

**FATE AND TRANSPORT OF NON-STEROIDAL ANTI-INFLAMMATORY  
DRUGS (NSAIDS) IN SOILS RECEIVING LAND APPLIED ALKALINE  
TREATED BIOSOLIDS IN NOVA SCOTIA**

by

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## ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat and prevent illness. Land application of biosolids is one potential source of NSAIDs into agricultural soils and enter as mixtures into the environment. Interactions between NSAIDs and biosolids can affect the physico-chemical characteristics of soil impacting the fate and transport of NSAIDs in the environment. A soil incubation study was conducted to measure the biodegradation rates and half-lives of individual and mixtures of naproxen (NPX), ibuprofen (IBF), and ketoprofen (KTF) with and without the addition of an alkaline treated biosolid (ATB) amendment. Results of the study showed a loss of target compounds in all treatments that was attributed to biodegradation following first-order kinetics. Soils that received the ATB amendment demonstrated inhibited dissipation of NPX in all treatments, as well as IBF and KTF in individual compound treatment over the 14-day incubation study. The dissipation of the NSAIDs in the mixture compound environment varied among the compounds.

The leaching potential of NPX, IBF, and KTF was evaluated using field-based lysimeter cells receiving three rates of ATB(0, 7, and 28 Mg ha<sup>-1</sup>). All three NSAIDs in the lysimeter cells migrated vertically in the soil after spiking, but only a small amount of target NSAIDs were detected in the leachate. A mass balance analysis indicated a low accumulation of these compounds in the soil by the end of the study (Day 34) in all treatments. Application of ATB significantly increased soil pH and organic matter (OM) content but did not impact the retention of the compounds in the soil profile. Overall, all three NSAIDs were found to have low mobility in the acidic loamy sand textured soil.

The fate and transport of the NSAIDs were modeled using the Root Zone Water Quality Model 2 (RZWQM2) and compared to data measured in the field-based lysimeter cells. The calibrated model adequately predicted the water seepage out of the soil profile and percent recovery of initial spiked NSAIDs in the soil at three depths and in water samples. The model performance could be further improved with more field-measured data and by considering macropore flows.

## LIST OF ABBREVIATIONS AND SYMBOLS USED

AA	Alkaline Admixtures
AASSAD	Advanced Alkaline Stabilization with Subsequent Accelerated Drying
Al	Aluminum
ATB	Alkaline Treated Biosolids
ANOVA	Analysis of Variance
BEEC	Bio-Environmental Engineering Center
C	Carbon
Ca	Calcium
CEC	Cation exchange capacity
CKD	Cement Kiln Dust
COX	Cyclooxygenase
R <sup>2</sup>	Linear regression coefficient of determination
DCF	Diclofenac
ESOCs	Emerging Substances of Concerns
k	First-order exponential degradation rate constants
GC	Gas Chromatograph
t <sub>1/2</sub>	Half-life
HRM	Halifax Regional Municipality
IBF	Ibuprofen
Fe	Iron
KTF	Ketoprofen
LKD	Lime Kiln Dust

LMB	Liquid Municipal Biosolids
LOD	Limit of Detection
LOQ	Limit of Quantification
NPX	Naproxen
N	Nitrogen
NSE	Nash-Sutcliffe model efficiency
NER	Non-extractable residues
NSAIDs	Non-Steroid Anti-Inflammatory Drugs
MSD	Mass-selective detector
Log $K_{ow}$	Octanol-water partition coefficient
OC	Organic carbon
OM	Organic matter
OTC	Over the counter
P	Phosphorus
PAR	Photosynthetically active radiation
PBIAS	Percentage bias
PEST	Parameter Estimation Software
PPCPs	Pharmaceuticals and personal care products
RBD	Randomized block design
RZWQM	Root Zone Water Quality Model
RZWQM2	Root Zone Water Quality Model 2
$K_{sat}$	Saturated hydraulic conductivity
SIM	Selected Ion Monitoring

SPE	Solid Phase Extraction
USDA-ARS	United States Department of Agriculture – Agricultural Research Service
$K_{oc}$	Sorption constant for soil organic matter
$K_d$	Sorption distribution coefficient
TOC	Total organic carbon
$K_{tscf}$	Transpiration stream concentration factor
VC	Variance Components
WWTPs	Wastewater Treatment Plants

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# Chapter 1 Introduction

## 1.1 Introduction

The ubiquitous use of antibiotics, pharmaceuticals, and personal care products (PPCPs) has resulted in the entry of these emerging substances of concern (ESOCs) and their metabolites into the environment, such as aquatic bodies and terrestrial systems, via various routes (Li, 2014). Wastewater treatment plants (WWTPs) are significant sinks for ESOCs like non-steroidal anti-inflammatory drugs (NSAIDs) with the concentration range from  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$ , which can be either unchanged or transformed during the treatment processes (Hydromantis, 2009, 2010; Xia et al., 2015). Among all the NSAIDs, ibuprofen (IBF) was the most frequently detected compound in the treated wastewater and surface water and the highest concentrations of naproxen (NPX) was measured in the Canadian treated wastewater (Rastogi et al., 2021; Tyumina et al., 2020). Agricultural practices, such as irrigation with reclaimed wastewater or land application of biosolids, becomes an important pathway for introducing NSAIDs into the aquatic environment and agricultural soil (Fenet et al., 2012; Gibson et al., 2010; Thomaidi et al., 2016). These pharmaceuticals may degrade or become persistent in soils, reach the surface or groundwater through runoff and leaching, or be taken up into plants, which creates concern of their risks to organisms, including human beings (Carter et al., 2014; Parolini, 2020; Rastogi et al., 2021; Zenker et al., 2014).

The fate and transport of NSAIDs in soil systems are determined by various processes, such as volatilization, reversible and irreversible sorption, and biotic and abiotic degradation. This thesis work only focused on measuring the dissipation of NSAIDs as parent compounds in the soil profile since the concentration of NSAIDs

metabolites formed by the degradation process were not measured. The complexity of the system lends itself to the use of computer simulation models as a means to attempt to understand the dynamic of NSAIDs in the environment (Ahuja et al. 2000). It is suggested by Pal et al. (2010) that fate and transport models need to be developed to better predict concentrations, exposure, and impacts of pharmaceuticals in the environment. The Root Zone Water Quality Model 2 (RZWQM2) is a comprehensive one-dimensional, numerical agricultural systems model used to predict the effects of agricultural management on crop production and environmental quality (Ahuja et al., 2000). The pesticide transport sub-model of the RZWQM2 has the ability to simulate the behavior of organic chemicals in agricultural systems and many studies have shown the model has adequate performance (Ma et al., 2004a; Malone et al., 2004a; Wauchope et al., 2004). The following section provides an overview of the background for the state of the research on NSAIDs in the environment and the model features of the RZWQM2.

### **1.1.1 Global consumption of NSAIDs**

Non-steroidal anti-inflammatory drugs are one of the most widely used classes of drugs that have anti-inflammatory and analgesic effects through the inhibition of the enzyme, cyclooxygenase (COX) (Pilotto et al., 2010). More than 30 million people are assumed to consume NSAIDs globally on a daily basis (Dulal and Khan, 2019). It has been reported that NSAIDs usage showed an increasing trend worldwide (Gómez-Acebo et al., 2018). In England, the total expenditure on NSAIDs was reported to increase by 57% from 1999 to 2004 (Barton et al., 2006). In the United States, NSAIDs accounted for approximately 11% of total costs of prescribed pain medication between 2000 and 2007 and the total number of NSAIDs prescriptions dispatched annually increased from 70 million to over

111 million in a decade (Kasciuškevičiūtė et al., 2018; Rasu et al., 2014; Russell, 2001). In Canada, NSAIDs sales as a percentage of total pharmaceutical sales decreased by 0.9% from 2012 to 2017, while the amount of consumption showed a trend of increasing during the same period (OECD, 2019). The Canadian Institute for Health Information (2019) listed out 100 top drug classes used in Canada in 2018 and found the rate of use of NSAIDs was 14.1%, whereas the class of propionic acid derivatives accounted for 9.2% and ranked 11<sup>th</sup> on the list. Beyond prescription, patients also have access to many types of NSAIDs over the counter (OTC). However, limited information is available about the OTC use of NSAIDs, which may result in the underestimating of the overall use of NSAIDs around the world.

### **1.1.2 NSAIDs in biosolids and biosolids-amended soil**

The latest report by CCME (2013) stated that Canada is producing more than 0.66 million dry tonnes (2.5 million wet tonnes) of biosolids annually, which is 70% higher than the total amount reported in 2009 (Banga et al., 2009). In Halifax Regional Municipality (HRM) of Nova Scotia, approximately 35000 tonnes of alkaline treated biosolids (ATB) are produced annually, with roughly 60% of the biosolids being used for agricultural purposes (N-Viro System Canada LP, 2009). Land application of biosolids is a prevalent alternative to other disposal methods, such as incineration and landfilling, to manage the increasing quantity of biosolids generated from WWTPs each year (Cooper 2005; Goodman and Goodman 2006). Land application of biosolids has many well-documented benefits, such as enhancement in soil fertility, soil physical conditions, soil bulk density, porosity, cation exchange capacity, water retention capacity, nitrogen (N) and phosphorus (P) availability, soil microbial activity and biomass, as well as soil

hydraulic conductivity (Akhtar et al. 2002; Singh and Agrawal 2008; Esteller et al. 2009). However, biosolids application is still a contentious public issue due to several potential risks, such as offensive odors and toxic metals (Krach et al. 2008; LongHua et al. 2012). Beyond all these issues, entry of the ESOCs and their metabolites into the environment through land application of biosolids is an increasing area of concern.

The studies related to the potential removal of ESOCs by different biosolids stabilization processes are limited, especially for NSAIDs (Hydromantis, 2009, 2010; Xia et al., 2015). The NSAIDs, such as naproxen (2S-2-(6-methoxynaphthalen-2-yl) propanoic acid), ibuprofen (2-[4-(2-methylpropyl) phenyl] propanoic acid), and ketoprofen (KTF, 2-(3-benzoylphenyl) propanoic acid), were reported to be frequently detected in the treated waters and biosolids generated from WWTPs (Dodgen et al. 2014; Hydromantis 2010). For example, it was reported that more than 80% of NPX will stay unchanged after administration in the human body and is excreted into municipal wastewater treatment plants (Carballa et al. 2004; Xia et al. 2005; Jiang et al. 2013). The concentration of NSAIDs in biosolids has been rarely studied up to the point and is quite variable. Guerra et al. (2014) studied the occurrence and fate of different PPCPs in 24 biosolids from six WWTPs in Canada over 3 years. This study detected NPX in 18 biosolids with the concentration ranging from 2.9 to 150 ng g<sup>-1</sup> dry weight (d.w) and IBF in 24 biosolids with the concentration ranging from 53 to 490 ng g<sup>-1</sup> d.w. Another report prepared by Hydromantis (2010) detected NPX in 25 out of 31 biosolids samples from 9 target Canadian municipal WWTPs with a median concentration of 98.1 ng g<sup>-1</sup> d.w and IBF in 21 out of 31 biosolids samples with a median concentration of 522 ng g<sup>-1</sup> d.w. The anaerobic digested biosolids used by Edwards et al. (2009) had NPX and IBF detected at

the concentration of 470 and 750 ng g<sup>-1</sup> d.w, respectively. The frequent occurrence of these NSAIDs may be due to the varied NSAIDs removal rates by different biosolids stabilization methods (Carballa et al. 2007; Hydromantis 2010).

Carballa et al. (2007) found that both mesophilic and thermophilic anaerobic digestion can result in high NPX removal efficiency (84%-88%) in sludge. A similar result was also reported by Hydromantis (2010), who conducted a field study on the effects of biosolids treatment processes on the concentration of ESOCs in the biosolids. Conversely, Hydromantis (2010) also found that NPX concentration increased during the aerobic composting of sludges, which may be due to the biotransformation from other compounds. This study also studied the ESOCs removal efficiency by alkaline stabilization methods, such as the patented N-Viro process. Alkaline stabilization is a biosolids treatment method combining high pH (>12) and temperature (between 52 and 62°C), as well as drying through mixing the sewage sludge and alkaline admixtures (AA) to kill pathogens. The patented N-Viro process, also known as Advanced Alkaline Stabilization with Subsequent Accelerated Drying (AASSAD), is one of the commonly accepted alkaline stabilization technologies (Hsiau and Lo 1997; N-Viro System Canada LP 2009). This process involves the mixture of dewatered sewage sludge with one or a combination of AA, including limestone, cement kiln dust (CKD), lime kiln dust (LKD), lime (CaO), carbide lime, hydrated lime, fly ash, wood ash, and other coal burning ashes, followed by accelerated drying (Logan and Harrison 1995). In this process, partially dewatered biosolids are mixed with 30% to 40% of AA on a wet basis of biosolids to raise the temperature and pH for pathogen reduction. The mixture is then dried to a total solids content between 60 and 65% to further reduce pathogens. After the drying stage,

the mixture is cured for 12 hours in a heat-pulse cell where the temperature is raised to between 52 and 62°C and the pH is kept above 12 to stabilize the product and to kill pathogens during the storage (Logan and Harrison 1995; N-Viro System Canada LP 2009). Although this process showed a moderate pharmaceutical removal efficiency, the concentration of some NSAIDs, such as IBF and NPX, increased by 82% and 17% at the end of the stabilization process, respectively (Hydromantis, 2010).

