P. Promelas | D. Magna | P. Promelas |
| 0                          | 63 (±9)    | A           | 65 (±5)    | A           | 64 (±10) | A           |
| 1                          | 54 (±12)   | AB          | 57 (±15)   | AB          | 60 (±12) | A           |
| 2                          | 32 (±8)    | BC          | 55 (±10)   | AB          | 67 (±8)  | A           |
| 3                          | 15 (±5)    | C           | 47 (±8)    | ABC         | 65 (±5)  | A           |
| 4                          | BDL        | 100         | 38 (±10)   | BC          | 63 (±5)  | A           |
| 5                          | BDL        | 100         | 44 (±5)    | BC          | 58 (±5)  | A           |
| 6                          | BDL        | 100         | 38 (±10)   | BC          | 58 (±2)  | A           |
| 7                          | BDL        | 100         | 33 (±12)   | C           | 54 (±7)  | A           |
| 8                          | BDL        | 100         | 32 (±11)   | C           | 56 (±4)  | A           |

BDL = below detection limit (<5 mg/L)
Figure 3.1 Schematic of photocatalytic reaction chamber.
Figure 3.2 Concentrations of Fluka naphthenic acids (mg/L) with time in photocatalysis, photolysis, and dark control treatments.

Note: Incident light intensity (LUX) on the secondary axis corresponds to photocatalysis and photolysis treatments.
CHAPTER FOUR

INFLUENCE OF COMMERCIAL (FLUKA) NAPHTHENIC ACIDS ON ACID VOLATILE SULFIDE PRODUCTION AND Divalent Metal Precipitation

Andrew D. McQueen¹,³; Ciera M. Kinley¹; John H. Rodgers Jr.¹ Vanessa Friesen², Jordyn Bergsveinson², Monique C. Haakensen²

¹ Department of Forestry and Environmental Conservation, 261 Lehotsky Hall, Clemson University, Clemson, SC 29634, USA

² Contango Strategies Limited, LFK Biotechnology Complex, 15-410 Downey Road, Saskatoon, SK S7N 4N1

³ Corresponding author: Andrew McQueen

Manuscript accepted in: Ecotoxicology and Environmental Safety
4.1 Abstract

Energy-derived waters containing naphthenic acids (NAs) are complex mixtures often comprising a suite of potentially problematic constituents (e.g. organics, metals, and metalloids) that need treatment prior to beneficial use, including release to receiving aquatic systems. It has been suggested previously that NAs can have biostatic or biocidal properties that could inhibit microbially driven processes (e.g. dissimilatory sulfate reduction) used to transfer or transform metals in passive treatment systems (i.e. constructed wetlands). The overall objective of this study was to measure the effects of a commercially available (Fluka) NA on sulfate-reducing bacteria (SRB), production of sulfides (as acid-volatile sulfides [AVS]), and precipitation of divalent metals (i.e. Cu, Ni, Zn). These endpoints were assessed following 21-d aqueous exposures of NAs using bench-scale reactors. After 21-days, AVS molar concentrations were not statistically different (p<0.0001; α=0.05) among NA treatments (10, 20, 40, 60, and 80 mg NA/L) and an untreated control (no NAs). Extent of AVS production was sufficient in all NA treatments to achieve $\sum\text{SEM:AVS} < 1$, indicating that conditions were conducive for treatment of metals, with sulfide ligands in excess of SEM (Cu, Ni, and Zn). In addition, no adverse effects to SRB (in terms of density, relative abundance, and diversity) were measured following exposures of a commercial NA. In this bench-scale study, dissimilatory sulfate reduction and subsequent metal precipitation were not vulnerable to NAs, indicating passive treatment systems utilizing sulfide production (AVS) could be used to treat metals occurring in NAs affected waters.
4.2 Introduction

Energy-derived waters containing naphthenic acids (NAs) are complex mixtures often containing a suite of potentially problematic constituents (e.g. organics, metals, and metalloids) that need treatment prior to beneficial use, including release to receiving aquatic systems (Seifert and Teeter, 1969; Dorn 1992; Tomczyzk et al., 2001; Allen 2008a). NAs are a class of organic acids (generally described by the formula C\textsubscript{n}H\textsubscript{2n+z}O\textsubscript{2}) that are sources of toxicity in these process-affected waters (Dorn 1992; Schramm, 2000), with adverse effects observed for fish, macroinvertebrates, macrophytes, and microorganisms (Nero et al., 2006; Frank et al., 2008; Armstrong et al., 2008; Jones et al., 2011; Kavanagh et al., 2012; Leclair et al., 2013; Swigert et al., 2015). To treat impacted waters, passive treatment approaches (e.g. constructed wetlands) are among technologies considered for transfer and transformation of problematic constituents (e.g. metals, organics; Allen 2008b; Foote 2012; Toor et al., 2013; Brown and Ulrich 2015). Microbial activity is an important contributor to biogeochemical processes and elemental cycling that occur in constructed wetlands (Rodgers and Castle 2008; Haakensen et al., 2015). Microbially mediated processes can be promoted in specifically designed constructed wetlands to treat problematic constituents contained in process-affected waters (Huddleston and Rodgers 2008; Spacil et al., 2011; Pham et al., 2011). Divalent metals (e.g. Cu, Cd, Pb, Ni, and Zn) can be sequestered as sulfide minerals with sulfides produced through dissimilatory sulfate reduction, altering the solubility and bioavailability of metals (Kanagy et al., 2008; Murray-Gulde et al., 2008; Rodgers and Castle 2008). To effectively design and implement constructed wetlands, potential
adverse effects of co-occurring constituents on microbially mediated pathways (e.g. sulfate reduction) must be understood. NAs occurring in process water can exhibit adverse effects on a laboratory strain of *Vibrio fischeri* commonly used in toxicity testing (i.e. Microtox EC\textsubscript{50} values ranging from 12 to 65 mg/L NAs; Rogers et al., 2002; Frank et al., 2008). There is evidence that sulfate-reducing bacteria (SRB) and microbial sulfate reduction occur in process waters and substrates (fine tailings) containing NAs (Holowenko et al., 2000; Salloum et al., 2002; Quagraine et al., 2005; Ramos-Padron et al., 2011; Liu et al., 2015); however, there are limited data measuring effects of NAs on production of acid volatile sulfides (AVS) and subsequent metal precipitation. To effectively design and implement passive or semi-passive water renovation strategies utilizing microbially driven AVS production, potential adverse effects on SRB (e.g. *Desulfovibrio*, *Desulfosporosinus*, *Desulfobulbus*) following exposures to NAs must be investigated.

Precipitation of metals via sulfides is a naturally occurring and microbially mediated pathway that has been successfully implemented in constructed wetlands for treatment of a variety of impaired waters (e.g. refinery effluents, oilfield produce waters, acid and neutral mine drainage; and urban and industrial stormwaters; Gillespie et al., 1999; Murray-Gulde et al., 2003; Johnson et al., 2008; Horner et al., 2012; Haakensen et al., 2015). Treatment is achieved by targeting the lithic biogeochemical sequestration of divalent metals through sulfide (i.e. S\textsuperscript{2-}, HS\textsuperscript{-}) precipitation as mineralized species (e.g. chalcocite [CuS], covellite [CuS\textsubscript{2}]). These sulfide bound species are relatively insoluble (CuS; k\textsubscript{sp}=10\textsuperscript{-16}; Stumm and Morgan 1996), and are transferred from the water column
into the hydrosol as non-bioavailable fractions (Murray-Gulde et al., 2003; Huddleston and Rodgers 2008). Anaerobic conditions with relatively low oxidation-reduction potentials (ORP; -250 to -100 mV) are necessary for promoting anaerobic metabolisms in bacteria which oxidize organic matter, producing electrons which reduce sulfate to hydrogen sulfide (H₂S) and other reduced sulfide species (i.e. bisulfide ion (HS⁻), sulfide ion [S²⁻]; Mitsch and Gosselink 2007). AVS is a commonly used operational measurement of the amount of sulfide in sediments (Allen et al., 1993; Rickard and Morse 2005). AVS is a measure of the reactive “pool” of sulfides available as ligands that can be compared to the molar ratio of simultaneously extracted metals (SEM; Di Toro et al., 1992). SEM and AVS ratios as a predictive measure have been used extensively for estimating ecological risk of divalent metal exposures (Di Toro et al., 1992; Ankley et al., 1996; Boothman et al. 2001; Burton et al. 2005). SEM:AVS ratios are useful for constructed wetland treatment design and monitoring, indicating the divalent metal treatment removal capacity which is likely to occur (Rodgers and Castle, 2008).

In this study, multiple lines of evidence were used to discern potential effects of NAs on SRBs and production of sulfides. Concentrations of AVS, SEM, and aqueous metals (Cu, Ni, and Zn) following exposures of NAs were measured. In addition, microbial analyses provided valuable information to discern changes in population densities, relative abundance, and diversity (Haakensen et al., 2015). Changes in total quantity, relative abundance, and types of SRBs may implicate shifts in target treatment processes (i.e. dissimilatory sulfate reduction). Commercially available (Fluka) NAs were used for this study to provide a reliable and reproducible source of NAs. Fluka NAs have been
well studied as a simplistic analogue to understand more compositionally complex mixtures of NAs present in energy-derived waters (Headley and McMartin 2004; Barrow et al., 2004; Scott et al., 2005; Rudzinski et al., 2002; Lo et al., 2006; Armstrong et al., 2008; Headley et al., 2010). In general, commercial NAs are more potently toxic to sentinel organisms (i.e. macroinvertebrates, fish, plants; Swigert et al., 2015; Kinley et al., 2016) as compared to NAs in process-affected waters. Therefore, in this study, commercial NAs offer a conservative exposure to evaluate the vulnerabilities of SRB.

The overall objective of this research was to measure responses of sulfate-reducing bacterial assemblages and microbially mediated treatment pathways (e.g. acid volatile sulfide concentrations) following a series of exposures to a commercial (Fluka) NA in bench-scale reactors. To achieve this overall objective, specific objectives were to: 1) measure relationships of acid-volatile sulfide (AVS), simultaneously extractable metal (SEM), and aqueous metal (copper, nickel, and zinc) concentrations following 21-d exposures to NAs, and 2) measure responses of sulfate-reducing bacterial (SRB) assemblages in terms of relative abundance, diversity, and density (most-probable number) in sediment to 21-d exposures of NAs.

4.3 Materials and Methods

4.3.1 Bench-scale exposure chambers

Bench-scale experiments were conducted in 1-L borosilicate glass jars with 500 g of sediment and 500 ml of reconstituted moderately hard water (pH 7.7 ± 0.5 SU, alkalinity 65 ± 8 mg/L as CaCO₃, hardness 88 ± 10 mg/L as CaCO₃, conductivity 350 ± 20 µs/cm;
Surficial sediment samples from 0-10 cm depth were collected from the Clemson Aquaculture Facility (34°40'6.14"N, 82°50'52.02"W) in a shallow wetland (water depth <30 cm) dominated by cattail (Typha latifolia). Prior to experiment initiation, sediment samples were added to the exposure chambers and amended with organic matter (5% wheat hay [w/w]) to support dissimilatory sulfate reduction by targeting a negative ORP (i.e. -100 to -250 mV; Brookins, 1988). Measurements of sediment characteristics included particle size distribution, percent solids, organic matter content, and cation exchange capacity (Table 4.1). Sediment used in this experiment was mostly silt (41%) and clay (32%), with a sand content of approximately 27%. Organic matter content, percent solids, and pH of the sediment were 10.8 %, 46 %, and 5.4 SU, respectively. Major cations in the sediment were 788 mg Ca/kg, 86 mg Mg/kg, and 46 mg Na/kg, with a cation exchange capacity of 8.2 mEq/100 g. Prior to initiation, divalent metals measured in the un-amended sediment included 5.4 mg Cu/kg, 1.18 mg Ni/kg, and 35.1 mg Zn/kg. In addition, there was no detectible AVS (MDL = 0.002 µmol/g) in sediments prior to test initiation. Response parameters (e.g. AVS, SEM, and aqueous metal concentrations) were measured at 3-d intervals for 21-days. All exposures were conducted at ambient room temperatures ranging from 22-24°C.

Experiments were designed as static/renewal systems to simulate the hydraulic retention time (HRT) of a constructed wetland treatment system. Every 3-days, water renewals were conducted for each treatment chamber by removing ~50% of the water volume with an electronic pipet (Fisher Scientific; Pittsburgh, PA) fitted with a 100 mL glass tip. Untreated controls received no amendments of NAs. Negative controls were
prepared using a SRB biocide, tetrakis (hydroxymethyl) phosphonium sulfate (THPS; Sigma-Aldrich; St. Louis, Mi), which is commonly used to inhibit growth of SRBs in the petroleum industry (Downward et al., 1997). THPS was added to negative controls (termed “SRB biocide”) at nominal concentrations of 25 mg THPS/L at each water renewal.

4.3.2 Naphthenic acid exposures and analyses

To achieve a series of nominal exposure concentrations of 10, 20, 40, 60, and 80 mg/L NA, a commercially available (Fluka) NA (Sigma-Aldrich; St. Louis, Mo) was pipetted into deoxygenated laboratory formulated moderately hard water adjusted to a pH of ~8.3 with 0.1 molar NaOH (Fisher Scientific; Pittsburgh, PA). The water accommodated fraction (WAF) method was used to prepare NA stock solutions, where NA solutions were mixed with magnetic stir bars for 24 hours at a speed sufficient to create a vortex which extended 30-50% of the solution depth (OECD, 2000; Swigert et al., 2015; Kinley et al., 2016 ; McQueen et al., 2016). Prior to analysis, samples and standards were adjusted to a pH of 8-10 SU using 3 M NaOH and added to 1.5 mL borosilicate amber glass vials. Methods for NA derivatization and subsequent analysis using high performance liquid chromatography (HPLC; Dionex, UltiMate-3000; Sunnyvale, CA) were based on Yen et al. (2004). The HPLC column was an Agilent LiChrospher 100 RP-18 (5 µm particle size, 125mm x 4 mm) with a guard column packed with 2 µm RP-18 solid phase material. Column temperature was maintained at 40° C with a sample injection volume of 60 µL mobilized with HPLC grade methanol.
(Fisher Scientific) at a flow rate of 60 µL/min. Recovery results ranged from 85-115%.

Naphthenic acid concentrations were reported as mean of triplicate analyses.

4.3.3 Acid volatile sulfide (AVS) and simultaneously extracted metal (SEM) analyses

AVS and SEM were measured using the diffusion method (Leonard et al., 1996). Sulfide ions trapped in sulfide anti-oxidant buffer (SAOB) were measured using an ion-selective electrode (ISE; Fisher Accumet 950 pH/ion meter) to determine the molar concentration of AVS. The SEM soil-acid extracts were vacuum-filtered through 0.45 µm nitrocellulose Millipore® membranes and metal concentrations were measured (as described above) and reported as molar concentrations. Quality assurance and quality control for AVS analyses included replicate measurements of AVS concentrations in sulfide standards. Reported AVS measurements were within ± 10% of the sulfide standards.

4.3.4 Statistical Analysis

Data were tested for normal distribution and homogeneity of variance using Chi-square and Bartlett’s tests, respectively. Normally distributed, homogeneous data were analyzed by one-way analysis of variance (ANOVA). Differences among treatments were determined using follow-up pairwise comparisons and contrasts using general linear models. Differences were considered significant at $p \leq 0.05$ (JMP v11; SAS Institute Inc., Cary, NC, USA).

4.3.5 Aqueous metal amendments and analyses

To achieve the nominal concentrations of divalent metals (i.e. Cu, Ni, and Zn), reagent grade salts (Fisher Scientific; Table 4.2) were added to WAF solutions and
magnetically stirred for 24-hrs (as described above) prior to renewals. Metals concentrations were measured using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Perkin Elmer; APHA, 2012). Quality assurance and control for analytical methods included comparisons to standard curves, replicate analysis, matrix spike recovery, and blank spike recovery.

Concentrations of metals (Cu, Ni, Zn) following 3-day HRTs were compared to United States Environmental Protection Agency (USEPA) and Environment Canada water quality guidelines (USEPA 1999; CCME 2005). Comparisons of metal concentrations to water quality guidelines provided context for achieving treatment performance in terms of extent of removal. Additionally, metal concentrations among treatments were compared to SRB biocide treatments to contrast potential confounding factors due to competing removal pathways (e.g. sorption to clay minerals and organic matter, microbial reduction, etc.).

4.3.6 Microbial Analyses

4.3.6.1 Most-probable number assay

Most-probable number (MPN) assays were performed for sulfate-reducing bacteria using a modified Sulfate API RP-38 broth with the following per liter distilled water: 4 mL 60% sodium lactate, 1 g yeast extract, 0.01 g K2HPO4, 0.2 g MgSO4*7H2O, 0.2 g Fe(NH4)2(SO4)2*6H2O, 0.1 g ascorbic acid, 10 mL 20% sodium acetate, and 0.1 g thioglycolic acid. Total anaerobic heterotrophs were also enumerated using R2A medium (HiMedia Labs, 2011). Sediment samples were diluted 1/100 with 0.1% peptone solution, and further serially diluted from 1/1,000 to 1/1,000,000,000 along a sterile 96-microwell
round-bottom plate containing the respective growth media. Plates were sealed with Breathe-Easy sealing membranes (Diversified Biotech) and placed in AnaeroPack Rectangular Jars with AnaeroPack-Anaero anaerobic gas generators (MGC). Plates were incubated without light at 20°C and monitored for visible growth (formation of a pellet, R2A media) and/or color change to black (SRB media) until no changes were observed for three subsequent days (34 days). The MPN of microbes was then calculated as described by Blodgett (2010).

4.3.6.2 Microbiome genetic sequencing and analyses

DNA was extracted from 0.25 g of sample using the MO BIO PowerLyzer PowerSoil DNA Isolation Kit as per the manufacturer’s protocol. Targeted DNA sequencing was used to identify bacteria present in samples via polymerase chain reaction (PCR) amplification of the v3/v4 region of the 16S ribosomal RNA gene (Klindworth et al., 2013). Library preparation and sequencing was performed as per the manufacturer’s instructions for MiSeq v3 paired-end 300 bp sequencing (Illumina). Library preparation included positive and negative controls, with the former consisting of mock communities, and the latter where no DNA is added to the PCR step, and the sample is carried through to sequencing. After sequencing, the forward and reverse reads were merged using PANDAseq (Masella et al., 2012), and all sequences then filtered to remove primer sequences and discard: low quality reads (Q < 30), reads containing N (unknown) bases, and reads shorter than 350 bp. Bioinformatics pipelines consisting of internally developed scripts and selected QIIME scripts (Caporaso et al., 2010; Edgar, 2010) were used to process the reads. Similar sequences were clustered into groups called
Operational Taxonomic Units (OTUs) using a 97% identity threshold and the QIIME pick\_de\_novo\_otus.py script. All OTUs with less than 10 representative sequences in at least one sample were discarded. Taxonomic classification of OTUs was performed using the Greengenes database version 13\_8 (DeSantis et al., 2006; McDonald et al., 2012).

**4.4 Results and Discussion**

**4.4.1 Extent and rate of acid volatile sulfide (AVS) production**

AVS concentrations were similar among NA treatments during the 21-day bench-scale experiment. With the exception of AVS concentrations measured on days 12 and 15 in the highest NA treatment (80 mg NA/L), there were no statistical differences among AVS concentrations in NA treatments compared to the untreated control (no NAs added; Figure 4.1). From day 3 to day 15, average AVS concentrations increased from 5.2 to 44.4 µmol/g and 7.7 to 24.4 µmol/g in the untreated control and the highest NA treatment (80 mg NA/L), respectively. There was a slight statistical difference in AVS concentrations measured on day 12 (p=0.0436; α=0.05) and day 15 (p=0.0238; α=0.05) in 80 mg NA/L treatments compared to the untreated control. However, no statistical differences of AVS concentrations in 80 mg NA/L treatments (as compared to the untreated control) were observed on days 18 or 21 (Figure 4.1). In contrast, significantly lower concentrations of AVS in SRB biocide treatments (as compared to untreated controls; p<0.0001; α=0.05) confirmed AVS production in treatments and untreated controls were due to microbially mediated sulfate reduction. After 9 days, black precipitates (presumably sulfide precipitates) were observed in the surficial water-
sediment interface in all NA treatments and untreated controls, but were absent in the surficial water-sediment interface in SRB biocide treatments. The rate of AVS increase was an apparent first order reaction, with AVS concentrations increasing at a rate of 0.225 and 0.158 day$^{-1}$ (measured from day 3 to day 12) for the untreated control and 80 mg NA/L treatment, respectively (Figure 4.2). Since AVS concentrations in NA treatments 10, 20, 40 and 60 mg/L were similar to the untreated control, rates of AVS production were not compared.

4.4.2 Simultaneously extracted metals (SEM) and acid volatile sulfide (AVS) ratios

In terms of $\Sigma$SEM:AVS ratios ($\Sigma$SEM = Cu, Ni, Zn), all NA treatments and the control (no NA added) achieved greater molar concentrations of AVS as compared to simultaneously extracted metals (i.e. $\Sigma$SEM:AVS <1; Figure 4.3) during the 21-day experiment duration. In contrast, SRB biocide controls had greater molar concentrations of SEM ($\Sigma$SEM ranged from 0.183 to 0.857 µmol/g) as compared to AVS, with $\Sigma$SEM:AVS ratios exceeding 1 following 12 days of the experiment. The $\Sigma$SEM:AVS results indicate NAs did not adversely affect the production of AVS to an extent that would alter metal binding and subsequent precipitation.

In specifically designed passive treatment systems (e.g. constructed wetlands), AVS are produced by microbially-catalyzed processes that use the plant biomass. Divalent metals, represented as $M^{2+}$ in equation 1, react with bisulfide or hydrogen sulfide (i.e. AVS) and precipitate as insoluble metal sulfides (Kosolapov et al., 2004).

$$H_2S + M^{2+} \rightarrow MS(\text{solid}) + 2H^+ \quad (\text{Eq. 1})$$
These metal sulfides are relatively insoluble (CuS; ksp=10^{-16}; NiS; ksp= 10^{-19}; ZnS ksp=10^{-25}; Stumm and Morgan 1996). The amount of AVS necessary for achieving treatment goals is site-specific, based on treatment goals and inflow water characteristics (e.g. M^{2+} concentrations). Comparison of molar ratios of SEM:AVS are useful parameters for predicting treatment capabilities for divalent metals (e.g. Cd, Cu, Ni, Pb, Zn, etc.) and can inform readiness and performance of constructed wetland treatment systems (Rodgers and Castle 2008; Haakensen et al., 2015). The goal for achieving transfer and transformation of divalent metals via dissimilatory sulfate reduction is to maintain an excess of molar concentrations of AVS as compared to the summation of the SEM (ΣSEM). In this study, excess molar ratios of AVS were achieved in all NA treatments tested; however, the question of the aqueous metal removal extent remains.

4.4.3. Copper, nickel, and zinc removal extent

In this study, Cu, Ni, and Zn exposure concentrations were renewed every 3 days at nominal concentrations of 0.5, 0.5, 1.0 mg/L, respectively. Following amendments, aqueous metal concentrations precipitously declined in untreated controls (no NA added) and NA treatments, with >95% removal of Cu, Ni, and Zn achieved on days 12 through 21 (Figure 4.4). Aqueous concentrations of Cu, Ni, and Zn were compared to water quality criteria established for the protection of aquatic life (USEPA 1999; CCME, 2005) to provide context for the environmental significance of the treatment extent. Following 12 days, metal removal extents for Cu, Ni, and Zn in untreated controls (no NA added) and all NA treatments were below targeted quality criteria for ambient water concentrations (Figure 4.4). In contrast, aqueous metal concentrations for Cu, Ni, and Zn
were significantly higher \((p<0.0001; \alpha=0.05)\) in SRB biocide controls as compared to untreated controls, and always in excess of the targeted guideline concentrations.

In SRB biocide controls, sulfate reduction was inhibited and AVS production was minimal; therefore, higher aqueous concentrations of metals were anticipated based on \(\Sigma\text{SEM}:\text{AVS}\) ratios previously discussed (Figure 4.3). In SRB biocide treatments, there was some metal removal observed (ranging from \(~40\) to \(80\%\)), likely due to formation of oxides of manganese and iron (Brookins 1988), as well as sorption and complexation with organic matter (Besser et al., 2003) and clay (Hoss et al., 1997). However, sorption sites in detritus and sediment are limited, as available sites “fill” with time (Machemer and Wilderman 1992). For this reason, sulfide precipitation is the preferred treatment process in passive systems (as compared to sorption) for achieving long-term treatment goals (Machemer and Wilderman, 1992; Murray-Gulde 2003). In this study, the range of NA concentrations tested had no apparent effect on dissimilatory sulfate production and precipitation of Cu, Ni, and Zn from the aqueous phase.

4.4.4 Water and Sediment Characteristics

Measured water and sediment characteristics were consistent among untreated controls and NA exposed treatments (Table 4.3). Exposures to NAs were confirmed analytically to be near nominal concentrations, with relative percent differences (RPD) ranging from 3.2 to 15.5%. NAs were added to moderately hard laboratory formulated water buffered with bicarbonate to minimize confounding exposure conditions that may influence solubility of NAs. Initial pH was approximately 8.15 at testing renewals, with overlying water pH declining slightly between water renewals (range 6.35 to 8.25).
Average *in situ* pH ranged from 7.57 to 7.62 during the 21-day experiment, thus pH was not likely a confounding treatment variable. During the 21 day experiment duration, average (n=6) sediment ORP among NA treatments ranged from -219 to -201 mV, indicating that reducing conditions were achieved to support the target biogeochemical processes. Average dissolved oxygen (DO) concentrations were slightly higher in biocide treatments (1.53 mg/L) as compared to NA treatments (0.61 mg/L [80 mg NA/L]) and untreated controls (0.93 mg/L; Table 4.3), supporting that microbial activity was decreased in biocide treatments (i.e. less biological oxygen demand). A slightly lower average (n=6) sediment ORP was observed in SRB biocide treatments (-184 mV), indicating a potential influence of THPS on microbial assemblages capable of oxidizing organic material and producing elections.

### 4.4.5 SRB density, diversity and relative abundance

Samples for microbial analyses were collected from all treatments on day 15, representing the treatment duration where the greatest differences of AVS concentrations among treatments were observed (i.e. RPD among control and 80 mg NA/L was 48%). Growth-based analysis of SRB and total anaerobic heterotrophs (as most probable number/gram) demonstrate no difference in density among untreated controls and NA exposures, and no measurable relationship between the concentrations of Fluka NA and SRB or total anaerobic heterotroph densities (Fig 5 and data not shown, respectively). In contrast, SRB biocide treatments impacted the abundance of SRB as well as total anaerobic heterotrophs, although some residual SRB and anaerobic heterotrophs were observed (Fig. 5 and data not shown). This may indicate that the SRB biocide treatment
was not completely biocidal, or was biostatic. Alternatively, it may be that the biocide (THPS) did not effectively penetrate below the surficial sediment-water interface, and there was carry-over from “deeper” sediments during sampling. Nonetheless, this control exhibited substantially less SRB density relative to experimental samples, which is consistent with the higher aqueous concentrations of metals and $\Sigma$SEM:AVS ratios in the SRB biocide control treatment.

Genetic sequencing was used to assess the community composition and diversity of bacterial communities in each NA treatment and control. Results demonstrate that NA exposure, regardless of concentration, did not notably alter the diversity or relative abundance of bacterial community members. Further, NA did not affect the presence, types, and relative abundance of SRB, with 5.6-6.2% of the bacterial community representing known SRB in the control and each NA concentration (Figure 4.6). In contrast, the biocide treatment resulted in a lower proportion of the bacterial community representing SRB (i.e. relative abundance was 1.5% in SRB biocide treatments as compared to 5.6-6.2% in NA treatments).

The dominant SRB in all samples including the SRB biocide treatment were *Desulfobulbus, Desulfovibrio*, and *Desulfosporosinus* (Fig. 6). In addition to SRB, several genera known to produce sulfides through reduction of other sulfur compounds such as thiosulfate and sulfur (e.g., *Fusibacter, Geobacter, Sulfurospirillum*) were present in all NA and control treatment samples and to a lesser extent in the biocide treatment. These organisms would be another source of sulfides for the precipitation of divalent metals (i.e. Cu, Ni, Zn), and were similarly not affected by the presence of NA.
Commercially available NAs contain compounds with relatively lower molecular weights (<< 500 Da) and numbers of ring structures than NAs found in energy-derived waters (Scott et al., 2005; Han et al., 2009; Headley et al., 2010), which can result in increased bioavailability and toxicity of commercial NAs to microorganisms (Holowenko et al., 2002; Clemente and Fedorak, 2005). Factors or conditions that could influence NA exposures, SRB densities, and AVS production (i.e. nutrient availability, aqueous sulfate and dissolved oxygen concentrations, pH, ORP, and temperature) were controlled in this study to focus on discerning the potential effects of NAs on SRB and consequent AVS production. Since no effects on SRB due to exposures of a commercial NA could be discerned within the constraints of this study, effects on SRB and subsequent AVS production due to exposures of more compositionally complex NA mixtures are not anticipated.