When NSAIDs are introduced into the soil through land application of biosolids, mechanisms like sorption and biological degradation can occur for these organic compounds. There are three common types of adsorption isotherms, including linear, Freundlich, and Langmuir. Xu et al. (2009a) found the adsorption of KTF in soils was well described by the Freundlich equation with  $R^2$  ranging from 0.915 to 0.979. The NPX and IBF were also reported to be well described by the Freundlich equation with  $R^2$  ranging from 0.95 to 0.98 and 0.9 to 1.00, respectively (Vulava et al., 2016). The sorption behavior of targeted NSAIDs in the current study, including NPX, IBF, DCF, and KTF, have been reported to be affected by the physicochemical characteristics of the receiving soil. For example, Xu et al. (2009b) found the sorption distribution coefficients ( $K_d$ ) of NPX and IBF have a positive relationship with the soil organic matter (OM) contents. Similarly, Vulava et al. (2016) found the  $K_d$  of NPX increased from 3.1 to 356 L kg<sup>-1</sup> when soil OM contents increased from 0.4 to 9%. Soil pH can be another factor that affects the sorption of NSAIDs. Compounds like NPX, IBF, and KTF all contain the pH-dependent carboxylic acid groups that will be deprotonated and repelled by the soil surface at soil between 5 and 8, which will decrease the sorption potential in the soil (Vulava et al., 2016). Beyond sorption, these targeted NSAIDs are rapidly dissipated in

soil through biological degradation. It was reported that mesophilic aerobic biodegradation is the primary mechanism of NPX dissipation in the soil, while mineral, pH, and OM content were not rate limiting factors (Topp et al., 2008). Xu et al. (2009a) also found a prolonged half-life of KTF after soil sterilization, which indicated that microbial degradation was the dominant pathway for KTF dissipation in soil. It was also reported the dissipation of KTF was fitted well in the first-order decay model with  $R^2$  ranging from 0.897 to 0.989 (Xu et al. 2009a). Lin and Gan (2011) found the dissipation of DCF, IBF, and NPX all followed the first-order decay model as well. The leaching of NSAIDs through the soil to water was reported significant (Xu et al., 2009b). Sui et al. (2015) conducted a comprehensive review of the concentration of PPCPs detected in groundwater between 2012 and 2014 in Serbia, Spain, Germany, China, Canada, Jordan, and Switzerland and found the maximum concentration of NPX, IBF, and DCF were 145, 988, and 380 ng L<sup>-1</sup>, respectively. Lapen et al. (2008) applied liquid municipal biosolids into the soil and detected both NPX and IBF in tile drainage after nine months with a maximum concentration of 1.05 µg L<sup>-1</sup> and 4.12 µg L<sup>-1</sup>, respectively. The main leaching mechanisms of contaminants are due to the advection along with dispersion in the soil. The leaching behavior of the NSAIDs can be significantly affected by soil properties and the characteristics of chemicals (Xu et al., 2009b). Chen et al. (2013) found higher concentrations of NPX, IBF, and DCF in drainage water in a loamy sand soil than in the sandy loam soil. The transport of organic compounds into tile drains or groundwater may also be due to the preferential flow through vertical macropores in the soil matrix (Gottschall et al., 2012). These large macropores reduce bulk ESOCs sorption, as well as biodegradation, and enhance both colloid and non-colloid contaminants transport to the

depth of tile drain or groundwater (Larsbo et al., 2009). The formation of transformation products is another dissipation pathway for NSAIDs in soils. According to Dodgen et al. (2014), a large amount of the extractable  $^{14}\text{C}$  NPX and DCF was transformed into degradation intermediates at the end of the 112 d incubation in different soils and the persistence of parent compound in soils was dependent on soil types and retention time.

Since the land application of biosolids is becoming a popular disposal approach, its effects on the fate and transport of ESOC like NSAIDs in the biosolids-amended soil is an increasing area of concern. Many studies have shown that the fate of NSAIDs will be affected in soils amended with different types of biosolids. For example, Monteiro and Boxall (2009) applied dewatered biosolids stabilized with mesophilic anaerobic digestion into soil and found that dissipation of NPX decreased. The half-lives of NPX increased from 3.1-6.9 d in the soil to 3.9-15.1 d in biosolids amended soil. The dewatered biosolids application increased the soil OM content that enhanced the sorption of NPX, which decreased the mobility of NPX in arable soil (Borgman and Chefetz, 2013; Grossberger et al., 2014; Li et al., 2013). Li et al. (2013) also stated that nutrients or carbon sources in biosolids may be more readily available than PPCPs which may result in the reduction in dissipation rates. However, Topp et al. (2008) found a contradictory result where land application of liquid municipal biosolids (LMB) accelerated NPX mineralization in a loam soil with higher  $^{14}\text{CO}_2$  measured. Depth of biosolids application also showed a significant effect on the dissipation of NSAIDs, where sub-surface application resulted in faster dissipation than surface biosolids application (Al-Rajab et al., 2015). Sabourin et al. (2009) studied runoff in soils receiving dewatered biosolids amendments and concluded runoff transport potential in soils can be limited for organic

compounds with log Kow values of 3.18 or higher (log Kow value of NPX is 3.18). Lapen et al. (2008) applied LMB into clay loam soil and found that the concentrations of NPX and IBF in tile water collected in the fall 2005 were below the limit of quantification (LOQ). Conversely, Edwards et al. (2009) found that NPX concentration in tile water collected after the land application of dewatered municipal biosolids in the summer of 2006 from the same study site was above the LOQ.

### **1.1.3 Risks of NSAIDs in the environment**

The entry of the NSAIDs into the environment has drawn concerns about their adverse effects on human and ecosystem health. NSAIDs have been frequently found in water bodies around the world in concentrations ranging from  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$  (Patel et al., 2019; Rastogi et al., 2021). The associated acute and chronic effects of NSAIDs on aquatic organisms and plants have been well studied (DeLorenzo and Fleming, 2008; Górný et al., 2019; Parolini, 2020; Rastogi et al., 2021; Xia et al., 2017). For example, freshwater catfish (*Rhamdia quelen*) exposed to aquatic environment concentrations of IBF (0.1, 1, and  $10 \mu\text{g L}^{-1}$ ) for 14 days displayed nephrotoxicity and immunosuppressive effects (Mathias et al., 2018). The chronic waterborne exposure of juvenile medaka fish (*Oryzias latipes*) to NPX caused decreased survival rates during the early life stages, as well as adverse effects on gene transcription (Kwak et al., 2018). The growth of marine phytoplankton *Dunaliella tertiolecta* was inhibited by DCF with concentrations of  $25 \text{ mg L}^{-1}$  and above (DeLorenzo and Fleming, 2008). Oaks et al. (2004) also reported that DCF is the main cause of the high mortality rate of vultures in the Indian subcontinent due to renal failure.

The risk assessment for pharmaceuticals is usually conducted for single

compounds. However, pharmaceuticals are always present in the environment as a multi-component mixture in reality (Kümmerer, 2009). Knowledge is still lacking about the fate and transport, as well as the toxicity of mixtures of pharmaceuticals in the environment, due to the more complex mode of action of mixture compounds than studying compounds singly (Topaz et al., 2020; Vasquez et al., 2014). Some literature have shown that mixtures might present more severe effects than individual compounds, even if each compound presents in low concentrations where toxic effects are unlikely (Cleuvers, 2004; Pomati et al., 2007; Vasquez et al., 2014). For example, a mixture of four NSAIDs, including DCF, IBF, NPX, and aspirin, has been reported to result in significant acute toxicity, where the limited effect was detected at the same concentration when considering these compounds individually (Cleuvers, 2004).

#### **1.1.4 Modeling fate and transport of organic contaminants in agricultural soils**

The fate and transport of pharmaceuticals in soil systems are determined by various processes, such as sorption, biotic and abiotic degradation, surface runoff, and leaching (Ahuja et al., 2000). The complexity of the system makes computer simulation modeling, which is an advanced tool in modern scientific research, a practical means to help researchers better understand the dynamics (Nance and Sargent 2002). Over 80 models are available for simulating the fate and transport of ESOC with applications mainly focused on pesticides and herbicides (Siimes and Kämäri, 2003). Some of the models, such as HYDRUS-1D/2D, Root Zone Water Quality Model (RZWQM), GLEAMS, MACRO, PRZM, and OpusCZ, have been well-reviewed and compared. Siimes and Kamari (2003) reviewed the available pesticide leaching models to simulate the fate of herbicide in Finnish sugar beet cultivation and concluded that MACRO and GLEAMS

were the most suitable models in that area and RZWQM is considered a highly regarded model. Zhang and Goh (2015) evaluated PRZM, RZWQM, and Opus CZ for simulating pesticide runoff from irrigated agricultural fields in semi-arid areas and found that OpusCZ and RZWQM were the most suitable models for simulation. Nolan et al. (2005) compared the capacities of seven unsaturated-zone solute-transport models to simulate the fate and transport of agricultural chemicals. They found the RZWQM presented a higher accuracy in predicting the concentration of atrazine at sites with heavily structured soils than other models, such as HYDRUS-2D and PRZM.

The latest version of RZWQM, the RZWQM2, is a comprehensive one-dimensional, numerical agricultural systems model used to predict the effects of agricultural management on crop production and environmental quality, which has over 200 publications related to its application in the agricultural environment (Ahuja et al., 2000). One advantage of RZWQM2 over other models is the availability of detailed theoretical documentation and strong technical support (Zhang and Goh, 2015). The model is designed to simulate conditions on a unit-area basis with the crop root zone as the primary zone of focus but can be extended to the deeper vadose zone. RZWQM is made up of six major components, including physical processes, soil chemical processes, nutrient processes, pesticide processes, plant growth processes, and management processes.

The hydrologic processes in RZWQM are simulated in the physical processes, which include water infiltration from rain or irrigation, redistribution of water throughout the soil profile, plant water uptake, and evaporation. Soil hydraulic properties are defined by the Brooks and Corey (1964) analytical models. Infiltration of the water into the soil

matrix is described by the Green-Ampt equation, where the soil is discretized into 1 cm increments (Malone et al., 2004a). The infiltration rate is considered equal to the rainfall rate if the infiltration rate is greater than the rainfall rate. Otherwise, the excess rainfall that does not infiltrate into the soil matrix will be available to macropores. Macropores are separated from the soil matrix in the RZWQM. The excess rainfall is routed into the macropores if present and is subject to the lateral water movement into the surrounding soil. The maximum macropore infiltration rate and lateral infiltration are calculated by Poiseuille's law and the lateral Green-Ampt equation, respectively (Malone et al., 2004a). Any excess rainfall over the maximum macropores infiltration rate or infiltration (if macropores are not present) is considered runoff. Between rainfall or irrigation events, soil water is redistributed using a mass conservation numerical solution to Richards' equation, which contains a sink term for root-water uptake and tile drainage. The Richards' equation in the RZWQM is solved by a mass-conservative, mixed form iterative finite-difference numerical solution. The evapotranspiration in RZWQM is simulated by the double-layer model of Shuttleworth and Wallace (1985), which is an extension of the Penman-Monteith (P-M) model that accounts for a partial canopy and surface residual, to predict daily potential rates of soil evaporation and crop transpiration.

Snow and soil freezing plays an important role in affecting the winter hydrology in areas like Canada. The RZWQM is able to conduct the over-winter simulation with the snowpack dynamics, which are simplified snow routines after the Precipitation Runoff Modeling System (PRMS) (Hanson et al., 1999). The daily initiation, accumulation, and depletion of a snowpack are simulated by this component. The surface temperature is assumed to equal the average air temperature for the day when snow is not present and

set to 0 °C when snow covers the surface. Both the snowpack water balance and energy balance are computed on a daily time scale. The snowpack is divided into two layers, including the upper 3 to 5 cm of the snowpack which is considered as the surface layer and the remaining snowpack is considered as the lower layer. The temperature within the snowpack can be calculated based on the conductivity of heat between the snow surface and the lower layers. Water is released as snow melt when the accumulation of free water due to the accumulation of energy exceeds the free water holding capacity. When more energy is brought into the system, the snowpack will keep accumulating water until the snowpack disappears completely (Hanson et al., 1999). Some of the snowmelt will infiltrate into the soil and excess flow is considered runoff as described before.

The fate and transport of organic chemicals in the unsaturated zone of soils can be simulated with the pesticide sub-model of the RZWQM. Malone et al. (2004a) did an extensive review of 22 validation studies that applied the RZWQM to simulate different parameters, such as soil moisture, runoff, and pesticide fate, and found the general performance of the model simulation was adequate with  $R^2$  ranging between 0.51 and 0.98. The location of application residues and dissipation/degradation pathways in the compartments of the RZWQM model is shown in Figure 1.1. As it is shown in Figure 1.1, the system is conceptually divided into four different compartments, including crop foliage, plant residue, soil surface, and soil sub-surface or root zone. Since the organic contaminants in the current research are mainly introduced by the land application of biosolids, the compartments of crop foliage and plant residue can be neglected. When the biosolids are land applied and incorporated into the soil, downward transport of the organic contaminants through the soil matrix to groundwater (leaching) may occur. The

hydrologic process described above computes the mass movement of water between soil layers, while the pesticide sub-model computes the concentrations of organic contaminants that are present in the water that is moved based on the convective-dispersive equation (Malone et al., 2004a). Any organic contaminants in water that move below the bottom of the soil profile are considered 'lost' from the system. The dispersion of the organic contaminants along the vertical direction during the leaching process is caused by three processes, including molecular diffusion, micropore-mesopore exchange, and macropore/micropore redistribution. It has been reported the transport of up to three pesticides can be simulated by the RZWQM simultaneously to the depth of 30 m for over 10 years period (Nolan et al., 2005).

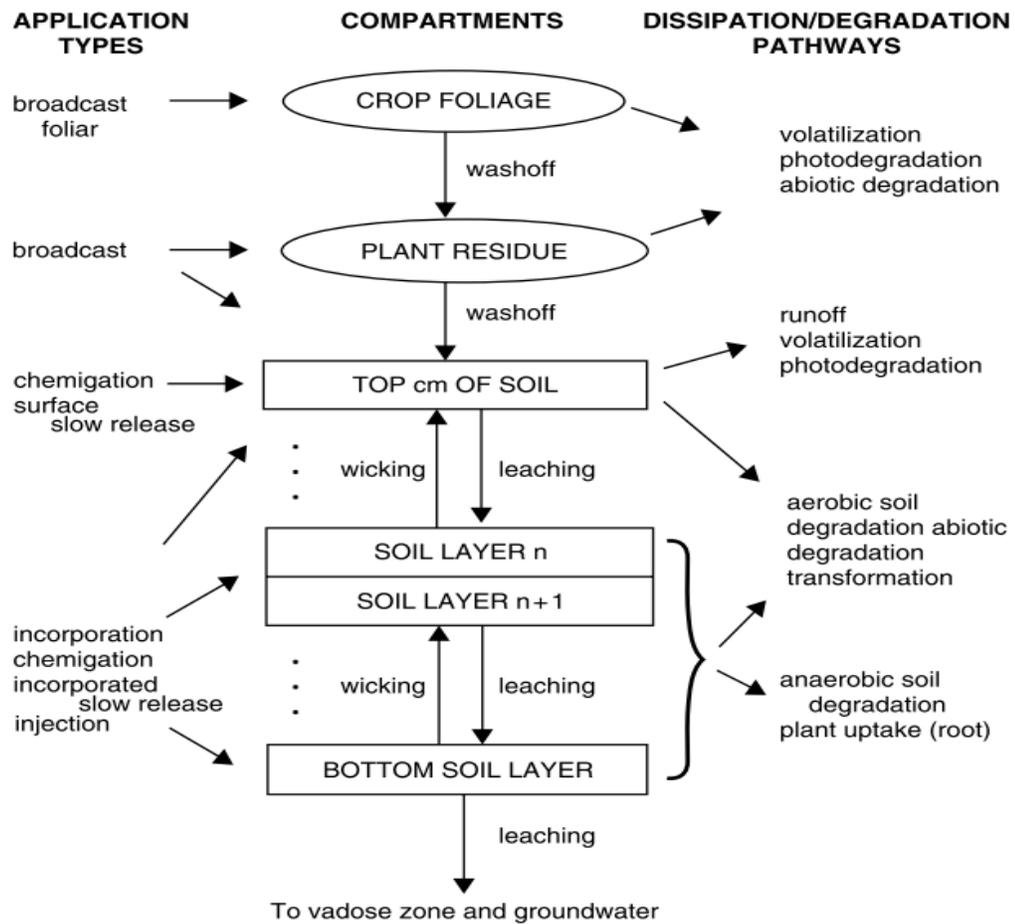


Figure 1.1. Location of application residues and dissipation/degradation pathways in the compartments of the RZWQM2 model (Wauchop et al., 2004).

Beyond the leaching through the soil layers, organic contaminants can be uptake by the plants growing in the soil. The RZWQM only simulates the plant uptake by roots (Wauchope et al., 2004). The plant uptake is determined by the amount of water uptake by the roots that are dependent on the growth stages and phenology of the plant, the concentration of organic contaminants in soil solution in the vicinity of the roots, and the ease of transport of the solute organic compound molecule through the root cell membranes, which can be measured by the transpiration stream concentration factor  $K_{tscf}$  (Wauchope et al., 2004). The  $K_{tscf}$  is related to the octanol-water partitioning coefficient ( $K_{ow}$ ) of the specific organic compound.

Sorption of the organic contaminants by soil is another important factor controlling the transport of these compounds in the soil. The RZWQM can model the soil sorption of organic contaminants by a linear or Freundlich instantaneous equilibrium model (Wauchope et al., 2004). The sorption can also be simulated with a two-site, equilibrium-kinetic sorption model in which a fraction of the soil sorption sites is in instantaneous equilibrium with a solution and first-order reversible adsorption kinetics are used to describe the remaining fraction of the sites (Ma et al., 2004a). For partial ionized organic contaminants, RZWQM models their sorption by calculating the degree of ionization based on either acids or bases ionization equilibrium constant. In the RZWQM, weak-acid or weak-base molecule sorption may be described by combining the linear, Freundlich, or two-site sorption model with ionization (Ma et al., 2004a). The RZWQM assumes the degradation of organic contaminants follows pseudo first-order kinetics, while the pseudo first-order rate constant for the soil surface and sub-surface compartments are adjusted for temperature and soil water content (Hanson et al., 1999).

The pseudo first-order rate constant is divided into separate processes like volatilization, photolysis, hydrolysis, anaerobic and aerobic biodegradation, oxidation, and complexation. For each time step, the model calculates the sum of the process rates.

Agricultural management practices, such as crop rotation, tillage operations, irrigation, and soil amendments application, can be simulated in the RZWQM concurrently or exclusively to determine the effects of these practices on the soil properties and conditions (Malone et al., 2004a). Soil tillage can affect the soil bulk density, macroporosity, and hydraulic properties, which will influence the fate and transport of the organic contaminants. RZWQM includes 29 different tillage implements with various tillage intensities depending on crop residue type. The tillage intensity will be incorporated into the EPIC model to predict soil bulk density after tillage. Tillage is also assumed to reduce all continuous macropore channels in the RZWQM and change them to dead-end macropores. Reconsolidation of the tillage soil is described based on the algorithm from the NTRM model (Linden and van Doren, 1987). The change in soil hydraulic properties after tillage is computed by Ahuja et al. (1998). Timing and application methods of manure, fertilizer, and pesticides can significantly affect their fate during the simulation. RZWQM provides both the specific data option and a system that is relative to the cropping system for timing. RZWQM also provides several types of application methods, including surface broadcast, broadcast with incorporation, mixed with irrigation water, or injected into deep soil layers.

The Richards' equation is solved by a mass-conservative, mixed form iterative finite-difference numerical solution in the RZWQM. A single Euler step is applied for the initial guess of the Richards' equation. The space mesh increments increase with depth

from 1 to 10 cm. In the RZWQM, the numerical solution of Richards' equation will achieve convergence by following predetermined criteria for the placement of the space mesh increments (Ahuja et al., 2000). These criteria include: 1) all layer thicknesses must be an integer number of centimeters; 2) a boundary of a numerical layer must coincide with a horizon boundary; 3) Numerical layer boundaries are centered between solution nodes to satisfy the solution technique; 4) the thickness of the first horizon must be greater than 2 cm. The time increment increases with time from 10<sup>-5</sup> h to 1 h, which can be also set back to aid the convergence of the Richards' equation. The Thomas algorithm is used to solve the linear system of equations at each iteration step. Erroneous solutions can occur if the solution is divergent, which can be prevented by setting the solution to the initial guess. The complexity of the RZWQM provides the flexibility to simulate many agricultural processes. However, this may result in the requirement of extensive data inputs (Nolan et al., 2005). Some of the parameters can be obtained from the system default values, while the model can also be parameterized using available site-specific input data and data from the literature or databases incorporated in the model (Malone et al., 2004a).

The studies related to the simulation of fate and transport of pharmaceuticals in soils are rare (Ellerbroek et al. 1998; Bakhsh et al. 1999; Bakhsh et al. 2004; Chinkuyu et al. 2005; Fox et al. 2006; Larsbo et al., 2009). Chen et al. (2013) applied HYDRUS-1D to simulate the fates and transport of pharmaceuticals in soil irrigated with reclaimed wastewater and suggested that this model has adequate capacity in simulation. Nolan et al. (2005) also found that RZWQM2 and HYDRUS-1D/2D has many similarities with respect to simulating the fate and transport of pesticides, including water flow, water

retention, heat transport, and solute processes. Based on a review conducted by Swanton et al. (2011), pesticides and pharmaceuticals undergo a similar registration process and share various commonalities like chemical properties. Therefore, the RZWQM2 is expected to be a potential tool to understand the fate and transport of pharmaceuticals like NSAIDs in the agricultural soil amended with biosolids.

## **1.2 Knowledge gaps and objectives**

The alkaline stabilization method to treat sewage sludges and generate an agricultural biosolid has been reported to increase the concentration of NSAIDs in the end-product ATB, which may result in a higher risk of NSAIDs contamination in the receiving environment. Land application of ATB as soil amendments may also change the soil characteristics, such as soil pH, OM, microbial communities, etc., that would be likely to affect the persistence of NSAIDs in the treated soil. However, limited studies have been conducted to explore the effects of land application of ATB on the behavior of NSAIDs in agricultural soil. Thus, studies related to land application of the ATB from the alkaline stabilization process to the agricultural soil need to be undertaken for a better understanding of the fate and transport of NSAIDs in the ATB receiving soil. Furthermore, literature available up to date were mostly reporting the behavior of individual NSAIDs in soil, while pharmaceuticals are normally presented in the environment as mixtures. Interactions between different compounds may also affect the fate and transport of NSAIDs in the environment. Additionally, fate and transport studies of NSAIDs have been extensively conducted using soil columns, with few field-based studies. Therefore, this thesis research is designed to be conducted using both laboratory and field lysimeter studies with the following objectives:

1. Determine the dissipation rate and half-life of the target NSAIDs (NPX, IBF, and KTF) in soils, with and without an ATB amendment.
2. Identify the effect of individual and mixture compound environments on the dissipation rate and half-life of the target NSAIDs.
3. Determine the fate and relative mobility of the target NSAIDs in soil profiles receiving different rates of ATB amendments under field conditions.
4. Parameterize the RZWQM2 model using empirical data from the lab and field studies.
5. Assess the capability of applying the pesticide module of the RZWQM2 in simulating the fate and transport of NSAIDs in soils receiving various rates of ATB amendments.

## **Chapter 2 Biodegradation kinetics of individual and mixture non-steroidal anti-inflammatory drugs (NSAIDs) in an agricultural soil receiving alkaline treated biosolids<sup>1</sup>**

### **2.1 Introduction**

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of pharmaceuticals with anti-inflammatory, analgesic, and antipyretic effects that have been ubiquitously prescribed or sold over-the-counter worldwide to treat pain, fever, and inflammation (Koumaki et al., 2017; Pilotto et al., 2010). Human excretion of NSAIDs, as the parent and metabolic transformation products, have made wastewater treatment plants (WWTPs) sinks for these compounds (Březinova et al., 2018; Larsson et al., 2014). Studies indicate the elimination of NSAIDs from WWTPs are incomplete, with highly variable removal rates for the various parent compounds (Jelic et al., 2011; Larsson et al., 2013). Biosolids are a by-product of the municipal wastewater treatment process. As a result, some NSAIDs such as naproxen (NPX) and ibuprofen (IBF) were frequently detected in biosolids generated from municipal WWTPs across Canada (Guerra et al., 2014; Hydromantis, 2010). Alkaline stabilization methods were also reported to increase the concentration of NPX and IBF at the end of the process, which suggests a higher risk of NSAIDs contamination in the receiving environment with the use of alkaline treated

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<sup>1</sup> A version of this chapter is published in Science of the Total Environment.

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biosolids (ATB). In the Halifax Regional Municipality (HRM), Nova Scotia, Canada, approximately 21,000 tonnes of ATB have been applied to agricultural soils as a liming amendment, which is a potential pathway to introduce NSAIDs into the aquatic environment and agricultural soils (Fenet et al., 2012; Gibson et al., 2010; N-Viro System Canada LP, 2009; Thomaidi et al., 2016). The entry of the NSAIDs into the environment has drawn concerns related to their adverse effects on animals, humans, and ecosystem health (Bilal et al., 2019; Klampfl, 2019; Petrie et al., 2015). It is important to understand the environmental fate of NSAIDs in agricultural soil as this represents several direct pathways to human and ecological receptors.

Soil adsorption of NSAIDs is an important mechanism that contributes to the abiotic loss of NSAIDs in soils (Bui and Choi, 2010; Durán-Álvarez et al., 2014; Zhang et al., 2017). A moderate sorption behavior has been reported for both NPX and ketoprofen (KTF) in different types of soils, while IBF was found to be sorbed weakly to soil (Biel-Maeso et al., 2019; Lin and Gan, 2011; Xu et al., 2009a, 2009b). Zhang et al. (2017) reported the adsorption affinity of the three target NSAIDs in a loam-textured soil, under an individual and mixture compound environment, to follow the order: NPX>KTF>IBF. Biotic and abiotic degradation (hydrolysis, oxidation, and photolysis) are also important pathways for NSAIDs in the environment (Cycon et al., 2016; Nakada et al., 2008; Waterman et al., 2002b, 2002a). Abiotic degradation of NSAIDs due to photochemical processes in soil has rarely been studied, but NPX and KTF are found to be highly susceptible to photodegradation in aquatic environments, with half-lives ranging from 0.7 min to 1.9 hours for NPX and from 0.54 min to 4.1 hours for KTF, respectively (Baena-Nogueras et al., 2017; Felis et al., 2007; Lin and Reinhard, 2005;

Matamoros et al., 2009; Packer et al., 2003; Vulava et al., 2016). IBF has high photostability and the dissipation of IBF in aquatic environments has been found to be primarily governed by biodegradation, with half-lives ranging from 1 to 5 days (Matamoros et al., 2009; Nakada et al., 2008; Packer et al., 2003). Multiple studies have reported contradictory results of NSAIDs dissipation kinetics in soils after biosolids application (Borgman and Chefetz, 2013; Carvalho et al., 2014; Sabourin et al., 2009; Topp et al., 2008). Currently, a paucity of research has been conducted comparing the dissipation of NSAIDs in soils when added as an individual or in a mixture compound environment, particularly in association with biosolids. Compound dissipation studies are typically conducted on individual compound systems, but pharmaceuticals enter into environmental matrices, such as soil and water, in a multi-compound mixture (Kümmerer, 2009; Mulkievics et al., 2021). The fate and transport of emerging contaminant mixtures in the environment is challenging to study, due to potentially more complex modes of action (Vasquez et al., 2014). The purpose of the present study was to explore the effect of individual and mixture compound environments on the dissipation rate, especially biodegradation, and half-life of three NSAIDs (NPX, IBF, and KTF) in a loamy sand textured agricultural soil, with and without an ATB amendment.