4.4.6 Implications to water renovation strategies

Lack of NA toxicity to SRB could be beneficial for water renovation strategies by passive or semi-passive methods such as constructed wetland treatment systems that incorporate transfer and transformation pathways for treating process-affected waters. NAs have been a primary source of toxicity in oil sands process-affected waters (Schramm, 2000; Clemente and Fedorak, 2005; Allen, 2008a) and refinery effluents (Dorn, 1992), and therefore, questions arise regarding the potential effects of NA exposures on SRB and subsequent sulfide production. In this conservative laboratory toxicity experiment, no adverse effects on SRB could be discerned. In addition, rates of AVS production were sufficient to achieve excess molar ratios of AVS to SEM,
indicating that available sulfide ligands were well in excess of aqueous metal concentrations regardless of NA exposure concentration within the range tested (10-80 mg NA/L). Further, SRB were relatively insensitive to commercial NA exposures. Thus, within the bounds of the experimental conditions in this study, effects on performance of metal-sulfide precipitation pathways due to co-occurring exposures of NAs are not anticipated in field situations.

4.5 Conclusions

In this controlled laboratory toxicity experiment using multiple lines of evidence (i.e. [AVS], ΣSEM:AVS, aqueous metal removal extents, and microbial analyses), dissimilatory sulfate reduction and metal precipitation were not influenced by exposures of a commercial (Fluka) NA. Following exposures of NAs, extent of AVS production were sufficient to achieve ΣSEM:AVS <1, indicating that available sulfide ligands were in excess of SEM (Cu, Ni, and Zn) concentrations regardless of NA exposure concentration (10-80 mg NA/L). In addition, no adverse effects to SRB populations in terms of density, diversity, or relative abundance were measured following exposures of a commercial NA. Since SRB were insensitive to exposures of a relatively potent (in terms of aquatic toxicity) commercial NA, adverse effects to SRB (and SRB-mediated pathways in wetlands) from exposures of more compositionally complex NAs (i.e. derived from oil sands process affected waters) are not anticipated. Further, lack of toxicity to the overall microbial population and absence of effect on diversity and community profile is a positive finding, given that maintaining diversity of microbially-
mediated pathways could be beneficial for passive wetland treatment systems. Passive systems that utilize these biogeochemical processes can be cost effective alternatives to traditional technologies, and having a robust microbial community capable of performing these processes can improve the efficiency and success of the system (Johnson and Hallberg, 2005; Nelson and Gladden, 2008; Haakensen et al., 2015). For effective design and operation of passive treatment systems (i.e. constructed wetlands) which utilize biogeochemical processes, it is useful to discern adverse effects due to co-occurring constituents on SRB which could alter the dissimilatory sulfate reduction and limit treatment of metals. In this study, dissimilatory sulfate reduction and subsequent metal precipitation were not vulnerable to NAs, indicating passive treatment systems could be used to treat metals occurring in NA affected waters.

4.6 Acknowledgments

Funding support for this research was provided by Shell Canada Ltd. and Suncor Energy. The authors are also grateful to Dr. Wayne Chao of Clemson University for providing analytical support and Jenny Liang and Ainsley Stewart of Contango Strategies Ltd. for providing support for microbial assays.
4.7 References


Sigma-Aldrich, 2015. MSDS, Naphthenic acid. St. Louis, Missouri.


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
<th>Method Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Direct Instrumentation: Orion Model 420A (Standard Methods 4500-H&lt;sup&gt;+&lt;/sup&gt; B) (APHA, 2012)</td>
<td>0.01 SU</td>
</tr>
<tr>
<td>Temperature&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Direct Instrumentation: Orion Model 420A</td>
<td>0.01 °C</td>
</tr>
<tr>
<td>Dissolved Oxygen&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Direct Instrumentation: YSI Model 52</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Conductivity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Direct Instrumentation: YSI 30 (Standard Method 2510 B) (APHA, 2012)</td>
<td>0.1 μS/cm</td>
</tr>
<tr>
<td>Alkalinity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Standard Methods: 2320 B (APHA, 2012)</td>
<td>2 mg/L as CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Hardness&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Standard Methods: 2340 B (APHA, 2012)</td>
<td>2 mg/L as CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Particle size&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hydrometer method (Gee and Bauder, 1986)</td>
<td>2%</td>
</tr>
<tr>
<td>Percent Solids %&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dry matter content: Standard Methods: 2540C</td>
<td>0.0001 g</td>
</tr>
<tr>
<td>Organic matter content&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Loss-on-ignition at 450°C (Nelson and Sommers, 1996)</td>
<td>0.0001 g</td>
</tr>
<tr>
<td>Cation exchange capacity (CEC)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Standard Methods: 2340 B (APHA, 2012)</td>
<td>0.1 meq/100g</td>
</tr>
<tr>
<td>Oxidation-Reduction Potential (ORP)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Modified standard method 2580B: Accumet ®calomel reference electrode, Fluke® 77 III voltage meter (Faulkner et al., 1989)</td>
<td>10 mV</td>
</tr>
<tr>
<td>AVS and SEM&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>Modified diffusion method (Leonard et al., 1996)</td>
<td>0.001 μmol sulfide/ g</td>
</tr>
<tr>
<td>Cd, Cu, Pb, Ni, Zn&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>Inductively Coupled Plasma-Atomic Emissions Spectrometry (ICP-AES): 200.7 (USEPA, 2001)</td>
<td>0.002-0.042 mg/L</td>
</tr>
<tr>
<td>Sulfate-Reducing Bacteria (SRB)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Most probable number (MPN; Blodgett, 2010)</td>
<td>202 MPN/g</td>
</tr>
<tr>
<td></td>
<td>Bacterial community profiling by 16S ribosomal RNA sequencing</td>
<td>0.002 – 0.0008 %</td>
</tr>
</tbody>
</table>

<sup>a</sup>Overlying water measurement  
<sup>b</sup>Sediment measurement  
<sup>c</sup>Surficial sediment depth (<1cm)
**Table 4.2** Chemical sources and nominal concentrations of constituents used for aqueous exposures.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Chemical Source</th>
<th>Nominal Concentration(s), mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial NA</td>
<td>Alkylated cyclopentane carboxylic acids (mixture)(^a)</td>
<td>10, 20, 40, 60, 80</td>
</tr>
<tr>
<td>Copper</td>
<td>CuSO(_4) (5)H(_2)O</td>
<td>0.5</td>
</tr>
<tr>
<td>Nickel</td>
<td>NiCl(_2) (6)H(_2)O</td>
<td>0.5</td>
</tr>
<tr>
<td>Zinc</td>
<td>ZnCl(_2)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\text{CAS # 1338-24-5; Sigma-Aldrich (2015)}\)
Table 4.3 Water and sediment characteristics measured during treatment periods 3-d to 21-d (n=21).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH (S.U.)</th>
<th>DO (mg/L)</th>
<th>Conductivity (mS/cm)</th>
<th>Alkalinity (mg/L as CaCO$_3$)</th>
<th>Hardness (mg/L as CaCO$_3$)</th>
<th>ORP (mV)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>(Range)</td>
<td>Mean</td>
<td>(Range)</td>
<td>Mean</td>
<td>(Range)</td>
</tr>
<tr>
<td>Control</td>
<td>7.58</td>
<td>(6.71-8.25)</td>
<td>0.93</td>
<td>(0.25-3.2)</td>
<td>1.45</td>
<td>(1.17-1.98)</td>
</tr>
<tr>
<td>10 mg NA/L</td>
<td>7.58</td>
<td>(6.57-8.15)</td>
<td>0.92</td>
<td>(0.05-2.87)</td>
<td>1.45</td>
<td>(1.21-1.82)</td>
</tr>
<tr>
<td>20 mg NA/L</td>
<td>7.55</td>
<td>(6.55-8.10)</td>
<td>0.83</td>
<td>(0.05-2.42)</td>
<td>1.49</td>
<td>(1.03-1.94)</td>
</tr>
<tr>
<td>40 mg NA/L</td>
<td>7.62</td>
<td>(6.58-8.20)</td>
<td>0.73</td>
<td>(0.25-2.47)</td>
<td>1.44</td>
<td>(1.1-1.95)</td>
</tr>
<tr>
<td>60 mg NA/L</td>
<td>7.59</td>
<td>(6.48-8.01)</td>
<td>0.76</td>
<td>(0.32-2.03)</td>
<td>1.53</td>
<td>(1.17-2.01)</td>
</tr>
<tr>
<td>80 mg NA/L</td>
<td>7.58</td>
<td>(6.4-8.18)</td>
<td>0.61</td>
<td>(0.16-1.78)</td>
<td>1.45</td>
<td>(1.23-1.92)</td>
</tr>
<tr>
<td>SRB Biocide</td>
<td>7.57</td>
<td>(6.39-8.11)</td>
<td>1.53</td>
<td>(1.05-2.43)</td>
<td>1.98</td>
<td>(1.65-2.05)</td>
</tr>
</tbody>
</table>

$^a$Temperature range (21.5-24.6°C)

$^b$Oxidation reduction potential (ORP) measured in situ, 2-3 cm below sediment water interface (n=6; collected at 12 and 21 day sampling periods)
Figure 4.1 Acid volatile sulfide (AVS) concentrations (µmol/g) measured during 21-day testing period (n=3). Error bars indicate standard deviations. Asterisks indicate AVS concentrations significantly different (p<0.05; α=0.05) from untreated controls.
Figure 4.2 Comparison of rate of acid volatile sulfide (AVS) production (µmol/g) in sulfate-reducing bacteria (SRB) biocide treatments, untreated control, and 80 mg/L naphthenic acid (NA) treatments (n=3) on days 3 through 15. Error bars indicate standard deviations.

Note: Treatments 10, 20, 40, and 60 mg NA/L were similar to the untreated control.
Figure 4.3 Simultaneously extracted metals (\(\Sigma\)SEM; Cu, Ni, Zn; \(\mu\)mol/g) and acid volatile sulfide (AVS) ratios among controls and highest NA treatment (80 mg NA/L) during 21-day testing period (\(n=3\)). Errors bars indicate standard deviations.

Note: Values below dashed line indicate “excess” molar ratios of AVS as compared to SEM. Treatments 10, 20, 40, and 60 mg NA/L were similar to the untreated control.
Figure 4.4 Copper (top), nickel (middle), and zinc (bottom) removal extent (mg/L) measured during 21-day treatment durations.

Note: Dashed line represents target removal goals based on water quality criteria (WQC; Cu = 0.011 mg/L; Ni = 0.052 mg/L; Zn = 0.120 mg/L; USEPA 1999; CCME 2005).
Figure 4.5 MPN of sulfate-reducing bacteria per gram of sample.

Error bars indicate standard error of the average results of samples \( (n = 3) \).
**Figure 4.6** Percentage of known SRB in the bacterial community based on genetic sequencing. Names of organisms are either genus (g) or family (f) level classification. Relative abundance (%) for each sample is the average across three replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
CHAPTER FIVE

PERFORMANCE OF HYBRID PILOT-SCALE CONSTRUCTED WETLAND
SYSTEMS FOR TREATING OIL SANDS PROCESS AFFECTED WATER FROM
THE ATHABASCA OIL SANDS AREA

Andrew D. McQueen1,3; Maas Hendrikse1, Daniel P. Gaspari2, Ciera M. Kinley1; John H.
Rodgers Jr.1, James W. Castle2

1 Department of Forestry and Environmental Conservation, 261 Lehotsky Hall, Clemson
University, Clemson, SC 29634, USA

2Department of Environmental Engineering & Earth Sciences, 455 Brackett Hall,
Clemson University, Clemson, SC 29634, USA

3Corresponding author: Andrew McQueen

Manuscript prepared for submission to: Ecological Engineering
5.1 Abstract

Mining leases in the Athabasca oil sands (AOS; near Ft. McMurray, Canada) produce large volumes of oil sands process-affected water (OSPW) that contain potentially problematic constituents requiring treatment prior to surface water discharge into receiving aquatic systems. The aim of this research was to identify constituents of concern (COCs) in OSPW sourced from an AOS external tailings facility and design a hybrid pilot-scale constructed wetland treatment systems (CWTS) to decrease concentrations of COCs and subsequently mitigate risks. COCs were identified based on comparisons to ambient water quality thresholds for the protection of aquatic life (i.e. Canadian Environmental Quality Guidelines [CEQGs], Alberta Environment Water Quality Guidelines [Alberta WQGs], and United States Environmental Protection Agency Water Quality Criteria [USEPA WQC]) and toxicity endpoints. Performance of the hybrid pilot-scale CWTS was evaluated by rate and extent of COC removal and change in toxicity as measured by an aquatic invertebrate *Ceriodaphnia dubia*. Following characterization of OSPW, specific COCs were identified as: naphthenic acids (NAs), oil and grease (O/G), metals/metalloids (Al, B, Cu, Ni, Se, and Zn), chloride, and total suspended solids (TSS). A hybrid pilot-scale CWTS was designed to promote treatment processes to alter (transfer and transform) COCs using sequential reducing and oxidizing wetland reactors and a solar photocatalytic treatment reactor using fixed film titanium dioxide (TiO$_2$). Performance criteria were achieved as the CWTS decreased concentrations of NAs, O/G, and metals below protective thresholds and decreased toxicity to *C. dubia*. Results from this study provide proof-of-concept data to inform
hybrid passive or semi-passive treatment approaches (i.e. constructed wetlands) that could be used to mitigate COCs contained in OSPWs.

5.2 Introduction

Oil sands process-affected water (OSPW) is produced during the extraction of bitumen, and in the Athabasca oil sands (AOS) presents considerable economic and environmental challenges due to the accumulation and composition of these waters (Mahaffey and Dubé 2016). OSPWs can contain a mixture of problematic constituents (e.g., naphthenic acids [NAs], metals, metalloids, and salts) that need to be treated prior to surface water discharge into receiving aquatic systems (Allen 2008a; McQueen et al., 2016a). A compositionally complex class of carboxylic acids (NAs; generally described by the formula $C_nH_{2n+2}O_2$) is present in many OSPWs that have been identified as sources of toxicity (Verbeek et al., 1994; Morandi et al., 2015), with adverse effects observed for fish (Nero et al., 2006; Kavanagh et al., 2012; Leclair et al., 2013; Marentette et al., 2015a,b), macroinvertebrates (Verbeek et al., 1994; Zubot et al., 2012; Anderson et al., 2012), macrophytes (Armstrong et al., 2008), and microorganisms (Frank et al., 2008). In addition to NAs, there are a number of other constituents in OSPW that may require treatment including trace metals/metalloids (Al, As, Cr, Cu, Fe, Pb, Ni, Zn), unrecovered bitumen (e.g., oil and grease [O/G]), cations and anions, and suspended and dissolved solids (Mackinnon and Boerger 1986; Allen 2008a; Zubot et al., 2012; McQueen et al., 2016a). To reclaim impacted waters, wet-landscape mitigation approaches (e.g., constructed wetlands) are among technologies considered for treatment.
of problematic constituents (e.g., metals, organics; Allen 2008b; Foote 2012; Toor et al., 2013; Brown and Ulrich 2015). To achieve reclamation goals in the AOS, OSPW will need to be treated and returned to aquatic receiving systems in addition to restoring disturbed areas to “equivalent land capabilities” of the pre-mined landscape (Allen 2008b; CEMA 2014; Province of Alberta 2014). Constructed wetlands may provide unique opportunities for achieving these AOS restoration goals. A constructed wetland treatment system (CWTS) could be designed to actively treat OSPW (achieving water return goals) and transition to serve as passive wetlands (achieving reclaimed wetland goals) following their operational lifespan. To be effective, CWTSs must be carefully designed and constructed with explicit consideration of OSPW composition and compatibility of materials (hydrosol and vegetation) to promote desired treatment processes in the AOS region.