The hypotheses were that i) mixture compound environments and ATB amendment would result in lower dissipation rates of the target NSAIDs and ii) biodegradation would be a dominant disappearance pathway for the three NSAIDs studied.

## **2.2 Materials and Methods**

### **2.2.1 Chemicals**

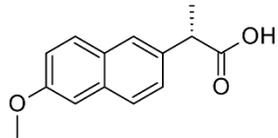
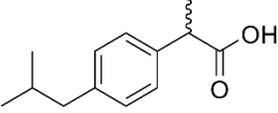
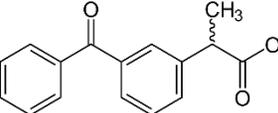
The NPX ( $\geq 98\%$  of purity), IBF ( $\geq 98\%$  of purity), and KTF ( $\geq 98\%$  of purity) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Deuterated forms of selected NSAIDs, including ( $\pm$ )-NPX- $d_3$  ( $\alpha$ -methyl- $d_3$ ) (99%  $d_3$ ), ( $\pm$ )-IBF- $d_3$  ( $\alpha$ -methyl- $d_3$ ) (99.6%  $d_3$ ), and ( $\pm$ )-KTF- $d_3$  ( $\alpha$ -methyl- $d_3$ ) (98.5%  $d_3$ ) were purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). Acetone (99.9%), ethyl acetate (99.9%), hexane (99.9%) and methanol (99.8%) were purchased from Fisher Scientific (Ottawa, ON, Canada). Derivatization agent, N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) was purchased from Chromatographic Specialties Inc (Brockville, ON, Canada). Stock solutions for each compound and corresponding internal standard were prepared in methanol and stored at  $-20\text{ }^\circ\text{C}$ . Chemical structures and properties for the target NSAIDs are listed in Table 2.1.

### **2.2.2 Soil and Alkaline Treated Biosolids (ATB)**

A loamy sand textured agricultural soil used for the dissipation study was collected from three control plots in a long-term agricultural lysimeter cell study site at the Bio-Environmental Engineering Center (BEEC) located in Bible Hill, Nova Scotia ( $45^\circ 23' 18''$  N,  $63^\circ 14' 15''$  W). The soil was collected from a depth of 0 to 15 cm, air dried, passed through a 2 mm sieve, and then stored in a temperature-controlled room ( $20^\circ\text{C}$ ). The ATB was collected from the N-Viro Systems Canada Biosolids Processing Facility in Halifax, N.S. (Walker Environmental Inc.). The ATB used in this study was treated using an Advanced Alkaline Stabilization with Subsequent Accelerated Drying

(AASSAD), which involved the combination of high pH (>12) and temperature (between 52 and 62 °C through mixing of dewatered sewage solids and cement kiln dust (CKD) to kill pathogens (N-Viro System Canada LP, 2009). The ATB was also air dried, passed through a 2 mm sieve, and then kept frozen at -20 °C until the beginning of the incubation study to slow down microbial decomposition of the organic matter (OM). Both soil and ATB were extracted and analyzed for the target compounds individually prior to the study in the Innovative Waste Management lab, Dalhousie University, Bible Hill, Nova Scotia, Canada. Additional physical and chemical parameters of soil and ATB were analyzed at the Nova Scotia Department of Agriculture's Analytical Services Laboratory, Bible Hill, Nova Scotia. Selected physical and chemical characteristics of soil and ATB are listed in Table 2.2.

Table 2.1. Chemical properties and structures of Naproxen (NPX), Ibuprofen (IBF), and Ketoprofen (KTF)<sup>a</sup> used in this study.

Compound	Structure	Molecular Weight (g mol <sup>-1</sup> )	Water solubility (mg L <sup>-1</sup> )	pK <sub>a</sub>	Log K <sub>ow</sub>	Vapor Pressure (mm Hg at 25 °C)	Henry's law constant (atm·m <sup>3</sup> /mole at 25 °C)
Naproxen		230.263	15.9	4.15	3.18	1.89x10 <sup>-6</sup>	3.39x10 <sup>-10</sup>
Ibuprofen		206.285	21	4.91	3.97	4.74x10 <sup>-5</sup>	1.5x10 <sup>-7</sup>
Ketoprofen		254.285	51	4.45	3.12	3.72x10 <sup>-7</sup>	2.12x10 <sup>-11</sup>

<sup>a</sup>Data are from PUBCHEM Open Chemistry Database.

Accessible at <https://pubchem.ncbi.nlm.nih.gov/>

Table 2.2. Soil physico-chemical characteristics and chemical properties (dry weight basis) of the alkaline treated biosolids (ATB) used for dissipation study.

<b>Soil</b>		<b>ATB</b>	
Clay (%)	6.3	pH (pH Units)	9.95
Sand (%)	78.2	OM (%)	34.97
Silt (%)	15.5	Dry Matter (%)	61.58
pH (pH Units)	5.92	Nitrogen (%)	0.97
OM (%)	2.1	Ammonium-N (%)	<0.01
Calcium (kg ha <sup>-1</sup> )	558.89	Calcium (%)	16.47
K <sub>2</sub> O (kg ha <sup>-1</sup> )	111.28	K <sub>2</sub> O (%)	0.96
P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> )	387.13	P <sub>2</sub> O <sub>5</sub> (%)	1.53
Magnesium (kg ha <sup>-1</sup> )	57.14	Magnesium (%)	0.31
Aluminum (ppm)	1702.33	Sodium (%)	0.08
Boron (ppm)	<0.5	Boron (ppm)	18.78
Copper (ppm)	1.08	Copper (ppm)	99.41
Iron (ppm)	234.67	Iron (ppm)	7659.51
Manganese (ppm)	19.67	Manganese (ppm)	218.46
Zinc (ppm)	1.03	Zinc (ppm)	207.55
Naproxen (ng g <sup>-1</sup> TS dw)	BDL <sup>a</sup>	Naproxen (ng g <sup>-1</sup> TS dw)	BDL
Ibuprofen (ng g <sup>-1</sup> TS dw)	BDL	Ibuprofen (ng g <sup>-1</sup> TS dw)	BDL
Ketoprofen (ng g <sup>-1</sup> TS dw)	BDL	Ketoprofen (ng g <sup>-1</sup> TS dw)	BDL

<sup>a</sup> BDL: Below Detection Limit

### 2.2.3 Incubation experiment

A soil incubation study was conducted to examine the dissipation kinetics of the target NSAIDs in an individual and mixture compound environment under sterile and unsterile soil conditions. In total, 384 experimental units were prepared in this incubation experiment (Table A.1). The incubation study included the following treatments: 1) NPX, 2) IBF, 3) KTF, and 4) NPX:IBF:KTF mixture in both soil only and soil with an ATB amendment, and 5) Control without soil or soil+ATB. The control group consisted of each NSAIDs treatment, i.e. NPX, IBF, KTF, and NPX:IBF:KTF mixture, spiked into empty centrifuge containers, which was used to determine the loss of the target NSAIDs during extraction and sample analysis. Unspiked soil, soil+ATB, and ATB had previously been extracted and analyzed for the target compounds and none were detected. The experimental units were set up in a randomized block design (RBD) in an incubator with three blocks to account for potential vertical differences in temperature. Experimental units were established by weighing 5 g of soil, or 5 g of soil with 0.07 g of ATB (based on a field equivalent rate of 28 t ATB ha<sup>-1</sup>), into 50 ml centrifuge tubes. The pH of the initial soil and soil+ATB were 5.92 and 6.85, respectively, before the incubation. Each sample was spiked with 0.5 ml from a 10 mg L<sup>-1</sup> corresponding NSAIDs compound/mixture stock solution dissolved in methanol to achieve a final concentration of 1 µg NSAIDs g<sup>-1</sup> dry weight soil for each compound treatment combination. The solution was dripped onto the soil surface using a dispenser micropipette and thoroughly mixed with soil using a spatula. After spiking the air dried soil, the soil was brought to a gravimetric soil moisture content of 18% using deionized water. The control group received same volume of spiked sample only into an empty

container. Each treatment was established in triplicate and incubated in an environmentally controlled chamber set to  $20\pm 2$  °C under aerobic conditions and close to 100% relative humidity. Destructive samples were collected at eight time intervals (Day 0, 1, 2, 3, 4, 16, 30, and 48). The centrifuge tubes were wrapped with aluminum foil to minimize photodegradation of the compounds, as well as the top covered by parafilm to prevent contamination but to allow for any air/gas exchange. Moisture content was checked every two days gravimetrically by weighing the samples and comparing to the original weight. Water was added to the experimental unit if the weight differed from day 0. At designated time intervals, samples were taken out of the incubator, extracted and analyzed immediately.

Sterile soil treatments were prepared following the method described above but with the addition of 0.4 ml of 0.1%  $\text{HgCl}_2$  per 5 g of soil (Xu et al., 2008). However, analysis of initial soil samples indicated that treatments that were intended to be unsterilized soils were displaying a strong sterilization effect, similar to and/or in most cases greater than the soils sterilized with 0.1%  $\text{HgCl}_2$  (Figure A.1). A series of additional aerobic respiration studies were conducted to identify the source of sterilization in soils that were expected to be biologically active. The results of these studies identified the sterilization effect to have been the result of unevaporated methanol used as a carrier for the NSAIDs after spiking (Figure A.2). Therefore, based on the effectiveness of sterilization from the unevaporated methanol with NSAIDs, it was decided to exclude results from  $\text{HgCl}_2$  sterilized soil and the NSAIDs spiked soils were assessed as if they were sterile soils.

On the basis of the results from the first incubation study, a modified method of

spiking was established in order to eliminate the sterilization effect of the methanol on the soil. A second incubation study was conducted using the modified compound spiking method, with the same treatments as previously described above, in order to determine the half-lives of NSAIDs in an unsterile soil. A six-day pre-incubation of the soil was conducted in the environmentally controlled chamber, in the absence of UV light, at  $20\pm 2$  °C and at a gravimetric soil moisture content of 18%. The experimental units were also set up in a RBD in the incubator with three blocks. Experimental units were established by weighing 4.5 g of soil, or 4.5 g of soil with 0.07 g of ATB (based on a field equivalent rate of 28 t ATB ha<sup>-1</sup>), into 50 ml centrifuge tubes. Samples were collected destructively at eight time intervals (Days 0, 1, 2, 3, 4, 7, 10, and 14). In total, 288 experimental units were prepared in the second incubation experiment. The modified compound spiking method involved placing a bulk soil (40 g) in a 1 L mason jar and mixing with 40 ml of a 10 mg L<sup>-1</sup> NSAIDs individual compound or mixture stock solution dissolved in methanol. The mason jars were then left in a darkened fume hood for 24 hours before the start of the incubation experiment to allow the methanol to evaporate completely. Then, 0.5 g of spiked soil was taken from each Mason jar into the pre-weighed centrifuge tubes to achieve a final concentration of 1 µg NSAIDs g<sup>-1</sup> dry weight soil for each treatment combination. Each treatment was established in triplicate and incubated at  $20\pm 2$  °C under aerobic conditions and approximately 100% relative humidity. The rest of the methods were followed as described above.

#### **2.2.4 Sample preparation and analysis**

Target compounds in each sample were extracted using an ultrasonication solvent extraction method (Xu et al., 2008). Each sample was extracted sequentially using 5 ml

of acetone, 4 ml of acetone, 5 ml of ethyl acetate, and 4 ml of ethyl acetate. With each addition of a solvent, the sample was ultrasonicated at 42 kHz for 15 mins then centrifuged at 6000 rpm for 10 mins. The supernatants were decanted and combined, then evaporated to 1 ml in a water bath at 40 °C under a gentle stream of nitrogen gas. The concentrated supernatants were re-dissolved into 500 ml of H<sub>2</sub>SO<sub>4</sub> acidified deionized water to a pH of 3 and extracted by solid phase extraction (SPE) using Strata™-X 33 µm Polymeric Reversed Phase cartridges (200 mg, 6mL, Phenomenex, Torrance, CA, USA). The cartridges were attached to a 12 position SPE vacuum manifold (Phenomenex, Torrance, CA, USA) and pre-conditioned sequentially with 3 ml of ethyl acetate, 3 ml of methanol, and 3 ml of H<sub>2</sub>SO<sub>4</sub> acidified deionized water at pH of 3. An aliquot of sample solution was then passed through the SPE cartridges at a rate of 7.5 ml min<sup>-1</sup>. The target NSAIDs were eluted out of the cartridges with 8 ml ethyl acetate at a rate of about 1 ml min<sup>-1</sup> into 15 ml disposable glass tubes. The SPE eluates were concentrated to 0.5 ml in a water bath at 50°C using a TurboVap LV evaporator (Zymark Corporation, Hopkinton, MA, USA). The concentrates were transferred into 2 ml snap cap centrifuge tubes and 100 µL of 5 mg L<sup>-1</sup> internal standard mixture was added. The aliquots were evaporated completely in the 50°C water bath with a gentle stream of nitrogen gas then spiked with 100 µL of MTBSTFA and 100 µL of ethyl acetate to get a final volume of 200 µL. The samples were then derivatized in a 70°C water bath for one hour.

The derivatized samples were then transferred to 2ml screw top vials and analyzed in an Agilent 7890 series gas chromatograph (GC) connected to an Agilent 5975 series mass-selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA). The GC was installed with a Zebron ZB-5MS (30 m length, 0.25 mm I.D., and

0.25  $\mu\text{m}$  film thickness) column attached to a 10 m guard column with the inlet temperature set at 250°C. Helium was used as the carrier gas at a rate of 1.2 ml/min. The sample was injected into the GC column with initial oven temperature set at 70°C under splitless mode for a volume of 1  $\mu\text{L}$ . The oven temperature was programmed to hold at 70°C for 1 min, ramped up at 20°C  $\text{min}^{-1}$  to 280°C and held for 3 mins, then ramped up at 20°C  $\text{min}^{-1}$  to 300°C and held for 1 min. The total run time of each sample was 16.5 mins. The post-run temperature was set to 70°C. The transfer line temperature was set at 290°C. Data were acquired under selected ion monitoring (SIM).

For method validation, seven replicates of 4.5 g of soil or 4.5 g of soil amended with 0.07 g of ATB were weighed into 50 ml centrifuge tubes and mixed with 0.5 g of spiked soil to achieve a final concentration of 1  $\mu\text{g}$  NSAIDs  $\text{g}^{-1}$  dry weight soil for each compound treatment combination. Each sample was then analyzed following the procedures described above. Average recoveries for NPX, IBF, and KTF in soil were 95.7%, 115.1%, and 91.7%, respectively, and 94.9%, 94.8%, 86.0% in ATB amended soil, respectively. The limit of detection (LOD) for each compound was calculated by  $\text{LOD}=3.3\sigma/S$  and limit of quantification (LOQ) was calculated by  $\text{LOQ}=10\sigma/S$ , where the  $\sigma$  is standard deviation of responses at the lowest concentration and S is the slope of the calibration curve (Shrivastava and Gupta, 2011). The LOD was 14.9  $\text{ng g}^{-1}$  for NPX, 15.8  $\text{ng g}^{-1}$  for IBF, and 25.4  $\text{ng g}^{-1}$  for KTF. The LOQ was 45.2  $\text{ng g}^{-1}$  for NPX, 48.1  $\text{ng g}^{-1}$  for IBF, and 76.8  $\text{ng g}^{-1}$  for KTF.

### **2.2.5 Data analysis**

The average loss of target NSAIDs during extraction and sample analysis was determined from our control group as: 6% for NPX, 4.8% for IBF, and 5.4% for KTF, respectively.

The recovery of each NSAID in soil and ATB amended soil was calibrated by accounting for the loss of target NSAIDs determined from each control group and plotted against time. A simple first-order dissipation model was applied to fit the data using the following equation:

$$C_t = C_0 e^{-kt} \text{ (FOCUS, 2006)}$$

where  $C_t$  is the concentration of NSAIDs remaining in soil or soil-biosolid matrix ( $\mu\text{g g}^{-1}$ ) after  $t$  (days),  $C_0$  is the initial concentration of NSAIDs measured at time 0 ( $\mu\text{g g}^{-1}$ ), and  $k$  is the rate of dissipation ( $\text{d}^{-1}$ ). The unsterilized samples for days 30 and 48 in the second incubation study were lost due to a fire in the building housing the laboratory. Soil were recovered up to day 14 and therefore, the recovery of target NSAIDs for day 30 and 48 were predicted based on the first-order dissipation model using data from the recovered samples period. The recovery rate on day 0 was considered as 100% and the data for each following time period was standardized through  $C_t / C_0$ . The half-life,  $DT_{50}$  was calculated from:

$$DT_{50} = \frac{0.693}{k}$$

SigmaPlot v.13 (Systat Software Inc., San Jose, CA) was used for non-linear regression model fitting and determination of the dissipation coefficient ( $k$ ) in this study.

The effect of three factors of interest, including compound (2 levels: individual compound vs. mixture compounds), ATB amendment (2 levels: soil only vs. soil with ATB amendment, and time (8 sampling times) on recovery of NSAIDs, either in unsterile or sterile soil conditions, during the incubation study was determined using a repeated measure analysis of variance (ANOVA) in a randomized block design with three blocks using the Mixed Procedure of SAS v.9.4 (SAS Institute, Cary, NC). The model adequacy

was tested prior to ANOVA to determine if the assumption of normality and constant variance was violated (Montgomery, 2009). If violated, proper data transformation was conducted on response variables. Inverse square root, square root, and square transformations were conducted on NPX, IBF, and KTF data, respectively, and data were back-transformed after analysis for final interpretation. The variance-covariance matrix of the residuals, selected based on the basis of Akaike's Information Criterion, was variance components (VC; Montgomery, 2009). If effects were significant ( $p < 0.05$ ), means were compared with the Least Square Means test in SAS.

## **2.3 Results and discussion**

### **2.3.1 Dissipation of NSAIDs as individuals or mixtures in sterilized vs. unsterilized soils**

To study the dissipation kinetics of target NSAIDs in unsterile and sterile soils, recoveries were measured at different time intervals of the incubation (Figure 2.1). Data for each compound in unsterilized soil were fitted to a first-order exponential decay model resulting in good coefficients of determination in all treatments, except for KTF (Table 2.3). In samples from sterile soil, loss of NPX, IBF, and KTF was still observed but the measured recovery plateaued after day 4. In contrast, these compounds degraded rapidly in the unsterilized soil with the measured recoveries decreasing past 14 days. The difference in compound recovery between sterile and unsterile soil conditions resulted in 29% of NPX, 52% of IBF, and 19% of KTF being biodegraded over 14 days. The amount of NSAID disappearance due to biodegradation was further expected to increase to 56% of NPX, 71% of IBF, and 40% of KTF over 48 days based on the first-order exponential

model. The effect of the sterile soil was more notable when comparing the dissipation rates in Table 2.3. Dissipation of NPX and IBF in unsterilized soil was 6.5 to 47-fold and 12.8 to 23.5-fold greater, respectively, than in sterile soil (Table 2.3). These results were comparable to values calculated by Xu et al. (2009b) in a Hanford loamy sand soil. Biodegradation has been previously reported as a primary pathway for the loss of KTF in soil (Xu et al., 2009a). Dissipation rates of KTF in unsterilized soil ranged from  $0.018 \pm 0.085$  to  $0.025 \pm 0.014 \text{d}^{-1}$  and decreased to lower than  $0.004 \pm 0.001 \text{d}^{-1}$  in sterilized soil, which indicated biodegradation of KTF occurred in our study. The distinct differences in measured recovery and dissipation rates between sterilized and unsterilized soil in our study indicated that biodegradation is the most important mechanism for the losses of NPX, IBF, and KTF (Girardi et al., 2013; Lin and Gan, 2011).

All target NSAIDs were not expected to volatilize from soil surfaces based upon their estimated vapor pressure and Henry's Law constant (Table 2.1). Other abiotic mechanisms may contribute to the loss of, or inability to extract, NSAIDs in sterilized soil, such as the formation of non-extractable residues (NER). For example, a 90-day incubation study conducted by Girardi et al. (2013) using  $^{13}\text{C}_6$ -labelled IBF found  $^{13}\text{C}$ -NER in soil under biotic and abiotic conditions was 30% and 15.4%, respectively. In the first incubation study, NSAID recovery decreased rapidly up to Day 4 in all treatments before reaching a plateau (Figure 2.1 & Figure A.1). Subsequent respiration studies determined that microbial respiration was significantly inhibited in soils sterilized with  $\text{HgCl}_2$  and soils receiving NSAIDs with unevaporated methanol carrier solvent (Figure A.2). Since soil incubations occur under unsterile conditions it is possible that microbial re-colonization occurred leading to some biodegradation of target compounds over time.

However, other processes cannot be excluded as potential disappearance pathways, including sorption to organic matter and anaerobic degradation, for the three target NSAIDs in our study. Differential oxygen status in soil has been reported to affect the dissipation of pharmaceuticals, although the behaviors varied in soils with different physico-chemical properties (Biel-Maeso et al., 2019; Carr et al., 2011; Lahti and Oikari, 2011; Lin and Gan, 2011). Lin and Gan (2011) found both NPX and IBF were persistent in a medium loam soil under anaerobic conditions, while apparent dissipation was observed for IBF in a very gravelly fine loamy sand soil under both aerobic and anaerobic conditions. In their study, IBF was found to have negligible sorption to either of the two soils, while in contrast NPX was determined to be strongly sorbed to the soils (Lin and Gan, 2011). This may explain the slower rate of dissipation of NPX in unsterile soil, relative to IBF, with and without ATB treatments in our study (Figure 2.1). Another study compared the aerobic and anaerobic degradation of NPX, IBF, and KTF in Asian rice paddy soils and found reduced dissipation of IBF and KTF under anaerobic conditions, while NPX presented a higher dissipation rate under reduced oxygen conditions (Nazhakaiti et al., 2019). However, the half-life of IBF calculated in an alkaline clay loam soil under anaerobic conditions (41.2 days) was 3 times shorter than under aerobic conditions (Carr et al., 2011). Based on the biodegradation rates and half-lives obtained in our study, the order in which the target NSAIDs disappeared in both soil and ATB amended soil was: IBF>NPX>KTF, for both individual and mixture compound treatments. IBF and NPX showed low to moderate persistence in unsterile soil and ATB amended soil, while KTF appeared to be highly persistent with an average half-life of 33 days (Gustafson, 1989; Topp et al., 2008; Xu et al., 2009a).

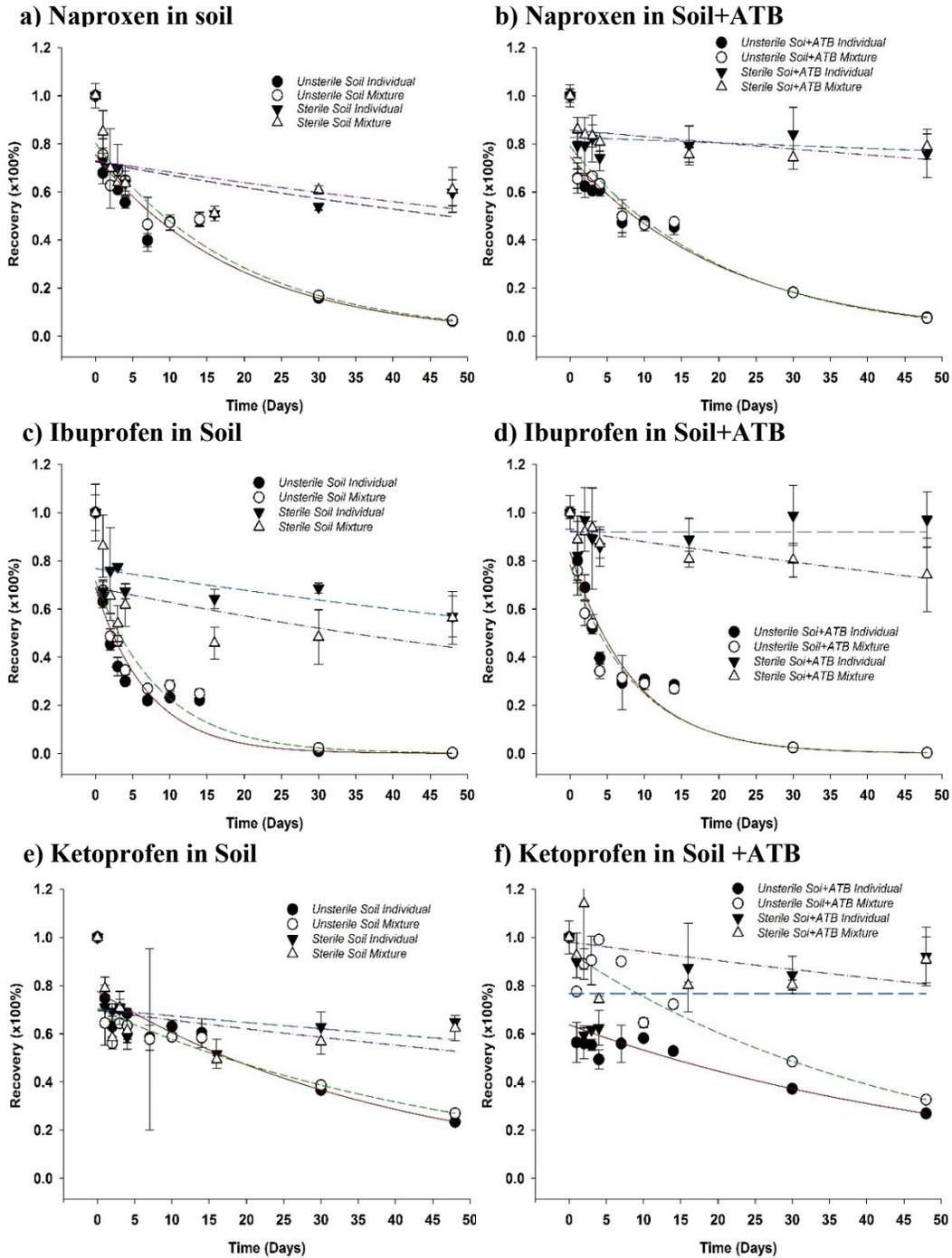


Figure 2.1. Dissipation of a) Naproxen in soil; b) Naproxen in alkaline treated biosolids amended soil (Soil+ATB); c) Ibuprofen in soil; d) Ibuprofen in Soil+ATB; e) Ketoprofen in soil; and f) Ketoprofen in Soil+ATB; in an individual vs. mixture compound environment, under unsterile and sterile soil conditions. Vertical bars represent the standard deviation (n=3). Lines were fitted to a first-order decay model (Days 30 and 48 in unsterile soil treatments were fitted based on the model).