Accurate characterization of OSPWs and identification of constituents of concern (COCs) is an initial step crucial for design and construction of successful wetlands used for treatment (McQueen et al., 2016a). In this study, COCs are elements, compounds, or parameters that interfere with the goal of releasing OSPW to freshwater receiving aquatic systems. OSPW can vary spatially, temporally, and with the extraction process employed (Mikula 2013; Frank et al., 2016). Thus the design basis will range widely with the constituents of OSPWs and will need to be robust (e.g., incorporate hybrid components) to ensure that wetlands can perform for a range of OSPWs. The treatment design approach taken here is based on an OSPW sourced from an external tailings facility in the AOS near Ft. McMurray, Canada.
CWTSs have been used successfully to treat constituents in a variety of problematic energy-derived process waters, (Knight et al., 1999; Gillespie et al., 2000; Eggert et al., 2008; Murray-Gulde et al., 2008), and typically cost less to construct, operate, and maintain than conventional water treatment systems (Halverson 2004; Mooney and Murray-Gulde 2008). To be successful for OSPWs stored in the AOS, CWTS will need to be passive (or semi-passive), requiring low energy inputs due to the volumes of water currently stored and requiring treatment. CWTS are designed to incorporate features (vegetation, hydrosoil, hydroperiods) facilitating treatment processes specifically designed to alter (transfer or transform) COCs contained in impacted waters (Rodgers and Castle 2008). Wetland systems are unique in offering a variety of transformation processes, including: photolysis, hydrolysis, speciation/ionization, oxidation, reduction, and biotransformation (Rodgers and Castle 2008). By choosing appropriate wetland macrofeatures (e.g., water depth, sediment type, plant species) that control biogeochemical conditions, modular reactors were designed to promote specific pathways for the transfer and transformation of organic (i.e. NAs and O/G) and inorganic (Al, B, Cu, Ni, Se, Zn, suspended solids) COCs. More recently, benefits of incorporating non-traditional “hybrid” components in CWTS in efforts to add or enhance treatment processes have been recognized (Murray-Gulde et al., 2003; Johnson et al., 2008; Haakensen et al., 2015).

Hybrid components (e.g., ozonation, solar catalysts) coupled with CWTS could augment performance of complex waters containing petroleum-derived organic constituents (e.g., NAs; Shi et al., 2015; Vaiopoulou et al., 2015; Leshuk et al., 2016;
McQueen et al., 2016b) potentially increasing the rate and extent of treatment. In the case of solar catalysts such as titanium dioxide (TiO₂), the combination of excitation energy and surface moisture produces hydroxyl- and superoxide radicals (Linsebigler 1995). To date, laboratory-scale photocatalytic degradation of both commercial and OSPW NA mixtures has been successful (Leshuk et al., 2016; McQueen et al., 2016b); however, larger-scale studies (i.e. pilot- or full-scale) are needed. Pilot-scale CWTSs offer the ability to experimentally test hypotheses under a variety of conditions prior to investing in full-scale CWTSs (Rodgers and Castle 2008). Factors such as treatment rates and extents can be evaluated to determine critical full-scale design parameters (e.g., loading rate, wetland area, and hydraulic retention time). In addition, the use of pilot scale CWTSs can decrease full-scale performance uncertainties.

This study uses multiple lines of evidence to measure performance of a pilot-scale CWTS, including analytical measurements of rates and extents of COC removal (e.g., NAs, metals) and changes in toxicity to sentinel aquatic species. A critical step for demonstrating performance of wetland systems will include the use of bioassays to monitor alterations in exposures, providing insight to changes in constituent bioavailability and mixture interactions (e.g., synergy, additivity, antagonism). The aquatic invertebrate (*Ceriodaphnia dubia* Richard) used in this study is sensitive to constituents (e.g., NAs, metals) contained in OSPW (Zubot et al., 2012; McQueen et al., 2016a). *C. dubia* are ecologically important to freshwater ecosystems (Pennak 1978; Carpenter et al. 1985) and routinely used by regulatory agencies for whole effluent...
toxicity testing as a part of discharge permit requirements (USEPA 1992; Environment Canada 2007).

The overall purpose of this research was to evaluate the performance of a specifically designed hybrid pilot-scale CWTS for treating OSPW. In order to achieve this overall objective, specific objectives were to: 1) characterize OSPW in terms of chemical composition and targeted constituents of concern, 2) design and assemble a hybrid pilot-scale CWTS to treat target constituents in OSPW, 3) measure performance of the pilot-scale CWTS for OSPW based on rates and extents of constituent removal, and 4) measure the performance of the pilot-scale CWTS using toxicity testing with the aquatic invertebrate Ceriodaphnia dubia.

5.3 Materials and Methods

5.3.1 Characterization of oil sands process waters (OSPW)

The OSPW used in this study was procured from Shell Canada Ltd.’s Muskeg River Mine External Tailings Facility (MRM-ETF; Shell Canada Limited 2016). MRM-ETF OSPW is produced from a surface mining operation in the AOS region (near Fort McMurray, AB, Canada) using a Clark caustic warm water floatation process to separate bitumen from ore in which NaOH is added to a heated (50-80°C) mixture of recycled OSPW and river water. OSPW at the MRM-ETF facility also receives: groundwater from depressurizing aquifers associated with the oil sands ore layer, surface water and shallow aquifer water from dewatering activities, precipitation and surface water runoff, water collected from dyke seepage control systems, and connate water.
NAs were derivatized and analyzed using high performance liquid chromatography (HPLC; Dionex, UltiMate-3000; Sunnyvale, CA) according to Yen et al. (2004) to quantify total concentrations of NA. Inductively couple plasma atomic emission spectrometer (ICP-AES; Spectro Flame Modula) was used to measure element concentrations: Ag, Al, As, B, Ba, Cd, Cr, Cu, Co, Fe, Pb, Mn, Mo, Ni, Se, and Zn following USEPA method 200.7 (USEPA 2001). General water chemistry parameters, including: pH, alkalinity, hardness, dissolved oxygen (DO), conductivity, total suspended solids (TSS) and dissolved solids (TDS), total ammonia, total phosphorus, and chemical oxygen demand (COD) were analyzed following methods described in *Standard Methods for the Examination of Water and Wastewater* (APHA 2012). O/G concentrations were measured using gravimetric methods with n-hexane extraction according to USEPA method 1664 revision A (USEPA 1999). Cations and anions were measured using a Dionex ISC-2000 ion chromatograph following APHA (2012) methods.

Identification of COCs was achieved by comparing chemical or physical parameters to water quality criteria limits (i.e. CEQGs, Alberta Environment WQG, and USEPA WQC; USEPA 2007; CCME 2011; ESRD 2014) or toxicity endpoint values (point estimates were chosen when available; e.g., LC50s). The toxicity testing species *Ceriodaphnia dubia*, *Daphnia magna*, rainbow trout (*Oncorhynchus mykiss*), and fathead minnow (*Pimephales promelas*) were chosen for this comparison because of their sensitivity to many constituents found in OSPW, availability of data, and use in developing and enforcing water quality criteria standards (USEPA 2007; CCME 2011).
COCs are parameters that exceeded the most conservative water quality standards or toxicity endpoint values selected (Equation 1).

\[ \text{COCs} = \text{OSPW Parameter} > WQC \text{ or Toxicity Endpoint} \]  \hspace{1cm} \text{Equation 1}

5.3.2 Design and Construction of a Hybrid Pilot-scale CWTS

The hybrid pilot-scale system included a 3780 L detention tank, five wetland reactors in replicate series, and a photocatalytic reactor, (Figure 1). The pilot-scale CWTS was built and housed in a greenhouse with natural (i.e., solar) photoperiod and regulated temperature. The photocatalytic reactor was located outside, using natural (solar) light as the energy source. OSPW was continually mixed in a detention basin with a 0.56 kW (¾ hp) sump pump for treatment in the hybrid pilot-scale CWTS. The flow of OSPW from the detention basis was maintained by FMI® piston pumps (Fluid Metering, Inc., NY) calibrated to a flow rate of 34 mL/min to achieve a nominal hydraulic retention time (HRT) of 13.5 days per wetland series. Wetland outflow was stored in a 1890 L detention tank prior to inflow to the photocatalytic reactor to allow for intermittent release during targeted solar conditions.

5.3.2.1 Wetland Reactors

Each wetland reactor was constructed using HDPE containers containing hydrosoil, plants, and treatment water connected by PVC pipe (Figure 1; Table 1). Reactors were gravity-fed following the first reactor receiving metered inflow water. Replicate treatment systems were defined as “Series A” and “Series B”. Two types of wetland reactors were designed to achieve “bulk” oxidative or reductive conditions and are operationally defined as “oxidizing” and “reducing” wetland reactors. These wetland
mesocosms incorporate design features that achieve criteria supportive of transfer and transformations targeting COCs present in OSPW and are compatible within the AOS region. The contributions of these design features are discussed in detail in section 5.4.2.

5.3.2.2 Photocatalytic reactor

The photocatalytic (PC) reactor was constructed using stainless steel sheets placed in a 60 cm wide by 182 cm long fiberglass container (Table 1). The reactor was a shallow depth (water depth of ~1 cm) flow-through reactor designed to achieve nominal HRTs during hours of direct sunlight. A metered FMI® piston pump was calibrated to achieve a HRT of 12 hours of direct sunlight using a timer switch. Epoxy resin and hardener (105 Epoxy Resin; 206 Hardener; West Marine Products, Inc., Watsonville, California) were applied as a <0.2 mm film on the stainless steel sheets followed by application of TiO₂ (Aeroxide™ P25; Fisher Scientific, Fairlawn NJ). TiO₂ used a mixture of 10-20% rutile and 80-90% anatase, with a mean particle diameter of approximately 21 nm and a specific surface area of 35-65 m²/g. Ultraviolet (UV [250-400nm]; Apogee SU-100, Logan, UT) and visible irradiance (HOBO Pendant® Temperature/Light 64K Data Logger, Bourne, MA) were measured continually throughout the treatments (supplementary material).

5.3.3 Performance Monitoring

The following parameters were measured to monitor performance of the hybrid pilot-scale wetland for removing COCs (e.g. organics, divalent metals, suspended solids). To monitor COC removal, as well as the rate of removal given different retention times, influent from the detention basin and outflows from reactors were sampled. In situ pH,
temperature, conductivity, and dissolved oxygen were measured bi-weekly in the water column of each wetland reactor (APHA 2012). Alkalinity and hardness were measured every two weeks in samples from wetland reactors (APHA 2012). Sediment oxidation-reduction potential (Eh [mV]) was measured every two weeks in the sediment at 2.5 cm depth in the middle of each wetland reactor using in situ platinum-tipped electrodes and an Accumet® calomel reference electrode with a Gardner Bender® GDT-311 voltmeter (Faulkner et al., 1989). Acid volatile sulfides (AVS) were measured monthly from surficial hydrosol samples using the diffusion method (Leonard et al., 1996), where sulfide ions trapped in sulfide anti-oxidant buffer (SAOB) were measured using an ion-selective electrode (ISE; Fisher Accumet 950 pH/ion meter) to determine the molar concentration of AVS.

To assess treatment performance of the pilot-scale systems, removal efficiency and rate coefficients were estimated using the following equations:

\[
\text{Removal efficiency (\%)} = \left( \frac{[C]_0 - [C]}{[C]_0} \right) \times 100 \quad \text{Equation 2}
\]

Where, initial concentration of COCs are denoted \([C]_0\) (mg/L), and \([C]\) (mg/L) is concentration of COCs in the outflow of the system. Removal rate coefficients \((k, \text{day}^{-1})\) were estimated using first order rate kinetics described by the following equation:

\[
\text{Removal Rate Coefficient } (k) = -\frac{\ln([C]/[C]_0)}{t} \quad \text{Equation 3}
\]

Where, \(t\) (days) is time, which for this purpose is at set intervals in the duration of the HRT. The removal rate coefficient (day\(^{-1}\)) represents the slope of the line by plotting – \(\ln([C]/[C]_0)\) versus time assuming first-order kinetics.
Performance was assessed over five “treatment periods” (treatment dates are provided in supplementary material). With the exception of the PC reactor, each “treatment period” is equivalent to the HRT of the wetland system (13.5 days). Performance was monitored by collecting samples following a plug flow to minimize potential influences of inflow variability.

5.3.4 Toxicity Testing

Toxicity experiments were performed with *C. dubia* exposed to untreated (inflow) and treated (outflow) OSPW. *C. dubia* were cultured based on USEPA methods (2002) at Clemson University’s Aquatic Animal Research Laboratory. Test organisms were ≤ 24 h old at the initiation of each experiment. *C. dubia* (n=20) were exposed to untreated (inflow) and treated (outflow) OSPW in 7-8 day static/renewal toxicity tests conducted following a USEPA and Environment and Climate Change Canada freshwater toxicity testing protocol (USEPA 2002; Environment Canada 2007). Toxicity to *C. dubia* was evaluated by measuring survival and reproduction. Statistical differences for mortality and reproduction in treatments relative to untreated controls were determined using one way analysis of variance (ANOVA) and Dunnett’s multiple range test (α = 0.05; JMP Pro V.11; SAS Institute Inc., Cary, NC, USA).
5.4 Results and Discussion

5.4.1 Characterization of OSPW

5.4.1.1 Organics

OSPW evaluated in this study had a slight hydrocarbon sheen, with oil and grease (O/G) concentrations ranging from 8 to 13 mg/L (Table 2). Concentrations of O/G (an aggregate measure of residual hydrocarbons) in OSPW exceeded narrative criteria for discharge water (i.e. no visible film or sheen of oil present; Table 2). OSPW NAs were measured at concentrations ranging from 80 to 128 mg NA/L (HPLC; n=10). Although there are currently no established numeric water quality criteria or guidance for NAs contained in OSPW, based on the concentrations and species of NAs present in OSPW as compared to reported toxicity endpoints, there is evidence that NAs may pose risk to aquatic biota, supporting the designation of NAs as a COC for this study (Table 2).