Table 2.3. First-order exponential dissipation rate constants (k), correlation coefficients (R<sup>2</sup>), and half-life (t<sub>1/2</sub>) of naproxen, ibuprofen, and ketoprofen in soil or alkaline treated biosolids amended soil (Soil+ATB) spiked as individual and mixture compound treatments, under unsterile and sterile soil conditions. Data for k and t<sub>1/2</sub> are presented as Mean ± SD (n=3).

Compound	Treatment	Model	Unsterile			Sterile		
			R <sup>2</sup>	k (d <sup>-1</sup> )	t <sub>1/2</sub> (d)	R <sup>2</sup>	k (d <sup>-1</sup> )	t <sub>1/2</sub> (d)
Naproxen	Soil Individual	Exponential	0.544	0.052±0.005	13.2±1.5	0.343	0.008±0.001	87.7±0.9
	Soil Mixture	Exponential	0.701	0.052±0.015	13.4±1.3	0.241	0.007±0.003	105±2.4
	Soil+ATB Individual	Exponential	0.646	0.047±0.006	14.8±0.8	0.075	0.001±0.001	495.1±204.2
	Soil+ATB Mixture	Exponential	0.696	0.049±0.011	14.1±2.9	0.338	0.003±0.001	216.6±26.7
Ibuprofen	Soil Individual	Exponential	0.675	0.141±0.014	4.9±0.5	0.405	0.006±0.003	111.8±17.5
	Soil Mixture	Exponential	0.719	0.115±0.032	6.1±1.3	0.264	0.009±0.003	73.7±9.3
	Soil+ATB Individual	Exponential	0.799	0.118±0.011	5.9±0.5	0.000	0.000	>500
	Soil+ATB Mixture	Exponential	0.761	0.115±0.006	6±0.3	0.788	0.005±0.002	138.6±42.9
Ketoprofen	Soil Individual	Exponential	0.412	0.025±0.014	28.2±6.2	0.116	0.004±0.001	169.1±32.4
	Soil Mixture	Exponential	0.222	0.02±0.006	35.4±6.1	0.165	0.006±0.002	119.5±3.7
	Soil+ATB Individual	Exponential	0.128	0.018±0.085	39.2±14.1	0.000	0.000	>500
	Soil+ATB Mixture	Exponential	0.456	0.021±0.004	32.4±2.3	0.101	0.004±0.001	169.1±39

### **2.3.2 Effect of mixture compound environment on the dissipation of NSAIDs**

A slightly higher average recovery was observed for NPX in soil as a mixture compound treatment but the dissipation rates calculated were not significantly different. The IBF in soil present as a mixture compound treatment had significantly higher average recovery and slower dissipation rates compared to the individual compound treatment (Figure 2.2).

In ATB amended soil, however, average recovery of NPX and IBF showed no differences between individual and mixture compound treatments with a faster dissipation observed for NPX (Figure 2.2). The individual KTF had a higher average recovery and slower dissipation in soil than KTF in mixture compound treatment, while the opposite result was observed in ATB amended soil. The extraction of KTF, especially from ATB amended soil, was very challenge and results were strange with large variability. Therefore, the results of KTF may not present the correct mixture compound effect in this study. The dissipation rates calculated for KTF were based on the model fitting with poor correlation coefficients, which were not considered to determine the effect of mixture compound treatment on dissipation. The mixture compound effect observed for selected NSAIDs in this study was likely due to the competitive interactions between the compounds. It has been reported that pharmaceutical with the octanol-water partition coefficient ( $\text{Log } K_{ow}$ ) value greater than 3 would reduce the biodegradation due to their low affinity to water and easier adsorption to soil OM (Bressler and Gray, 2003). The  $\text{Log } K_{ow}$  value of IBF (3.97) was higher than NPX (3.18) and KTF (3.12), which means the IBF is probably more competitive in partitioning into soil OM in the mixture compound environment (Table 2.1). Zhang et al. (2017) studied the sorption-desorption behavior of four NSAIDs, including NPX, IBF, KTF, and DCF, in a mixture compound

environment. The authors found NPX and KTF present in the mixture compound environment had a decreased sorption distribution coefficient ( $K_d$ ), while a higher  $K_d$  value was observed for IBF. The enhanced sorption of IBF could reduce bioavailability in a mixture compound treatment, which may explain the reduced dissipation rate observed in the soil and ATB amended soil. For KTF, the low hydrophobicity and reduced sorption in the mixture compound treatment explain the lower recovery found in the unamended soil treatment. Other factors, such as multilayer cooperative adsorption, cationic bridging, or the formation of organo-clays from the soil, have also been reported to affect the sorption behavior of compounds that would correspondingly alter the bioavailability (Bui and Choi, 2010; Durán-Álvarez et al., 2014; Zhang et al., 2017). The dissipation behavior of NPX and IBF in the mixture compound treatment were likely the result of a combination of factors discussed above, although these mechanisms were not determined in this study.

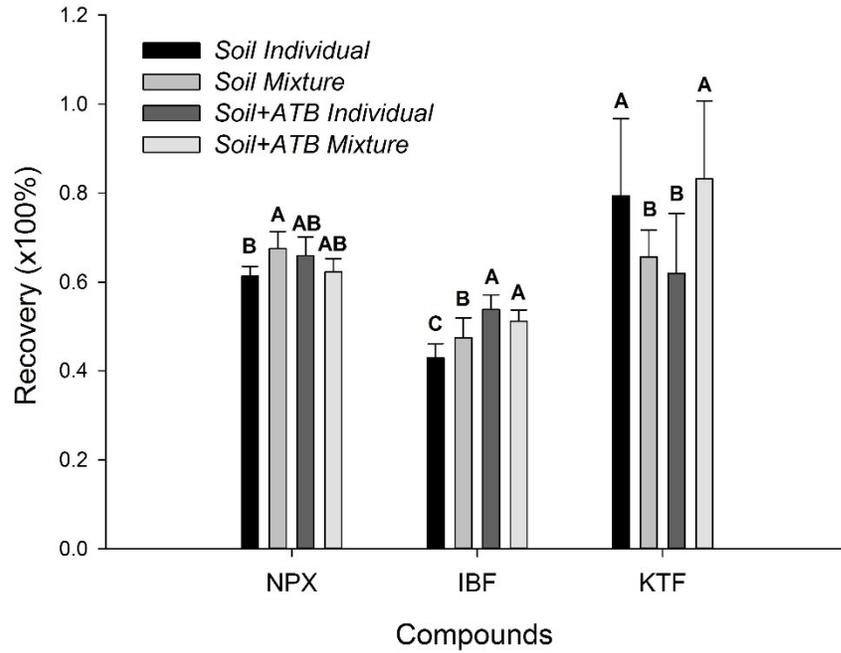


Figure 2.2. Mean recovery values of individual and mixture compound treatments, including naproxen (NPX), ibuprofen (IBF), and ketoprofen (KTF), from a two-way interaction of Compound x Soil over all sampling periods in an unsterile soil and soil+ATB. Vertical bars represent the standard deviation (n=3).

### **2.3.3 Effect of alkaline treated biosolids on the dissipation of NSAIDs in soil**

A two-way interaction of Compound x ATB amendment for recovery of NSAIDs was found to be significant ( $p < 0.05$ ) for all three NSAIDs in the unsterilized soil but not in the sterilized soil (Table 2.4; Figure 2.2). Compared to unamended soil treatments, ATB amended to soil did not significantly alter the measured recovery for NPX spiked as either an individual or mixture compound (Figure 2.2). For IBF, the overall measured recovery over the whole incubation period in ATB amended soil was significantly higher than in unamended soil treatments for both individual and mixture compounds. A higher recovery was determined for KTF in the mixture compound treatment in ATB amended soil, while the opposite result was observed for individual KTF. The opposite ATB effect found for KTF was due to large variability in measured recoveries for this compound in the study. In unsterilized soil, a significant two-way interaction of Time x ATB amendment was also observed for IBF ( $p = 0.001$ ) (Table 2.4). Recovery of IBF measured in ATB amended soil, regardless of individual or mixture compound treatment, was higher than in unamended soil for the first three days of the incubation study. The ATB effect disappeared after day 4 and the measured recovery subsequently plateaued at around 27% recovery. With NPX and KTF, the ATB effect on measured recovery was not found over time. The biodegradation rates listed in Table 2.3, however, were lengthened in the ATB amended soil for NPX, in both individual and mixture compound treatments, and for IBF and KTF as individual compounds.

Table 2.4. Repeated measures analysis of variance for the effects of compound, ATB amendment, and time on recovery of naproxen (NPX), ibuprofen (IBF), ketoprofen (KTF) in unsterile and sterile soils. The p values are shown in the table. Significant effects at  $p < 0.05$  are shown in bold.

Source	NPX (Unsterile)	IBF (Unsterile)	KTF (Unsterile)	NPX (Sterile)	IBF (Sterile)	KTF (Sterile)
Compound	0.510	0.280	0.329	0.334	0.002	0.000
Time	<b>0.000</b>	0.000	<b>0.002</b>	0.000	0.000	0.000
ATB amendment	0.895	0.000	0.986	0.000	0.000	0.000
Compound x Time	0.104	0.350	0.784	0.449	<b>0.007</b>	0.000
Compound x ATB amendment	<b>0.011</b>	<b>0.000</b>	<b>0.000</b>	0.801	0.583	0.000
Time x ATB amendment	0.257	<b>0.001</b>	0.999	<b>0.000</b>	<b>0.001</b>	0.000
Compound x Time x ATB amendment	0.109	0.501	0.212	0.417	0.097	<b>0.000</b>

In sterilized soil, the Time x ATB amendment effect was significant for NPX and IBF ( $p < 0.05$ ), while the recovery of KTF was significantly affected by a three-way Compound x Time x ATB amendment interaction ( $p = 0.000$ ) (Table 2.4). All three NSAIDs in sterilized ATB amended soil reached a plateau earlier than in soil only, with overall higher recoveries measured over time (Data not shown). The dissipation rate of all NSAIDs in sterilized soil amended with ATB was found to be considerably lower than in sterilized unamended soil treatment, which was in line with the ATB effect found in unsterilized soil (Table 2.3).

Excluding for microbial activity in the soil, the significantly higher recovery and reduced dissipation rate of NSAIDs observed in the sterilized soil with ATB in this study may have been due to enhanced sorption of the compounds to the OM matrix. Lin and Gan (2011) reported significantly reduced disappearance of NPX over an 84 day aerobic incubation in a medium loam textured soil, which had half the organic carbon (OC) content than a sandy textured soil that was also tested (0.33% OC vs. 0.16% OC). Biosolids application can increase the OC content of soils and has also been reported to increase the potential for enhancing sorption and decreasing the bioavailability and mobility of NSAIDs in arable soils (Borgman and Chefetz, 2013; Grossberger et al., 2014; Li et al., 2013). A study conducted by Wu et al. (2009) found increased sorption of triclosan and triclocarban in a silt clay soil and a sandy loam soil amended with aerobic digested biosolids. The authors suggested the increase of soil OM due to biosolids application governed the overall sorption of those compounds in the amended soil. Acidic pharmaceutical compounds, such as NPX and IBF, containing deprotonated carboxylic functional groups, are expected to sorb to protonated functional groups available on soil

OM (Vulava et al., 2016). The labile carbon sources in biosolids are also more likely to be used by microorganisms first before accessing carbon in the NSAIDs, which would further decrease the dissipation rate of the target compounds (Li et al., 2013). The behavior of NSAIDs in ATB amended soils may also be affected by the change in soil pH from an acidic soil into a more neutral range. Ionic molecules usually have more complex behavior as their fate changes with the different pH conditions. Field application of ATB has been reported to increase the soil pH in agricultural soils (Price et al., 2015). The ATB amendment (pH=9.95) neutralized the acidic soil (pH=5.92) to a pH of 6.85 at the beginning of our incubation study. The pK<sub>a</sub> values of NPX, IBF, and KTF are 4.15, 4.91, and 4.45, respectively. Therefore, these compounds would be deprotonated and negatively charged based on the pH of soil and ATB amended soil in this study. As shown in Table 2.1, calcium (Ca) had the highest concentration in soil, followed by aluminum (Al) and iron (Fe). At soil pH 5.92, these minerals would mainly be present as Al<sup>3+</sup>, Al(OH)<sup>2+</sup>, Al(OH)<sub>2</sub><sup>+</sup>, Fe(OH)<sup>2+</sup>, Fe(OH)<sub>2</sub><sup>+</sup>, Ca<sup>2+</sup>. The increased cation exchange capacity (CEC) and the introduction of more Ca, magnesium (Mg), and Fe due to the ATB amendment will change the behavior and balance of metal cations present in the soil and affect the sorption potential NSAIDs (Bui and Choi, 2010). Based on the pH of ATB amended soil, minerals present would be present mainly as Al(OH)<sub>2</sub><sup>+</sup>, Fe(OH)<sup>2+</sup>, Fe(OH)<sub>2</sub><sup>+</sup> and a higher concentration of Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Ca(OH)<sup>+</sup>. The adsorption of IBF and KTF have been reported to increase in the presence of Mg<sup>2+</sup> and Ca<sup>2+</sup> in soil, which may explain the decreased biodegradation observed in ATB amended soil (Bui and Choi, 2010). The increased concentration of Fe(OH)<sub>2</sub><sup>+</sup> would offer more simple ligand exchange sites for NPX and IBF (Vulava et al., 2016). Furthermore, the Al(OH)<sub>2</sub><sup>+</sup> can

bridge the negatively charged functional groups in NSAIDs and negatively charged sites on soil and ATB surfaces through electrostatic forces, which would also result in the increased adsorption and decreased bioavailability of acidic pharmaceuticals such as NPX and IBF. Shifts in the microbial community due to the ATB amendment can also affect the dissipation of NSAIDs. Price et al. (2020) conducted a metagenomic study from a long-term research site with the same soil and receiving the same ATB as in the present study and found the addition of ATB significantly shifted the abundance of various bacterial communities, including Alpha-, Beta-, and Gamma-proteobacteria, Actinobacteria, and Bacteroidetes. Several studies have reported dissipation of the target NSAIDs in our study by these bacterial populations (Caracciolo et al., 2015; Jiang et al., 2017). However, we did not conduct the same microbial analysis in this incubation study and this remains an area requiring further investigation.

## **2.4 Conclusions**

Biotic and abiotic degradation are important natural attenuation pathways that affects the fate of pharmaceuticals in soil systems. This study evaluated the biodegradation behavior and disappearance of three commonly used NSAIDs, including NPX, IBF, and KTF, in a sandy loam textured soil, with and without an ATB amendment, using an unsterilized and sterilized soil. The studied NSAIDs were introduced to soil as either an individual or mixture compound treatment. Comparison of results from sterile vs. unsterile soil indicated that biodegradation of NSAIDs was the main cause of compound loss for both individual and mixture compound treatments. The biodegradation of two target compounds, NPX and IBF, modeled well using a first-order exponential decay model. The amendment of soil with ATB in both unsterilized and sterilized soils reduced the

dissipation rate of all three NSAIDs, in either the individual or mixture treatments, except for IBF and KTF in a mixture compound environment. Mixture compound effects on the dissipation of NSAIDs were inconsistent for the different compounds. In mixture compound treatments, IBF dissipation was inhibited in both soil and ATB amended soil. The dissipation rate of KTF in mixture compound environment in soil was lower, while the opposite effects were observed in ATB amended soils. For NPX, the dissipation was enhanced in mixture compound environment in ATB amended soil, while the same dissipation rate of NPX was calculated in soil. The mechanism of interaction among studied NSAIDs in the multi-substance environment was not determined in the current study. This study has demonstrated the complexity of NSAIDs dissipation in a soil system, either as an individual or mixture compound treatment, and highlights the need to further investigate the behavior and mechanisms of dissipation of compound mixtures in soils.

## **2.5 Supplementary Information**

Supplementary Information related to this article are included in Appendix A and B.

## **Chapter 3 Effect of biosolids amendment on the fate and mobility of non-steroidal anti-inflammatory drugs (NSAIDs) in a field-based lysimeter cell study<sup>2</sup>**

### **3.1 Introduction**

More than 30 million people globally consume NSAIDs, one of the most widely used class of pharmaceuticals with anti-inflammatory, analgesic, and antipyretic effects, on a daily basis (Dulal and Khan, 2019; Pilotto et al., 2010). NSAIDs, such as naproxen (NPX), ibuprofen (IBF), and ketoprofen (KTF) are transported into wastewater treatment plants (WWTPs) through human excretions and accumulate in the biosolids generated from WWTPs due to the limited removal efficiency of those pharmaceuticals by typical biosolids stabilization processes (Carballa et al., 2008; Dodgen et al., 2015; Hydromantis, 2010). Approximately 35,000 tonnes of alkaline treated biosolids (ATB) are produced annually in Nova Scotia, Canada and land applied as soil amendments (N-Viro System Canada LP, 2009). There is some evidence to suggest that alkaline stabilization can increase the concentration of NPX and IBF in ATB, which could create environmental risks associated with elevated NSAIDs in agricultural soils (Hydromantis, 2010).

Once NSAIDs are introduced into soil they can be sorbed to soil particles, transported into aquatic environments through leaching or runoff, or they can decompose into secondary metabolites. Some studies have detected these compounds in surface

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<sup>2</sup> A version of this chapter has been submitted to Environmental Science & Technology.

Shu, W., Price, G.W., Jamieson, R., Lake, C., 2022. Effect of biosolids amendment on mobility of non-steroidal anti-inflammatory drugs (NSAIDs) in a field-based lysimeter cell study. Environ. Sci. Technol. (submitted on March 28<sup>th</sup>, 2022)

water and groundwater at concentration levels from  $\text{ng L}^{-1}$  to  $\text{ug L}^{-1}$  (Sui et al., 2015; Xu et al., 2010). The mobility of NSAIDs in soil is governed by several mechanisms, including sorption or biotic and abiotic degradation. These attenuation mechanisms depend on each compound's chemical characteristics and soil properties, such as texture, organic matter (OM) content, pH, and microbial community (Ascar et al., 2017; Shu et al., 2020; Xu et al., 2010). Xu et al. (2010) conducted a column study with three cropland soils (a sandy loam soil, a silty clay soil, and a silt loam soil) and determined a high leaching potential for three NSAIDs, including NPX, IBF, and KTF. However, incorporation of ATB as a soil amendment has also been reported to increase soil fertility and to positively change other soil physico-chemical properties, which might affect the presence or mobility of NSAIDs in soil (Price et al., 2020; Shu et al., 2016).

A study was conducted to explore the effect of three ATB application rates on the fate and mobility of three target NSAIDs (NPX, IBF, and KTF) in a loamy sand textured agricultural soil using field-based lysimeters. The objectives of the study were to determine the fate and relative mobility of NSAIDs through the soil profile and transport out with drainage water.

The hypotheses were that i) biodegradation would be a dominant disappearance pathway for the three NSAIDs studied in the field-based lysimeter cells; ii) the target NSAIDs would have low mobility in the soil profiles; and iii) higher rates of ATB amendment would result in lower mobility of the target NSAIDs in the soil profiles.

## 3.2 Materials and Methods

### 3.2.1 Chemicals

The NPX ( $\geq 98\%$  of purity), IBF ( $\geq 98\%$  of purity), and KTF ( $\geq 98\%$  of purity) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Deuterated forms of selected NSAIDs, including ( $\pm$ )-NPX- $d_3$  ( $\alpha$ -methyl- $d_3$ ) (99%  $d_3$ ), ( $\pm$ )-IBF- $d_3$  ( $\alpha$ -methyl- $d_3$ ) (99.6%  $d_3$ ), and ( $\pm$ )-KTF- $d_3$  ( $\alpha$ -methyl- $d_3$ ) (98.5%  $d_3$ ) were purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). Acetone (99.9%), ethyl acetate (99.9%), hexane (99.9%) and methanol (99.8%) were purchased from Fisher Scientific (Ottawa, ON, Canada). Derivatization agent, N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) was purchased from Chromatographic Specialties Inc (Brockville, ON, Canada). Stock solutions for each compound and corresponding internal standards were prepared in methanol and stored at  $-20\text{ }^\circ\text{C}$ . Chemical structures and properties for the target NSAIDs are listed in Table 2.1 (Shu et al., 2020).

### 3.2.2 Lysimeter cell study

#### 3.2.2.1 Site description and biosolids application

A long-term agricultural lysimeter cell study site at the Bio-Environmental Engineering Center (BEEC), located in Bible Hill, Nova Scotia ( $45^\circ 23' 18''$  N,  $63^\circ 14' 15''$  W) was used to study the transport of the target NSAIDs. Lysimeter cells were 6.1 meters in length and 3.66 meters in width, with a  $1.44\text{ m}^2$  square shaped inner cell installed at the centre of each cell. Each inner plot of the lysimeter cells was connected to a separate drain line attached to an ISCO 6700 Full-size Portable Sampler (Teledyne Isco, Lincoln, NE, USA) and a calibrated tipping bucket system for flow measurement (Appendix C).

The lysimeters were established in 2009 by excavating the area and laying out a gravel base with drain lines to a sampling hut. Concrete highway dividers were used to create discrete cells and a structure was built in the center of the cell, over top the drain line, with an impermeable geotextile membrane to separate it from the rest of the cell. Soil was packed in layers to achieve a bulk density similar to agricultural soils. Additional information has been published in Sayyad et al. (2016). Lysimeter cells were amended with ATB at three different rates, including 0 Mg ha<sup>-1</sup> (Control), 7 Mg ha<sup>-1</sup>, and 28 Mg ha<sup>-1</sup> (wet weight basis). The ATB was collected from the N-Viro Systems Canada Biosolids Processing Facility in Halifax, N.S. (Walker Environmental Inc.). The ATB used in this study was treated using an Advanced Alkaline Stabilization with Subsequent Accelerated Drying (AASSAD), which involved the combination of high pH (>12) and temperature (between 52 and 62 °C) through mixing of dewatered sewage solids and cement kiln dust (CKD) to kill pathogens (N-Viro System Canada LP, 2009). Each rate was established in triplicate for a total of nine lysimeter cells. Lysimeter cells were weeded and tilled using a small push rototiller and the ATB was applied then tilled in to a depth of 5 cm on May 29<sup>th</sup>, 2018. Perennial ryegrass was broadcast seeded uniformly across each lysimeter cell on May 31<sup>st</sup>, 2018. Soil samples were collected from three depths using an Oakfield soil probe (0-15 cm, 15-30 cm, and 30-55 cm soil depths) before spiking the soil surface of the inner plot of each lysimeter cell with NSAIDs. ATB samples were collected from different locations in a stockpile obtained for field application. Both soil and ATB were air dried, passed through a 2 mm sieve, and extracted and analyzed for the target compounds individually prior to the study in the Innovative Waste Management laboratory, Dalhousie University, Bible Hill, Nova

Scotia, Canada. Additional physical and chemical parameters of soil and ATB were analyzed at the Nova Scotia Department of Agriculture's Analytical Services Laboratory, Bible Hill, Nova Scotia. Selected physical and chemical characteristics of soil and ATB are provided in Table 2.2.

### **3.2.2.2 Water and soil sampling**

Selected NSAIDs, including NPX, IBF, and KTF, for spiking of the lysimeter cells were weighed, 1.149 g of each compound, and dissolved in 100 ml methanol as a mixture compound solution. The solution mixture was combined with 2 L water then applied evenly to the inner plot of each lysimeter cell using a hand sprayer on August 24<sup>th</sup>, 2018. The spraying regime was established in order to reach a target concentration of 1  $\mu\text{g}$  of NSAID  $\text{g}^{-1}$  soil, based on a 55 cm soil depth. Precipitation events were simulated by manual irrigation of the inner plot using 50 L of water, which was equivalent to 35 mm of rainfall a day, to guarantee a sufficient volume of water to generate drainage through the soil profile of the lysimeter cell. Each inner plot was covered with a tarp to avoid entry of natural rainfall. The water left in the drain line and the PVC pipe containers were emptied before each irrigation. Five irrigation events were conducted over 34 days (Day 1, 7, 13, 23, and 32) between August 24<sup>th</sup>, 2018 (Day 1) and September 26<sup>th</sup>, 2018 (Day 34). The autosampler was triggered by five tips of the tipping buckets to draw an 800 ml subsample of water flowing out of the lysimeter. The autosamplers were outfitted with 24 individual 1 L collection bottles to collect subsamples over the entire flow event. The collection bottles were removed from the autosamplers 24 hrs after each irrigation event. The water collected in the 24 containers, for each autosampler, was combined into a composite sample using a 20L bucket. A 2.5 L water subsample was taken from each 20

L bucket, after mixing thoroughly, and frozen for analysis at a later date. The composited water sample represented concentrations over the total flow event. Soil samples from each lysimeter cell were also collected using an Oakfield soil probe (2 cm in diameter) from three soil depths (0-15 cm, 15-30 cm, and 30-55 cm) on Day 3, 18, and 34. The soil samples were air dried at room temperature to avoid significant losses from volatilization or degradation and to allow samples to pass through a 2 mm sieve easily. The sieved soil samples were then stored at -20°C until analysis.

### **3.2.2.3 Sample preparation and analysis**

Soil samples were prepared by weighing 5 g of soil into 50 ml centrifuge tubes. Target compounds in each sample were extracted using an ultrasonication solvent extraction method (Xu et al., 2008). Each sample was extracted sequentially using 5 ml of acetone, 4 ml of acetone, 5 ml of ethyl acetate, and 4 ml of ethyl acetate. With each addition of a solvent, the sample was ultrasonicated at 42 kHz for 15 mins then centrifuged at 6000 rpm for 10 mins. The supernatants were decanted and combined, then evaporated to 1 ml in a water bath at 40 °C under a gentle stream of nitrogen gas. The concentrated supernatants were re-dissolved into 500 ml of H<sub>2</sub>SO<sub>4</sub> acidified deionized water to a pH of 3 and extracted by solid phase extraction (SPE) using Strata™-X 33 µm Polymeric Reversed Phase cartridges (200 mg, 6mL, Phenomenex, Torrance, CA, USA). Water samples were removed from the freezer the night before analysis and thawed at room temperature. Each sample was filtered through a Buchner funnel with a G6 glass fiber filter to remove soil particles in the water and acidified to pH of 3 with H<sub>2</sub>SO<sub>4</sub> and extracted by SPE using Strata™-X 33 µm Polymeric Reversed Phase cartridges (200 mg, 12 mL, Phenomenex, Torrance, CA, USA). The cartridges were attached to a 12 position

SPE vacuum manifold (Phenomenex, Torrance, CA, USA) and pre-conditioned sequentially with 3 ml of ethyl acetate, 3 ml of methanol, and 3 ml of H<sub>2</sub>SO<sub>4</sub> acidified deionized water at pH of 3. An aliquot of sample solution was then passed through the SPE cartridges at a rate of 7.5 ml min<sup>-1</sup>. The target NSAIDs were eluted out of the cartridges with 8 ml ethyl acetate at a rate of about 1 ml min<sup>-1</sup> into 15 ml disposable glass tubes. The SPE eluates were concentrated to 0.5 ml in a water bath at 50°C using a TurboVap LV evaporator (Zymark Corporation, Hopkinton, MA, USA). The concentrates were transferred into 2 ml snap cap centrifuge tubes and 100 µL of 5 mg L<sup>-1</sup> internal standard mixture was added. The aliquots were evaporated completely in the 50°C water bath with a gentle stream of nitrogen gas then spiked with 100 µL of MTBSTFA and 100 µL of ethyl acetate to get a final volume of 200 µL. The samples were then derivatized in a 70°C water bath for one hour.