5.4.1.2 Water characteristics (e.g. pH, suspended solids) and nutrients (ammonia, TP)

OSPW is well buffered (alkalinity ranges from 320 to 340 mg/L as CaCO$_3$) due to bicarbonate concentrations (HCO$_3^-$; ranges from 300 to 320 mg/L) and as a result pH of OSPW is relatively stable, ranging from 7.91 to 8.45 (Table 2). OSPW hardness and conductivity range from 160 to 178 mg/L (as CaCO$_3$) and 1791 to 1800 $\mu$S/cm, respectively. Total suspended solids concentrations range from 130 to 400 mg/L in OSPW, and exceed narrative WQC, which are based on the potential for increasing background turbidity in receiving systems (CCME 2011). Water characteristic parameters observed for this source OSPW are generally representative of other OSPWs located in the AOS (Allen 2008a; Mahaffey and Dubé 2016).
Based on maximum concentrations of ammonia present in OSPW in this study (0.099 mg/L), ammonia was not identified as a COC. Published concentrations of ammonia in other OSPWs have been reported as high as 18.4 mg/L (± 1.2; Lai et al., 1996), exceeding toxicity values or water quality criteria guidelines (CCME 2011; ESRD 2014). Ammonia toxicity is dependent upon pH and temperature (Thurston and Russo 1981). At 14.1°C and pH of 8.29, the 96 h LC50 of ammonia to rainbow trout is 0.563 mg/L (Thurston and Russo 1981), approximately 5.5x greater than ammonia concentrations observed in the source OSPW. In this study, the maximum total phosphorus concentration (0.082 mg/L) observed in OSPW was above CCME (2011) guidelines (Table 4). However, in the context of passive treatment, nutrient concentrations (e.g., TP) can promote microbial activity and enhance microbially mediated wetland biogeochemical processes (e.g., biodegradation of NAs [Herman et al., 1994; Lai et al., 1996]; dissimilatory sulfate-reduction). Thus, in the context of measuring treatment performance of CWTS, total phosphorus was not carried forward in monitoring rate and extent of removal.

5.4.1.3 Cations/ Anions

Predominant ions in OSPW are: bicarbonate (HCO$_3^-$), sodium (Na$^+$), and chloride (Cl$^-$). The ionic strength and balance (or imbalance) of impaired waters has implications for risks to receiving system biota (Goodfellow et al., 2000). The ionic balance, which is the ratio of the sum of cations to anions, of OSPW is near neutral, ranging from 0.85 to 1.0. Chloride concentrations in OSPW are elevated (~240 mg/L) in comparison to regional background (Athabasca River concentrations range from 2 to 50 mg Cl/L;
RAMP 2001), exceed ambient water quality criteria, and were thus identified as a constituent of concern (CCME 2011; ESRD 2014). Sodium ions (Na\(^+\)) were ~360 mg/L in OSPW (Table 2); however, the contributions of Na\(^+\) to aquatic toxicity are minor in comparison to the Cl\(^-\) anion, with *C. dubia* median effects thresholds (48 h LC\(_{50}\)) occurring at concentrations of 1770 mg Na\(^+\)/L (Mount et al., 1997; Goodfellow et al., 2000).

### 5.4.1.4 Inorganics

OSPW was analyzed for 16 elements (Table 4). Elements that were not measured above the method detection limit (MDL), and therefore were not identified as COCs, included: cadmium (MDL = 0.0002 mg/L), chromium (MDL = 0.004), cobalt (MDL = 0.0002 mg/L), and silver (MDL = 0.0002 mg/L). Based on the chemical-specific characterization approach, COCs for metals/metalloids in OSPW included: Al, B, Cu, Fe, Pb, Ni, Se, and Zn (Table 3). These elements measured as “total” exceed conservative concentrations in water quality criteria or toxicity endpoints for sensitive sentinel aquatic biota. Although metal speciation and concentrations in tailings ponds differ due to geologic heterogeneities and recycling of tailing pond water, concentration ranges of elements contained in OSPW are generally consistent with other OSPWs from the AOS (MacKinnon and Boerger 1986; Siwik et al., 2000; Allen 2008a).

### 5.4.2 Design of Hybrid Pilot-scale CWTS for OSPW

#### 5.4.2.1 Design Basis

The design basis was accomplished using COC fate processes and removal rate data (e.g., Horner et al., 2013; Del Rio et al., 2005; Hwang et al., 2013; McKenzie et al.,
and batch reactor trials (unpublished data). For this pilot-scale study, two types of wetland reactors were chosen to achieve “bulk” oxidative or reductive conditions and are operationally defined as “oxidizing” and “reducing” wetland reactors. An advanced oxidation process followed the wetland reactors using a solar catalyst (TiO2) to target degradation of “residual” organic compounds (i.e. NAs).

5.4.2.2 Treatment Processes for Organic Constituents (e.g. petroleum hydrocarbons and NAs)

Microbial transformation is a primary removal process in wetlands for organic constituents (e.g., NAs). Microbial transformation in wetlands is facilitated by organisms present in or on substrate (plants, detritus, hydrosol) capable to degrading organic constituents (Bishay 1996; Knight et al., 1999; Pham et al., 2011; Horner et al., 2012; Toor et al., 2013). NAs contained in OSPWs are putatively the primary contributors to toxicity (Verbeek et al., 1994; Headley and McMartin 2004; Marinette et al., 2015; Morandi et al., 2015; McQueen et al., 2016a). Therefore, targeting treatment of NAs is critical for achieving narrative performance goals (e.g., “no toxics in toxic amounts”; USEPA 2007). NA biodegradation rates are more favorable in aerobic than in anaerobic conditions (Del Rio 2006), where β-oxidation is a primary pathway by which aerobic microorganisms degrade carboxylic acids (Taylor et al., 1978; Quagraine et al., 2005; Han et al., 2008). To achieve β-oxidation, molecular oxygen is necessary to facilitate microbial transformation of aliphatic and alicyclic carboxylic acids (Taylor and Trudgill 1978; Trudgill et al., 1984). Promoting aerobic conditions (e.g., 4-8 mg DO/L) in
wetlands is achieved by selecting hydrosoil with low organic matter content and high porosity (Rodgers and Castle 2008), minimizing water depth, and selecting plants which can translocate diatomic oxygen through rhizosphere radial oxygen loss (ROL). Cattail (T. latifolia) provides ~0.3 nmol O₂ g⁻¹ root dry weight s⁻¹ ROL (Inoue and Tsuchiya 2008). Additionally, the presence of cattail aid in altering the structural composition of NAs contained in OSPW presumably due to transformation of NAs by microbial communities on roots or by cometabolic properties of root exudates (e.g., sugars, enzymes, inorganic ions; Armstrong et al., 2009).

In this study, “oxidizing” wetland reactors were designed using cattail (Typha latifolia; initial density ~30 shoots/ m²), quartz sand hydrosoil containing <1% organic matter content, and water depths ~20 cm. These combined features in the pilot-scale system provided bulk aerobic conditions (DO ~3-4 mg/L) in the initial oxidizing wetland reactors and in reactors 2-5 (DO > 4 mg/L; Table 4). Bulk hydrosoil redox indicated that aerobic conditions were maintained in the oxidizing reactors (>50 mV; supplementary material).

For residual petroleum hydrocarbons (e.g., O/G), transfer processes in wetlands include sorption, volatilization, precipitation, and bioconcentration in plants (Rodgers and Castle 2008). Phase-separation and sorption can be an important process for higher molecular weight hydrophobic chemicals (O/G). Although sorption of hydrocarbons offers an incomplete removal mechanism, it allows additional contact time for transformation processes (e.g., microbial degradation). In this pilot-scale design, sorption
was promoted by the target flow rate (<1 cm/s) coupled with macrophyte densities (
*T. latifolia* mean shoot density 48 shoots/ m²; n=90; supplementary material).

Solar photocatalysis was used as an advanced oxidation step following outflows from wetland reactors. Wetland reactors provide treatment of suspended solids (and turbidity) which can influence UV attenuation (supplementary data) and therefore efficiency of photocatalysis. The placement of the PC reactor followed wetlands reactors to minimize the influence of suspended particles (inflow TSS ranged from 130 to 400 mg/L). Photocatalysis of NAs can be achieved within environmentally relevant rates (half-lives hours to days) and extents (achieving [NA] <5 mg/L), and is effective at decreasing toxicity of commercial and OSPW-extracted NAs (Mishra et al., 2010; Leshuk et al., 2016; McQueen et al., 2016b). Paring fixed-film catalyst (e.g., TiO₂) irradiated with natural sunlight would eliminate the need for an energy source and recovery of catalyst, thus decreasing construction and operating costs. Based on reported NA degradation rates of solar-driven photocatalysis using settled or fixed-film TiO₂ (Leshuk et al., 2016; McQueen et al., 2016b), the design basis for the photocatalytic reactor targeted cumulative solar UV (250-400 nm) of 1.0 to 3.0 MJ/m² or insolation (400-800 nm) of 30 to 40 MJ/m² (photoperiod ranging from 12-36 h; Leshuk et al., 2016; McQueen et al., 2016b; Table 4; supplementary material).

5.4.3 Treatment Processes for Inorganic Constituents (e.g., metals and metalloids, and suspended particles)

The treatment pathway targeted for divalent metals in OSPW is precipitation with sulfide forming relatively insoluble and non-bioavailable forms (Brookins 1988).
Hydrosoil conditions with redox in the range of -50 to -250 mV can facilitate microbially-mediated dissimilatory sulfate reduction, creating reduced sulfide species (e.g., bisulfide ions (HS\(^-\)), sulfide ions \([S^{2-}]\)) for complexation with divalent metals that are precipitated as sulfide minerals (e.g., CuS and ZnS; Murray-Gulde et al., 2003). These sulfide-bound species are relatively insoluble (e.g., CuS; \(k_{sp} = 10^{-16}\); Stumm and Morgan 1996) and are transferred from the water column to the hydrosoil as non-bioavailable fractions (Murray-Gulde et al., 2003; Huddleston and Rodgers 2008). The initial “reducing” wetland reactors were designed with features to promote anoxic conditions and precipitation of divalent metals as sulfides. To date, there is no evidence that NAs adversely affect sulfate-reducing bacteria (and hence dissimilatory sulfate reduction); therefore, the sequence (or placement) of reducing reactors within the hybrid CWTS can be flexible (McQueen et al., 2016a).

To maintain bulk reducing conditions, reactors were designed with greater water depths (34 cm) as compared to the oxidizing reactors. In addition, hydrosoil was amended with 2-3% organic matter (vol/vol; as wheat hay), and 0.5-1% (vol/vol) pelletized gypsum (CaSO\(_4\cdot2H_2O\)) was added as an additional sulfate source. Softstem bulrush (Schoenoplectus tabernaemontani) was the vegetation selected for the “reducing” reactors based on the ability of Schoenoplectus spp. to tolerate and maintain reducing hydrosoil conditions (Kantrud 1989; Murray-Gulde et al., 2005).

Targeted ranges of ORP, DO, and pH were maintained throughout the duration of the 10-week experiment in the “reducing” reactors (Table 4 and supplemental data). Surficial sediment AVS concentrations in the reducing reactors ranged from 0.54 to 13.6
µmol/g. Wetland reactors were designed to maintain flow velocities <1cm/s to allow suspended sediments and precipitated minerals to settle from the water column to the hydrosoil. Shoot density in the reducing reactors reached >100 shoots/m² (*S. tabernaemontani*), further promoting settling of suspended (inorganic) solids (Karathanasis et al., 2003).

5.4.3.1 Organic COCs

Organic COCs identified in OSPW included residual petroleum hydrocarbons (as O/G) and NAs. Inflow water had a visible but slight hydrocarbon sheen. Following a 4.5-day HRT in the wetland reactors, hydrocarbon sheens were no longer visible. Inflow O/G concentrations ranged from 6 to 15 mg/L and declined to non-detect (MDL = 2 mg/L) within 1.5 to 4.5 day HRTs for all five treatment periods evaluated (Figure 2). Residual hydrocarbons (as O/G) have been treated successfully by CWTS, with inflow concentrations of 20 mg O/G /L declining to <1.4 mg/L following a 1 to 2 day HRT in free-water surface wetlands planted with *T. latifolia* (Horner et al., 2012). Spacil et al. (2011) achieved treatment of low molecular weight petroleum hydrocarbons in pilot-scale CWTS planted with *T. latifolia* with removal rate coefficients of 0.8 day⁻¹ (T₁/₂ = ~0.9 days). OSPWs can contain O/G concentrations as high as 92 mg/L (Allen 2008a). Oil-water separators prior to inflows into CWTS may be warranted for waters containing higher concentrations of hydrocarbons (e.g., >50 mg/L O/G; Pardue et al., 2014); however, based on the inflow O/G concentrations in OSPW (6 to 15 mg/L), concentrations declined to achieve narrative guidelines within 1.5 to 3.5 days of treatment.
“Total” NA concentrations (quantified by HPLC) did not significantly decrease in outflows from wetland reactors after 13.5 days (Figure 3). Other studies using unplanted wetland bench-scale microcosms have demonstrated an average 40% decrease in NAs (mean inflow concentrations of 80 mg NA/L) following a 40-day HRT (Toor et al., 2013). Additionally, Toor et al. (2013) observed complete removal of acute toxicity (100% to 0% mortality) to rainbow trout (96 h bioassays) following a 40-day HRT in the bench-scale wetland microcosms.

The rate and extent of biodegradation of naphthenic acids is highly dependent on molecular weight and structure (Han et al., 2008, Holowenko et al., 2002, Scott et al., 2005). Holowenko et al. (2002) found that NAs with a carbon number ≥22 are less degradable than those with a carbon number <22. Han et al. (2008) demonstrated the importance of molecular structures of NAs in predicting their biodegradability. Increased cyclicity and alkyl branching (typical of NAs in weathered OSPW) decrease degradability of NAs by microbial β-oxidation. Following wetland reactors, photocatalysis provided an additional process for degrading “recalcitrant” organic fractions (NAs).

Following photocatalysis, NA concentrations decreased from initial concentrations of 75 to 122 mg/L to outflow concentrations of 8 to 65 mg/L (47 to 93% removal efficiencies). Flow-through solar photocatalysis experiments indicate that NAs in OSPW are degrading at environmentally relevant rates, with ~50% removal achieved within ~12h of direct sunlight exposures (~1.3 to 2.3 MJ/m² cumulative UV; Figure 3). Mishra et al. (2010) achieved degradation of NAs extracted from OSPW using TiO₂ and
artificial UV$_{254}$, with half-lives of 1.55 and 4.8 h for deionized water and South Saskatchewan River water, respectively. Leshuk et al. (2016) degraded acid extractable organics (NAs) in OSPW using natural sunlight to an extent of $<1$ mg/L (initial [NA] of 40 mg/L) following 30 MJ/m$^2$ (400-700 nm) insolation (2-3 days of sunlight exposure). In this study, degradation of NAs were achieved to an extent of 10 to 16 mg/L (week 4 treatment) following an average daily cumulative radiant exposure of 1.65 MJ/m$^2$.

5.4.3.2 Inorganic COCs

Concentrations of Al, Cu, Ni, and Zn following a 13.5 day HRT in wetland reactors significantly declined from inflow to outflow (Figure 4). With the exceptions of B and Se, extents of removal (i.e. outflow concentrations) reached targeted performance goals for all metals and metalloids identified as COCs (Figure 4). Average removal efficiency (n=5 treatment periods) for Al and Cu ranged from 88 to 90%. Removal extents for Al and Cu ranged from 0.019 to 0.043 mg/L and 0.005 to 0.011 mg/L, respectively. OSPW inflow concentrations of Ni ranged from 0.01 to 0.015 (n=5), slightly above the numeric water quality guideline of 0.0096 mg/L (CEQGs; CCME 2011). Following treatment in wetland reactors, Ni concentrations were below the CEQGs, with an average removal efficiency of 49 and 44% for wetland Series A and B, respectively. Inflow concentrations of Zn ranged from non-detect (MDL = 0.002 mg/L) to 0.064 mg/L. Outflow concentrations of Zn were below the target removal extent of 0.03 mg/L (based on CEQGs), with average removal efficacies of 65 and 58% (n=5) for Series A and B, respectively.
Inflow Se concentrations ranged from non-detect (MDL = 0.0002 mg/L) to 0.0066 mg/L, above regional background (Athabasca River; range <0.001 to 0.0014; RAMP 2001; Athabasca River upstream of Muskeg River) and guidelines of 0.001 mg/L (CEQGs) and 0.0015 mg/L (USEPA WQC; USEPA 2007; CCME 2011). Following 13.5 day HRT in wetland reactors, Se concentrations declined, with outflow concentrations ranging from 0.0004 to 0.0045 mg/L. Average Se removal efficiencies for Series A and B were 42 and 85%, respectively. However, outflow concentrations of Se following wetland reactors were above the CEQGs target of 0.001 mg/L (Figure 4). Development of site- or regionally-specific Se criteria are forthcoming, which may incorporate exposure modifying factors (e.g. sulfate) to the interpretation and use of current guidelines. Exposure modifying factors are crucial to understanding bioavailability and ecological risk of Se (Chapman et al., 2009). Nonetheless, Se removal efficiencies can be enhanced in CWTS by including longer retention times in reducing biogeochemical conditions (Spacil et al., 2011). Se removal in CWTS is primarily achieved by microbial reduction from oxidized forms of Se (SeO₃²⁻) to elemental selenium (Spacil et al. 2011). Spacil et al. (2011) reported Se removal efficiencies measured in a pilot-scale system from 86-99%, with removal rate coefficients ($k$) ranging from 0.16 to 0.99 day⁻¹ by promoting reducing conditions (e.g. < 2 mg DO/L and ORP < -50mV). In this study, boron concentrations did not decline from inflow to outflow, with outflow concentrations ranging from 1.8 to 2.7 mg B/L, greater than the target numeric criterion of 1.5 mg/L (CCME 2011). However, boron concentrations in OSPW indicate concentrations are below toxicity thresholds to sentinel aquatic species (T. latifolia 7-day NOEC [seedling
root growth] = 10.4 mg B/L; *C. dubia* 7-day NOEC [reproduction] = 16.4 mg B/L [Damiri 2009]; rainbow trout 28-day LC50 = 79 mg/L [Birge and Black 1977]).

Al and Zn had the greatest removal rate coefficients of the elemental COCs, with mean rate coefficients of 0.35 day\(^{-1}\) or (T\(_{1/2}\) = ~2 days; n = 5; Series A and B) and 0.37 (T\(_{1/2}\) = ~2 days; n = 5; Series A), respectively. Removal rates were slower for Cu and Ni, with mean rate coefficients ranging from 0.20 day\(^{-1}\) (Cu, T\(_{1/2}\) = ~3.5 days Series A and B) to 0.07 (Ni; T\(_{1/2}\) = ~10 days; Series A and B). Se mean rate coefficient was 0.17 day\(^{-1}\) (n = 5 treatment periods) in wetland Series A; however, Series B concentrations increased slightly (e.g. 0.001 to 0.004 mg/L) from 7.5 to 13.5 days, therefore rates were not estimated.

Rates of removal observed in this study parallel rates of removal measured in sequential reducing and oxidizing wetlands reactors for Al, Cu, Ni and Zn contained in other energy-derived waters (Johnson et al., 2008; Murray-Gulde et al., 2008; Eggert et al., 2008; Spacil et al., 2011; Horner et al., 2012). In CWTS studies where sulfide or iron-manganese co-precipitation is the targeted biogeochemical removal process, aqueous removal of Al, Cu, Ni, and Zn (to the extent of achieving target water quality guidelines) in CWTS is achieved on the order of days (i.e. k = 0.1 to 1.0 day\(^{-1}\); Johnson et al., 2008; Murray-Gulde et al., 2008; Eggert et al., 2008; Spacil et al., 2011; Horner et al., 2012). Removal rates and extents are essential for scaling similar system designs to other locations (Huddleston and Rodgers 2008). In this study, pilot-scale wetland reactor experiments were conducted in a covered greenhouse, with no influence of dilution by
rainfall; therefore, rates and extents of removal reported for all constituents are conservative.

5.4.3.3 Toxicity Experiments

Survival and reproduction of *C. dubia* in 7-8 day exposures were used to evaluate the ability of the pilot-scale CWTS to mitigate risks associated with OSPW. *C. dubia* mortality ranged from 18 to 80% (n=5 treatment periods) in untreated (inflow) OSPW. Reproduction of *C. dubia* was impaired in all but one (treatment period 3) of the inflows tested as compared to a laboratory control. Following 13.5 day HRT in the wetland reactors, *C. dubia* toxicity was eliminated (Figure 5). For treatment periods 1 and 2, reproduction increased from inflow to outflow of wetland reactors with a mean of 27 and 30 neonates/live adult (no statistical difference from laboratory control; p= 0.56; α = 0.05). Toor et al. (2013) achieved decreases in rainbow trout mortalities to OSPW following treatment in unplanted simulated wetland hydrosols (wetland sediment and aerated microcosms with input of OSPW collected from Mildred Lake settling basin). Presumably, toxicity in OSPW is influenced by “classical” NAs (Verbeek et al., 1994; Morandi et al., 2015). In this study, total NAs concentrations (as quantified by HPLC) did not appreciably decline in the wetland reactors following 13.5 day HRT; however, toxicity was eliminated (Figure 5). These observations support that adverse effects of OSPW (and specifically the organic acid fraction) are influenced by composition of NAs and not concentration alone (Brown and Ulrich 2015, Mahaffey and Dubé 2016). Results from this study also support the hypothesis that wetlands can be effective for removing
acute OSPW toxicity (Armstrong et al., 2009; Toor et al., 2013) within environmentally relevant timeframes (e.g., hydraulic retention times of ~2 weeks).

5.5 Conclusions

Results from this hybrid pilot-scale study provided evidence that problematic constituents contained in OSPW could be treated to extents necessary to remove toxicity to a sentinel organism (C. dubia). Based on characterization of an OSPW from an active settling basin (OSPW), COCs needing treatment were identified as: NAs, petroleum hydrocarbons (as O/G), Al, B, Cu, Ni, Se, Zn, and TSS. The hybrid pilot-scale CWTS used passive (i.e. low energy) biogeochemical and advance oxidation processes to transfer or transform COCs to achieve numeric and narrative treatment goals. Results from this study provide proof-of-concept data to inform hybrid passive or semi-passive treatment approaches (i.e. constructed wetlands) that could be used to mitigate COCs contained in OSPWs.

5.6 Acknowledgements

Funding support for this research was provided by Shell Canada Ltd. and Suncor Energy. The authors are also grateful to Dr. Wayne Chao of Clemson University and Dr. John Headley and Kerry Peru of Environment and Climate Change Canada for providing analytical support.
5.7 References


biodegradability and biofilm formation characteristics in bioreactors. *Bioresource technology* 130: 269-277.


document for water quality-based toxics control. EPA/505/2-90-001. Washington,
D.C.

extractable material (HEM; oil and grease) and silica gel treated n-hexane
extractable material (SGTHEM; non-polar material) by extraction and gravimetry,
Method 1664. Washington, D.C.

United States Environmental Protection Agency (USEPA). (2001). EPA Method 200.7,
Trace elements in water, solids, and biosolids by inductively coupled plasma-
atomic emission spectrometry, Revision 5.0, EPA-821-R-01-010. Washington,
D.C.

United States Environmental Protection Agency (USEPA). (2002). Short-term Methods
for Estimating Chronic Toxicity of Effluents and Receiving Water to
Freshwater Organisms, EPA-821-R-02-013. Washington, D.C.

Recommended Water Quality Criteria., Washington, D.C.

reduction of naphthenic acids by ozonation and combined ozonation-aerobic

fundamentals and applications in the petroleum industry. Cambridge University
Press. N.Y.

Microbiology 70: 93-125.

acids concentrations in aqueous environmental samples by liquid

source of recalcitrant naphthenic acid mixtures in oil sands tailing pond waters?
Table 5.1 Hybrid pilot-scale CWTS design features.

<table>
<thead>
<tr>
<th>Reactor Type</th>
<th>Vol. (L)\textsuperscript{a}</th>
<th>HRT (time/reactor)</th>
<th>Flow-rate (mL/min)</th>
<th>Reactors per series</th>
<th>Reactor Dimensions (cm)</th>
<th>Hydrosoil\textsuperscript{c,d}</th>
<th>Vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing Wetland Reactors</td>
<td>74</td>
<td>1.5d</td>
<td>34</td>
<td>1</td>
<td>26 radius, 73 height</td>
<td>30 cm medium to coarse grained quartz sand; 2% v/v organic matter\textsuperscript{e}, 1% v/v gypsum (CaSO\textsubscript{4}·2H\textsubscript{2}O)</td>
<td>Schoenoplectus tabernaemontani (softstem bulrush); Initial density 20-30 shoots/m\textsuperscript{2}</td>
</tr>
<tr>
<td>Oxidizing Wetland Reactors</td>
<td>148</td>
<td>3d</td>
<td>34</td>
<td>4</td>
<td>61 height, 122 length, 64 width</td>
<td>30 cm medium to coarse grained quartz sand</td>
<td>Typha latifolia (broadleaf cattail); Initial density 20-30 shoots/m\textsuperscript{2}</td>
</tr>
<tr>
<td>Fixed-film TiO\textsubscript{2} Solar Photocatalysis Reactors</td>
<td>7</td>
<td>12h\textsuperscript{f}</td>
<td>10</td>
<td>1</td>
<td>15 height, 182 length, 60 width</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a}volume by direct measure  
\textsuperscript{b}flow-rate maintained by metered piston pump (Fluid Metering Inc. [FMI®])  
\textsuperscript{c}slow release fertilizer (Osmocote®) added to all reactors on initiation  
\textsuperscript{d}hydrosoil bulk porosity = 0.25  
\textsuperscript{e}organic matter = wheat hay  
\textsuperscript{f}HRT based on hours of direct sunlight
Table 5.2 Comparison of water quality characteristics and organic constituents in OSPW to water quality guidelines (USEPA 2007; CCME 2011, ESRD 2014) and toxicity values for *C. dubia* (Cd), rainbow trout (*O. mykiss* [Om]), and fathead minnow (*P. promelas* [Pp]). COCs (i.e. concentration > guideline) are bolded.

<table>
<thead>
<tr>
<th>Parameter mg/L</th>
<th>OSPW (n=10)</th>
<th>Water Quality Guidelines (mg/L)</th>
<th>Reported Toxicity Values (mg/L)</th>
<th>COC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(unless noted)</td>
<td>Min</td>
<td>Max</td>
<td>Alberta WQG</td>
<td>CEQG</td>
</tr>
<tr>
<td>pH (SU)</td>
<td>7.91</td>
<td>8.4</td>
<td>Chronic</td>
<td>Chronic</td>
</tr>
<tr>
<td>Alkalinity (CaCO₃)</td>
<td>320</td>
<td>340</td>
<td>6.5-9.0</td>
<td>No</td>
</tr>
<tr>
<td>Total Ammonia (N)</td>
<td>0.051</td>
<td>0.099</td>
<td>0.17-1.5 b</td>
<td>2.9</td>
</tr>
<tr>
<td>Total Phosphorus (TP)</td>
<td>0.037</td>
<td>0.082</td>
<td>0.01-0.02</td>
<td></td>
</tr>
<tr>
<td>Total Suspended Solids (TSS)</td>
<td>130</td>
<td>400</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Total Naphthenic Acids (NAs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAs (HPLC)</td>
<td>80</td>
<td>128</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>NAs (Orbitrap MS)</td>
<td>93</td>
<td>103</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Oil and grease (O/G)</td>
<td>8</td>
<td>13</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (HCO₃⁻)</td>
<td>300</td>
<td>320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (Ca⁺)</td>
<td>29</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride (Cl⁻)</td>
<td>240</td>
<td>245</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Sodium (Na⁺)</td>
<td>360</td>
<td>364</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfate (SO₄²⁻)</td>
<td>150</td>
<td>175</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Narrative statement; no visible sheen, or unreasonable turbidity (no more than >25mg/L TSS than background in a 24-hr period)
**"No toxics in toxic amounts"

Note: NAs quantified by different methods do not necessarily measure the same suite of compounds (Headley et al., 2015)

---

*a Minimum
*b Temperature range from 5-20°C and pH of 8.0-8.5
*c Thurston and Russon 1981; @ 14.4°C and pH of 8.3
*d Kavanagh et al. 2011; 5 d larvae; NAs quantified by ESI-MS
*e Marentette et al. 2015; ”fresh” OSPW; embryos; NA quantified by LC/QToF
*f Hoke et al. 1992
*g Mount et al. 1997
*h Adelman et al. 1976
*i Soucek and Kennedy 2005
Table 5.3 Comparison of metals and metalloids in OSPW to water quality guidelines (USEPA 2007; CCME 2011; ESRD 2014) and toxicity values for *C. dubia* (Cd), *D. magna* (Dm), fathead minnow (*P. promelas* [Pp]), and rainbow trout (*O. mykiss* [Om]). Identified COCs (i.e. concentration > guideline) are bolded.

<table>
<thead>
<tr>
<th>Parameter (mg/L)</th>
<th>OSPW (n=10)</th>
<th>Water Quality Guidelines (mg/L)</th>
<th>Reported Toxicity Values (mg/L)</th>
<th>COC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Alberta WQG</td>
<td>CEQG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chronic</td>
<td>Chronic</td>
</tr>
<tr>
<td>Aluminum (Al)</td>
<td>0.36</td>
<td>10.9</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>0.0006</td>
<td>0.0032</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Barium (Ba)</td>
<td>0.21</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boron (B)</td>
<td>2.09</td>
<td>2.12</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>&lt;0.0002</td>
<td>&lt;0.0002</td>
<td>0.00016&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00038&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>&lt;0.004</td>
<td>&lt;0.004</td>
<td>0.0089</td>
<td>0.0089</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>&lt;0.0002</td>
<td>&lt;0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.003</td>
<td>0.12</td>
<td>0.007</td>
<td>0.0024&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>1.2</td>
<td>1.5</td>
<td>0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>&lt;0.0002</td>
<td>0.0014</td>
<td>0.0032&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00318&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.137</td>
<td>0.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>0.041</td>
<td>0.062</td>
<td>0.073</td>
<td>0.073</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>0.0056</td>
<td>0.01</td>
<td>0.052</td>
<td>0.0096</td>
</tr>
<tr>
<td>Selenium (Se)</td>
<td>0.002</td>
<td>0.004</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Silver (Ag)</td>
<td>&lt;0.0002</td>
<td>&lt;0.0002</td>
<td>0.001</td>
<td>0.00025</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.014</td>
<td>0.113</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>pH: ≥6.5  
<sup>b</sup>Hardness: 100 mg/L as CaCO₄  
<sup>c</sup>Dissolved fraction  
<sup>d</sup>Freeman and Everhart 1971; biomass  
<sup>e</sup>Biesinger and Christensen 1972  
<sup>f</sup>OPP, 2000  
<sup>g</sup>Suedel et al. 1997  
<sup>h</sup>Kimball 1978  
<sup>i</sup>Mount, 1968  
<sup>j</sup>Pickering and Henderson 1966  
<sup>k</sup>Boucher and Watzin 1999  
<sup>l</sup>Birge 1977  
<sup>m</sup>Keithly et al. 2004
### Table 5.4 Targeted treatment processes, operational metrics, and measured ranges in hybrid CWTS designed for OSPW.

<table>
<thead>
<tr>
<th>Target Treatment Process</th>
<th>Operational Metric</th>
<th>Target Range</th>
<th>Measured Range&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorption/ Settling</td>
<td>shoot density and root mass&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>&gt;100 shoots m² (bulrush)</td>
<td>165 shoots/m²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;30 shoots m² (cattail)</td>
<td>48 shoots/m²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>low flow rate (&lt;10 cm/s)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;1 cm/s</td>
</tr>
<tr>
<td></td>
<td>HRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction</td>
<td>ORP</td>
<td>ORP -150 to -250 mV&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ORP &lt; -150 mV</td>
</tr>
<tr>
<td></td>
<td>acid volatile sulfides (AVS)</td>
<td>excess molar ratio of AVS to SEM (AVS:SEM&gt;1)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>AVS:SEM, 1.5 to 4.6</td>
</tr>
<tr>
<td>Oxidation</td>
<td>ORP</td>
<td>ORP &gt; -50 mV</td>
<td>ORP &gt; -28 mV</td>
</tr>
<tr>
<td></td>
<td>plant density (estimation of rhizome radial oxygen loss)</td>
<td>&gt;30 shoots·m²</td>
<td>48 shoots/m²</td>
</tr>
<tr>
<td></td>
<td>aqueous DO concentration</td>
<td>&gt;2.0 mg/L</td>
<td>2.65-6.8 mg/L</td>
</tr>
<tr>
<td>Aerobic biodegradation</td>
<td>ORP</td>
<td>ORP &gt; -50 mV</td>
<td>ORP &gt; -28 mV</td>
</tr>
<tr>
<td></td>
<td>aqueous DO concentration</td>
<td>&gt;2.0 mg/L</td>
<td>2.15-6.32 mg/L</td>
</tr>
<tr>
<td></td>
<td>SOD&lt;sub&gt;5-day&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&gt;100 mg/L O₂&lt;sup&gt;g&lt;/sup&gt;</td>
<td>333-546 mg/L</td>
</tr>
<tr>
<td></td>
<td>shoot density (indicator of rhizome radial oxygen loss)</td>
<td>&gt;30 shoots/m²</td>
<td>48 shoots/m²</td>
</tr>
<tr>
<td>Advanced oxidation</td>
<td>daily cumulative UV (250-400nm)</td>
<td>&gt;1.0 MJ/m²&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.86 to 1.5 MJ/m²</td>
</tr>
<tr>
<td>(photocatalysis)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>ORP = oxidation reduction potential (mV); surficial sediment (2-6 cm)
<sup>b</sup>HRT = hydraulic retention time
<sup>c</sup>SOD<sub>5-day</sub> = 5-day sediment oxygen demand
<sup>d</sup>Conditions measured during experiment duration (mean [n=5] or range)
<sup>e</sup>ROD = oxidation reduction potential (mV); surficial sediment (2-6 cm)
<sup>f</sup>Di Toro et al., 1992
<sup>g</sup>Di Toro et al., 1992
<sup>h</sup>Di Toro et al., 1992
<sup>i</sup>Rodgers and Castle 2008
<sup>j</sup>Li et al., 2010
<sup>k</sup>Reduction based on glucose+glutamic standard with no added nutrients
<sup>l</sup>Based on batch reactor fixed-film photocatalysis trials using OSPW (unpublished)

Note: shoot density can be an indicator of root mass (typically 1:1 biomass ratio; Li et al., 2010)
Table 5.5 Inflow concentrations, outflow concentrations, removal efficiencies, and removal rate coefficients of COCs for each CWTS replicate series.

<table>
<thead>
<tr>
<th>Treatment Period</th>
<th>[Inflow mg/L]</th>
<th>Removal extent (mg/L)</th>
<th>Removal efficiency (%)</th>
<th>Rate coefficient (day⁻¹), (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Series A</td>
<td>Series B</td>
<td>Series A</td>
</tr>
<tr>
<td><strong>Aluminum (Al)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>0.879</td>
<td>0.023</td>
<td>0.021</td>
<td>97</td>
</tr>
<tr>
<td>Period 2</td>
<td>0.102</td>
<td>0.031</td>
<td>0.036</td>
<td>70</td>
</tr>
<tr>
<td>Period 3</td>
<td>0.363</td>
<td>0.022</td>
<td>0.026</td>
<td>94</td>
</tr>
<tr>
<td>Period 4</td>
<td>0.524</td>
<td>0.019</td>
<td>0.022</td>
<td>96</td>
</tr>
<tr>
<td>Period 5</td>
<td>0.702</td>
<td>0.037</td>
<td>0.043</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td><strong>Average (n=5)</strong></td>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td><strong>Boron (B)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>2.05</td>
<td>2.02</td>
<td>1.80</td>
<td>1</td>
</tr>
<tr>
<td>Period 2</td>
<td>2.04</td>
<td>2.20</td>
<td>1.99</td>
<td>-8</td>
</tr>
<tr>
<td>Period 3</td>
<td>2.10</td>
<td>2.16</td>
<td>2.00</td>
<td>-3</td>
</tr>
<tr>
<td>Period 4</td>
<td>2.12</td>
<td>2.54</td>
<td>2.19</td>
<td>-20</td>
</tr>
<tr>
<td>Period 5</td>
<td>2.16</td>
<td>2.77</td>
<td>2.60</td>
<td>-29</td>
</tr>
<tr>
<td></td>
<td><strong>Average (n=5)</strong></td>
<td></td>
<td></td>
<td>-12</td>
</tr>
<tr>
<td><strong>Copper (Cu)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>0.13</td>
<td>0.007</td>
<td>0.008</td>
<td>94</td>
</tr>
<tr>
<td>Period 2</td>
<td>0.058</td>
<td>0.007</td>
<td>0.007</td>
<td>88</td>
</tr>
<tr>
<td>Period 3</td>
<td>0.062</td>
<td>0.006</td>
<td>0.005</td>
<td>90</td>
</tr>
<tr>
<td>Period 4</td>
<td>0.123</td>
<td>0.011</td>
<td>0.005</td>
<td>91</td>
</tr>
<tr>
<td>Period 5</td>
<td>0.043</td>
<td>0.006</td>
<td>0.007</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td><strong>Average (n=5)</strong></td>
<td></td>
<td></td>
<td>88</td>
</tr>
<tr>
<td><strong>Nickel (Ni)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>0.014</td>
<td>0.0056</td>
<td>0.008</td>
<td>60</td>
</tr>
<tr>
<td>Period 2</td>
<td>0.010</td>
<td>0.0052</td>
<td>0.0048</td>
<td>48</td>
</tr>
<tr>
<td>Period 3</td>
<td>0.015</td>
<td>0.0092</td>
<td>0.0094</td>
<td>35</td>
</tr>
<tr>
<td>Period 4</td>
<td>0.015</td>
<td>0.0060</td>
<td>0.0068</td>
<td>60</td>
</tr>
<tr>
<td>Period 5</td>
<td>0.011</td>
<td>0.0062</td>
<td>0.0070</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td><strong>Average (n=5)</strong></td>
<td></td>
<td></td>
<td>49</td>
</tr>
<tr>
<td><strong>Selenium (Se)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>0.0066</td>
<td>0.0045</td>
<td>0.0004</td>
<td>32</td>
</tr>
<tr>
<td>Period 2</td>
<td>0.005</td>
<td>0.0032</td>
<td>0.0015</td>
<td>36</td>
</tr>
<tr>
<td>Period 3</td>
<td>0.0058</td>
<td>0.0025</td>
<td>0.0006</td>
<td>57</td>
</tr>
<tr>
<td>Period 4</td>
<td>BDL¹</td>
<td>BDL</td>
<td>BDL</td>
<td>-</td>
</tr>
<tr>
<td>Period 5</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><strong>Average (n=3)</strong></td>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td><strong>Zinc (Zn)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>0.051</td>
<td>0.002</td>
<td>0.002</td>
<td>96</td>
</tr>
<tr>
<td>Period 2</td>
<td>BDL²</td>
<td>BDL</td>
<td>BDL</td>
<td>-</td>
</tr>
<tr>
<td>Period 3</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>-</td>
</tr>
<tr>
<td>Period 4</td>
<td>0.064</td>
<td>0.018</td>
<td>0.027</td>
<td>72</td>
</tr>
<tr>
<td>Period 5</td>
<td>0.042</td>
<td>0.02</td>
<td>0.021</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td><strong>Average (n=3)</strong></td>
<td></td>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>

BDL = below method detection limit
Treatment periods = 13.5 day HRT

¹Selenium analytical method detection limit = 0.0002 mg/L
²Zinc analytical method detection limit = 0.002 mg/L
Fig 5.1 A schematic diagram of the pilot-scale experiment.
Fig 5.2 O/G concentrations in inflow and reactor outflows for Series A (solid line) and Series B (dashed line).
Fig 5.3 Total NA concentrations in wetland inflow and outflows for Series A (solid lines) and Series B (dashed lines), and inflow and outflow from photocatalysis treatments.

Note: Photocatalysis (PC) represents inflow and outflow from thin-film TiO₂ reactors conducted outdoors during the months of May, June, and July. Photocatalysis nominal HRT = 12h. Average daily cumulative UV (200-400 nm) ranged from 0.86 to 1.5 MJ/m² (supplementary material). PC treatments are presented as mean (n=3) NA concentration with error bars representing standard deviation.
Fig 5.4 Inorganic COC (Al, B, Cu, Ni, Se, Zn, and TSS) concentrations in inflow and reactor outflows for Series A (solid line) and Series B (dashed line) for treatment periods 1-5.

Note: “Water Quality Criteria Target” represented by dashed line:
Al = 0.087 mg/L (USEPA WQC)
B = 1.5 mg/L (CEQG)
Cu = 0.007 mg/L (Alberta WQG)
Ni = 0.0096 mg/L (CEQG)
Se = 0.001 mg/L (CEQG)
Zn = 0.03 mg/L (CEQG)
Fig 5.5 Survival (a) and reproduction (b) of *Ceriodaphnia dubia* exposed to inflow (untreated) and wetland outflow samples of OSPW treated by pilot-scale CWTS. (n=20 per treatment). Note: 7-8 day durations with static/renewals. Bars with asterisks are significantly different (α = 0.05; p<0.05) as compared with control. Error bars represent standard deviation.

Note: Treatment period timelines and conditions are provided in supplementary data.
APPENDIX A

CHAPTER 5

Supplemental Data
Table 5.1.A. Treatment dates and conditions for hybrid pilot-scale CWTS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reactor(s)</th>
<th>Location</th>
<th>Treatment Dates</th>
<th>Start</th>
<th>End</th>
<th>Average Daily Cumulative UV (MJ/m²)</th>
<th>Weekly Rainfall (mm)</th>
<th>Evaporation Rate (mm h⁻¹)</th>
<th>Ambient Air Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>Wetland</td>
<td>Greenhouse</td>
<td>7-Mar-16 - 21-Mar-16</td>
<td>-</td>
<td>-</td>
<td>0.2 to 2.0 d</td>
<td>11</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Period 2</td>
<td>Wetland</td>
<td>Greenhouse</td>
<td>23-Mar-16 - 6-Apr-16</td>
<td>-</td>
<td>-</td>
<td>0.2 to 2.0 d</td>
<td>13</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Period 3</td>
<td>Wetland</td>
<td>Greenhouse</td>
<td>10-Apr-16 - 24-Apr-16</td>
<td>-</td>
<td>-</td>
<td>0.2 to 2.0 d</td>
<td>12</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Period 4</td>
<td>Wetland</td>
<td>Greenhouse</td>
<td>25-Apr-16 - 9-May-16</td>
<td>-</td>
<td>-</td>
<td>0.2 to 2.0 d</td>
<td>12</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Period 5</td>
<td>Wetland</td>
<td>Greenhouse</td>
<td>10-May-16 - 24-May-16</td>
<td>-</td>
<td>-</td>
<td>0.2 to 2.0 d</td>
<td>12</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Week 1</td>
<td>Photocatalysis</td>
<td>Outdoor</td>
<td>11-May-16 - 18-May-16</td>
<td>1.36</td>
<td>5.00</td>
<td>0.3 to 0.5 e</td>
<td>17</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>Week 2</td>
<td>Photocatalysis</td>
<td>Outdoor</td>
<td>1-Jun-16 - 7-Jun-16</td>
<td>1.45</td>
<td>9.14</td>
<td>0.3 to 0.5 e</td>
<td>21</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>Week 3</td>
<td>Photocatalysis</td>
<td>Outdoor</td>
<td>13-Jun-16 - 20-Jun-16</td>
<td>0.85</td>
<td>25.40</td>
<td>0.3 to 0.5 e</td>
<td>19</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>Week 4</td>
<td>Photocatalysis</td>
<td>Outdoor</td>
<td>11-Jul-16 - 18-Jul-16</td>
<td>1.65</td>
<td>9.91</td>
<td>0.3 to 0.5 e</td>
<td>22</td>
<td>22</td>
<td>36</td>
</tr>
<tr>
<td>Week 5</td>
<td>Photocatalysis</td>
<td>Outdoor</td>
<td>20-Jul-16 - 27-Jul-16</td>
<td>1.33</td>
<td>22.86</td>
<td>0.3 to 0.5 e</td>
<td>21</td>
<td>21</td>
<td>35</td>
</tr>
</tbody>
</table>

aEach treatment period represents a 13.5 HRT
bUV = 250-400 nm; Apogee SU-100 and HOBO Pendant® Temperature/Light 64K Data Logger
cData collected from outdoor weather station accessed for Clemson, SC; accessed via: https://www.wunderground.com/history/
dReported range of evapotranspiration coefficients for outdoor pilot-scale reactors planted with Typha latifolia (Beebe, 2013)
eRange of average daily evaporation rates measured in the photocatalytic reactor
fTemperature data measured via HOBO Pendant® Temperature/Light 64K Data Logger, Bourne, MA

Table 5.2.A Mean water characteristics (n=5) measured in hybrid pilot-scale CWTS.
(Ranges are in parenthesis)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>Alkalinity (mg/L CaCO₃)</th>
<th>Hardness (mg/L CaCO₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wetland Series</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflow</td>
<td>20.2 (15.4-23.8)</td>
<td>8.30</td>
<td>8.16 (7.98-8.38)</td>
<td>2006 (1791-2210)</td>
<td>364 (340-394)</td>
<td>169 (160-178)</td>
</tr>
<tr>
<td>Series A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>20.1 (15.1-23.5)</td>
<td>7.72</td>
<td>2.29 (1.99-2.61)</td>
<td>1892 (1782-1987)</td>
<td>344 (280-394)</td>
<td>139 (120-152)</td>
</tr>
<tr>
<td>A2</td>
<td>20.2 (15.3-23.4)</td>
<td>7.66</td>
<td>3.47 (3.01-3.68)</td>
<td>1939 (1790-2061)</td>
<td>344 (248-405)</td>
<td>152 (145-160)</td>
</tr>
<tr>
<td>A3</td>
<td>20.0 (15.5-23.4)</td>
<td>7.67</td>
<td>4.21 (3.25-4.65)</td>
<td>1973 (1801-2163)</td>
<td>397 (264-488)</td>
<td>141 (112-184)</td>
</tr>
<tr>
<td>A4</td>
<td>19.9 (15.2-23.5)</td>
<td>7.69</td>
<td>5.10 (4.45-5.55)</td>
<td>2078 (1901-2204)</td>
<td>368 (228-440)</td>
<td>148 (130-180)</td>
</tr>
<tr>
<td>A5</td>
<td>20.2 (15.3-23.5)</td>
<td>7.82</td>
<td>6.11 (5.88-6.25)</td>
<td>2245 (1910-2678)</td>
<td>374 (236-448)</td>
<td>167 (142-184)</td>
</tr>
<tr>
<td>Series B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>20.3 (15.6-23.8)</td>
<td>7.73</td>
<td>2.29 (1.97-2.6)</td>
<td>1945 (1787-2059)</td>
<td>357 (288-424)</td>
<td>168 (152-176)</td>
</tr>
<tr>
<td>B2</td>
<td>20.2 (15.5-23.6)</td>
<td>7.70</td>
<td>3.32 (2.65-4.51)</td>
<td>1939 (1801-2104)</td>
<td>374 (272-430)</td>
<td>161 (146-178)</td>
</tr>
<tr>
<td>B3</td>
<td>20.2 (15.4-23.4)</td>
<td>7.80</td>
<td>4.33 (3.32-5.21)</td>
<td>1957 (1845-2083)</td>
<td>376 (240-488)</td>
<td>154 (132-178)</td>
</tr>
<tr>
<td>B4</td>
<td>20.2 (15.5-23.5)</td>
<td>7.76</td>
<td>5.64 (3.74-6.35)</td>
<td>2030 (1910-2181)</td>
<td>367 (232-450)</td>
<td>166 (138-200)</td>
</tr>
<tr>
<td>B5</td>
<td>20.2 (15.6-23.6)</td>
<td>7.79</td>
<td>6.32 (5.55-6.8)</td>
<td>2129 (1979-2301)</td>
<td>368 (256-440)</td>
<td>176 (128-224)</td>
</tr>
<tr>
<td>Photocatalysis Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflow</td>
<td>8.15 (7.78-8.25)</td>
<td>7 ± 1</td>
<td>3312 (3198-3557)</td>
<td>403 (380-420)</td>
<td>149 (120-170)</td>
<td></td>
</tr>
<tr>
<td>Week 1 - Outflow</td>
<td>8.35</td>
<td>8 ± 1</td>
<td>6810</td>
<td>570</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Week 2 - Outflow</td>
<td>8.52</td>
<td>8 ± 1</td>
<td>4421</td>
<td>490</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>Week 3 - Outflow</td>
<td>8.34</td>
<td>8 ± 1</td>
<td>2476</td>
<td>385</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Week 4 - Outflow</td>
<td>8.35</td>
<td>8 ± 1</td>
<td>4177</td>
<td>484</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Week 5 - Outflow</td>
<td>8.5</td>
<td>8 ± 1</td>
<td>5256</td>
<td>520</td>
<td>178</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 5.1.A** Mean plant shoot density measured in reducing reactors (top graph; n=4) and oxidizing reactors (bottom graph; n=10) during CWTS maturation and treatment periods for replicate wetland Series A and B. Error bars indicate standard deviations.

Note: Reducing reactors = softstem bulrush *S. tabernaemontani*; oxidizing reactors = broadleaf cattail (*T. latifolia*)
Figure 5.2.A Mean plant shoot height measured in reducing (n=6) and oxidizing (n=36) reactors during CWTS maturation and treatment periods. Error bars indicate standard deviations.
Note: Reducing reactors = softstem bulrush *S. tabernaemontani*; oxidizing reactors = broadleaf cattail (*T. latifolia*)

Figure 5.3.A Mean oxidation-reduction potential (ORP; mV) measured in reducing (n=4) and oxidizing reactors (n=10) during CWTS maturation and treatment periods. Error bars indicate standard deviations.
Note: Reducing reactors = softstem bulrush *S. tabernaemontani*; oxidizing reactors = broadleaf cattail (*T. latifolia*)
Figure 5.4.A Heteroatom classes present in untreated (inflow) OSPW using high-resolution Orbitrap MS.

Note: The chemical composition and profile of NAs contained in OSPW were identified by use of direct injection linear ion trap-orbitrap mass spectrometer (Orbitrap MS; Orbitrap Elite, Thermo Fisher Scientific, San Jose, CA, USA) in negative (ESI−) electrospray according to the method described by Headley et al. (2016).
Figure 5.5.A. UV/visible transmittance (250 to 700 nm) at 1.0 cm water depth of untreated (inflow) OSPW and post-wetland treatment outflow. Distilled water included as a reference point.

Note: UV/visible light data were collected using a Spectrofluorometer (SpectraMax M2; microplate reader [Molecular Devices Corp. Sunnyvale, Ca])
Figure 5.6.A. Light extinction coefficient (Kd cm$^{-1}$) of untreated OSPW.

Note: Light intensity (250-700 nm), both incident and submersed, was recorded at each water depth (n=20) using a LI-1400 data logger with a LI-190 photometric sensor (incident light) and a LI-192 submersible sensor (LICOR Biosciences, Lincoln, NE, USA).
CHAPTER SIX

CONCLUSIONS

6.1 Objectives

The rational of this research was to provide a viable approach using hybrid constructed wetland treatment systems for the mitigation of constituents in OSPW. Four major objectives were completed and are presented in Chapters 2 through 5 of this dissertation:

6.2 Identify Constituents of Concern in OSPW and Discern Potential Treatment Processes

The second chapter of this dissertation focused on the identification of specific COCs in OSPW that need to be targeted to effectively mitigate ecological risk. The goal of this risk-based approach was to characterize a site-specific OSPW for the purpose of identifying specific COCs needing treatment and informing CWTS processes necessary for altering risks to aquatic biota. COCs identified in OSPW include organics (NAs, O/G), metals/metalloids, and suspended solids. Toxicity testing confirmed that COCs were in sufficient forms and concentrations to have measurable adverse effects on sentinel aquatic species. Sensitivities of aquatic organisms to OSPW indicated that fish ≥ aquatic invertebrates > macrophytes. The sensitivity distribution of organisms to OSPW in addition to strategic bench-scale manipulations indicate organic constituents are contributing to the observed toxicity. Alteration of the organic fraction of OSPW (i.e. H₂O₂ + UV₂₅₄ and GAC treatments) significantly increased survival and reproduction of C. dubia as compared to untreated OSPW.
Based on multiple lines of evidence, these data indicate that organic fractions (i.e. O/G, NAs) of OSPW are sources of toxicity. In addition, numeric exceedances of metals/metalloids indicate the need to decrease concentrations to achieve WQC thresholds. Results from this study provide critical information to inform mitigation strategies using passive or semi-passive treatment processes (i.e. constructed treatment wetlands) to mitigate ecological risks of OSPW to aquatic organisms.

6.3 Photocatalysis of a Commercial Naphthenic Acid using Fixed-film TiO₂

The third chapter of this dissertation focused on measuring performance of fixed-film photocatalysis for degradation of a commercial NA. Greater than 90% removal of Fluka NAs (initial concentration 63 mg/L) was achieved in 4-hr with photocatalysis in fixed-film (TiO₂) reactors in direct sunlight. Photocatalysis also eliminated acute toxicity to sentinel species, with mortality decreasing from 100% to 0% after 5-hr of photocatalytic treatment for fathead minnow (Pimephales promelas) and after 4-hr for the freshwater invertebrate (Daphnia magna). In this experiment, measuring responses of aquatic organisms concomitantly with analytical quantification of Fluka NAs over time confirmed alteration of exposures as well as mitigation of risk. Fixed-film TiO₂ application may provide an alternative solution for scaling the technology for larger treatment systems. Photocatalytic degradation using fixed-film TiO₂ irradiated with sunlight achieved efficacious rates and extents of removal of Fluka NAs, indicating the potential for application of this technology for mitigating ecological risks associated with NAs.
6.4 Influence of Commercial Naphthenic Acids on Acid Volatile Sulfide (AVS) Production and Divalent Metal Precipitation

The fourth chapter of this dissertation focused on potential vulnerabilities of a microbially mediated treatment process (dissimilatory sulfate reduction). Lack of NA toxicity to SRBs could be beneficial for the passive or semi-passive renovation of process-affected waters using constructed wetland treatment systems. Following exposures of NAs, extent of AVS production were sufficient to achieve $\Sigma$SEM:AVS < 1, indicating that available sulfide ligands were in excess of SEM (Cu, Ni, and Zn) concentrations regardless of NA exposure concentration (10-80 mg NA/L). In addition, no adverse effects to SRB populations in terms of density, diversity, or relative abundance were measured following exposures of a commercial NA. Since SRB were insensitive to exposures of a relatively potent (in terms of aquatic toxicity) commercial NA, adverse effects to SRB (and SRB-mediated pathways in wetlands) from exposures of more compositionally complex NAs (i.e. derived from oil sands process affected waters) are not anticipated. Further, lack of toxicity to the overall microbial population, and absence of effect on diversity and community profile is a positive finding, given that maintaining diversity of microbially-mediated pathways could be beneficial for passive wetland treatment systems. Passive systems that utilize these biogeochemical processes can be cost effective alternatives to traditional technologies, and having a robust microbial community capable of performing these processes can improve the efficiency and success of the system (Johnson and Hallberg, 2005; Nelson and Gladden, 2008; Haakensen et al., 2015). In this study, dissimilatory sulfate reduction and subsequent
metal precipitation were not vulnerable to NAs, indicating passive treatment systems
could be used to treat metals occurring in NA affected waters.

6.5 Performance of a Hybrid Pilot-scale Constructed Wetland System for Treating
Oil Sands Process-affected Water from the Athabasca Oil Sands

Using the information gained from the preceding experiments (Chapters 2-4), a
process-based design approach was used to design and construct a hybrid pilot-scale
CWTS and facilitate conditions conducive for mitigating risks associated with OSPW.
Results from this experiment provided evidence that problematic constituents contained
in OSPW could be treated to an extent necessary to remove toxicity to a sentinel
organism (C. dubia). Based on characterization of an OSPW from an active settling
basin, COCs needing treatment were identified as: NAs, petroleum hydrocarbons (as
O/G), Al, B, Cu, Ni, Se, Zn, and TSS. The hybrid pilot-scale CWTS used in this study
provided passive (i.e. low energy) biogeochemical and advance oxidation processes to
transfer or transform COCs to achieve numeric and narrative treatment goals. Results
from this study provide proof-of-concept data to inform hybrid passive or semi-passive
treatment approaches (i.e. constructed wetlands) that could be used to mitigate COCs
contained in OSPWs.

6.6 Conclusion

Results from this study demonstrate that specifically designed hybrid CWTS are a
viable treatment option for OSPWs located in the AOS. Sulfate-reducing bacteria, and
hence metal treatment via the production and precipitation of acid volatile sulfides are not
likely to be adversely influenced by the low molecular weight NA fraction of OSPWs
(i.e. presumably the most potent fraction). Fixed-film photocatalysis was effective at decreasing concentrations of commercial NAs and subsequently eliminating toxicity in environmentally relevant rates (i.e. hours). Results from this research provide approaches to identify problematic constituents contained in complex energy derived waters (e.g. OSPW) and strategies for mitigating risks by altering exposures using passive (low-energy) treatment systems. Results from this research provide proof-of-concept data to inform hybrid passive or semi-passive treatment approaches (i.e. constructed wetlands) that could be used to mitigate COCs contained in OSPWs.
6.7 References