The derivatized samples were then transferred into 2 ml screw top vials and analyzed in an Agilent 7890 series gas chromatograph (GC) connected to an Agilent 5975 series mass-selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA). The GC was installed with a Zebron ZB-5MS (30 m length, 0.25 mm I.D., and 0.25 µm film thickness) column attached to a 10 m guard column with the inlet temperature set at 250°C. Helium was used as the carrier gas at a rate of 1.2 ml min<sup>-1</sup>. The sample was injected into the GC column with an initial oven temperature set at 70°C under splitless mode for a volume of 1 µL. The oven temperature was programmed to hold at 70°C for 1 min, ramped up at 20°C min<sup>-1</sup> to 280°C and held for 3 mins, then ramped up at 20°C min<sup>-1</sup> to 300°C and held for 1 min. The total run time of each sample was 16.5 mins. The post-run temperature was set to 70°C. The transfer line temperature

was set at 290°C. Data were acquired under selected ion monitoring (SIM).

The limit of detection (LOD) for each compound was calculated by  $LOD=3.3\sigma/S$  and limit of quantification (LOQ) was calculated by  $LOQ=10\sigma/S$ , where the  $\sigma$  is the standard deviation of responses at the lowest concentration and S is the slope of the calibration curve (Shrivastava and Gupta, 2011). The LOD was 24.5 ng g<sup>-1</sup> for NPX, 39.3 ng g<sup>-1</sup> for IBF, and 16.1 ng g<sup>-1</sup> for KTF. The LOQ was 80.3 ng g<sup>-1</sup> for NPX, 119 ng g<sup>-1</sup> for IBF, and 48.9 ng g<sup>-1</sup> for KTF.

The soil OM was determined using the dry-ashing method in a temperature-regulated muffle furnace, which involved drying 2 g of soil samples at 105 °C, followed by increasing and maintaining temperature to 370 °C for 1 hour, then ashing the sample at 550 °C for 20 hours. The pH was measured in a 2:1 deionized water to soil ratio using a Fisher Scientific Accumet Excel XL50 pH (Ottawa, ON, Canada).

### **3.2.3 Statistical analysis**

The effect of three factors of interest, including ATB amendment (3 levels: 0, 7, 28 Mg ha<sup>-1</sup>), soil depth (3 levels: 0-15, 15-30, and 30-55 cm), and time (3 sampling times) on the recovery of NSAIDs, OM, and pH in soil samples was determined using a repeated measures analysis of variance (ANOVA) in a completely randomized design with three replicates using the Mixed Procedure of SAS v.9.4 (SAS Institute, Cary, NC). The effect of two factors of interest, including ATB amendment (3 levels) and time (5 sampling times) on the recovery of NSAIDs in water samples was also determined using a repeated measures ANOVA in a completely randomized design with three replications using the Mixed Procedure of SAS v.9.4 (SAS Institute, Cary, NC). The model adequacy was tested before ANOVA to determine if the assumption of normality and constant variance

was violated (Montgomery, 2009). If violated, proper data transformation was conducted on response variables. Square root, fourth root, and third root transformations were conducted on NPX, IBF, and KTF recovery data in soil, respectively, and data were back-transformed after analysis for final interpretation. The variance-covariance matrix of the residuals, selected based on Akaike's Information Criterion, was variance components (VC) (Montgomery, 2009). If effects were significant ( $p < 0.05$ ), the means were compared with the Least Square Means test in SAS.

### **3.3 Results and discussion**

#### **3.3.1 Transport of NSAIDs in the lysimeter cells**

All NSAIDs measured in the soil were significantly affected by a two-way interaction of Time x Soil Depth and Time x ATB amendment rate (Table 3.1). After the initial spiking of NSAIDs on Day 1, all the compounds migrated down to the 30-55 cm depth in the lysimeter cells by Day 3, with the highest recoveries measured at the 0-15 cm depth (Figure 3.1). The total mass of NPX, IBF, and KTF recovered from all soil depths on Day 3 were 87%, 77.5%, and 72.9%, respectively. On day 34, the recoveries of NPX and IBF measured at a soil depth of 0-15 cm were significantly greater than at the 30-55 cm soil depth, while the recovery of KTF was not different at any of the three soil depths. The total NPX residue, at all soil depths, was reduced to 4.8% of the initial spiking mass by the end of the study and the overall recoveries of IBF and KTF were both less than 1% (Figure 3.1).

NSAIDs were detected in all the leachate water samples collected from every irrigation event, indicating a vertical movement of these compounds through the soil

profile (Table 3.2). The NSAID recoveries in the leachate were not significantly affected by the two-way interaction of Time x ATB amendment ( $p>0.05$ ). Recovery of NPX was significantly affected by ATB amendment only, while both IBF and KTF were affected by Time ( $p<0.05$ ). However, the total mass of NPX, IBF, and KTF detected in the leachate, from all the plots over all irrigation events, were very low, averaging only 0.03% (72  $\mu\text{g}$ ), 0.02% (46  $\mu\text{g}$ ), and 0.04% (93  $\mu\text{g}$ ) of the initial spiking mass (1.142 g) to the surface soil, respectively, or 0.06  $\mu\text{g l}^{-1}$ , 0.038  $\mu\text{g l}^{-1}$ , and 0.077  $\mu\text{g l}^{-1}$ , by concentration, respectively (Table 3.2).

Table 3.1. Repeated measures analysis of variance for the effects of Time, Depth, and ATB amendment on recovery of naproxen (NPX), ibuprofen (IBF), and ketoprofen (KTF) measured in soil. The p values are shown in the table. Significant effects at  $p < 0.05$  are shown in bold.

Source	NPX	IBF	KTF
Time	0.000	0.000	0.000
Depth	0.000	0.000	0.000
ATB amendment	0.000	0.002	0.000
Time x Soil Depth	<b>0.000</b>	<b>0.003</b>	<b>0.000</b>
Time x ATB amendment rate	<b>0.003</b>	<b>0.023</b>	<b>0.000</b>
Depth x ATB amendment rate	0.070	0.073	<b>0.026</b>
Time x Depth x ATB amendment	0.300	0.584	0.411

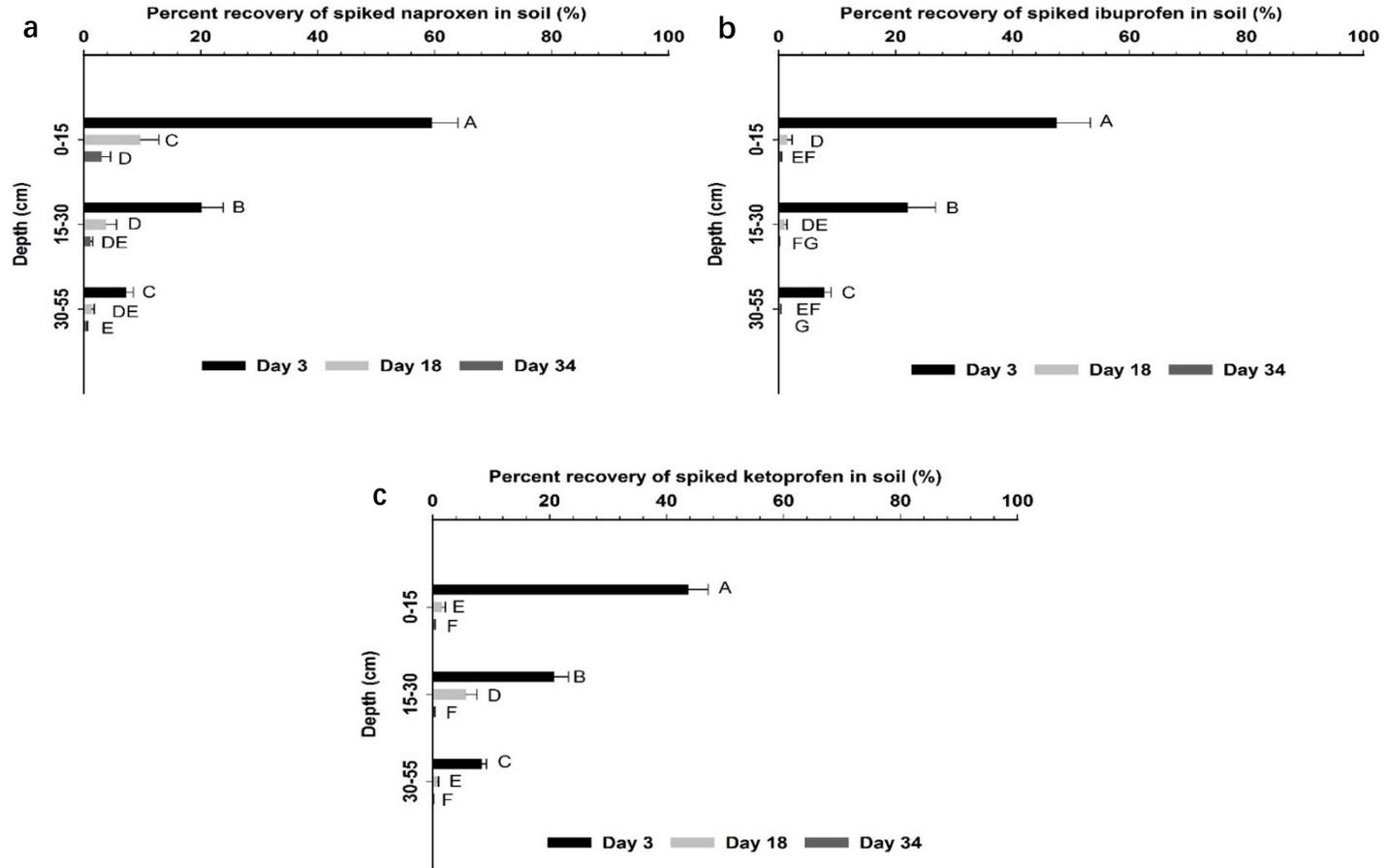


Figure 3.1. Interaction of soil depth and time on the recovery of a) Naproxen (NPX); b) Ibuprofen (IBF); and c) Ketoprofen (KTF); in the soil from lysimeter cells. Vertical bars represent standard deviation (n=9). Means followed by the same letter are not significantly different ( $p < 0.05$ ).

A mass balance of each NSAID was calculated by summing the compound residue in the soil on day 34 and the mass of NSAIDs in the leachate recovered over the irrigation events and subtracting from the initial spiking mass. The results showed that approximately 95% of initial spiked NPX and over 99% of IBF and KTF were not recovered from the soil or drainage water, regardless of treatments. The fate of NSAIDs in soils is affected by various processes, such as volatilization, hydrolysis, sorption, and degradation. According to the estimated low vapor pressure, Henry's Law constant, and chemical structure of individual NSAIDs, volatilization into the air or hydrolysis were not expected to take place in this study (Table 2.1). Both NPX and KTF were reported to be moderately/strongly sorbed to OM once being introduced into soil, while weak sorption behavior has been reported for IBF in various soils (Biel-Maeso et al., 2019; Lin and Gan, 2011; Xu et al., 2009a, 2009b). The small percentage of NSAIDs recovered from the soil at the end of the present study indicates that sorption may have had a limited effect on the fate of these target chemicals in the field-based lysimeter cells. Shu et al. (2020) conducted a laboratory-based incubation study for NPX, IBF, and KTF in the soil sampled from the same lysimeter cells used in this study and found the disappearance of NSAIDs all followed first-order kinetics, with biodegradation as the primary loss mechanism. Based on the half-lives obtained for the target NSAID from Shu et al. (2020), the predicted disappearance in the lysimeter cells, to a depth of 15 cm over 34 days, were calculated to be 83% for NPX, 98% for IBF, and 59% for KTF, respectively. This field-based study was conducted in a soil system that would be considered more biologically dynamic than under laboratory conditions. The half-lives of the target NSAIDs calculated by Shu et al. (2020) were based on 15 cm of soils, while the

lysimeter cells used in this study have a soil depth of 55 cm, and the irrigation events during the experiment were expected to exceed the soil field capacity and generate drainage. The deeper depth of soil profile could generate a temporary shift from aerobic to anaerobic conditions due to decreased oxygen availability (Wright et al., 2012). Nazhakaiti et al. (2019) found NPX showed an enhanced dissipation under anaerobic conditions in Asian rice paddy soils, which makes anaerobic degradation a potential disappearance pathway for NPX in the lysimeter cell. Overall, it can be concluded that biodegradation played an important role in the disappearance of selected NSAIDs in the field-based lysimeter cells.

In our study, only a small proportion of NSAIDs were recovered in the leachate samples. This result supports our previous finding that these NSAIDs would be expected to have low mobility within agricultural soils. The  $\log K_{ow}$  values of NPX and IBF shown in Table 2.1 were greater than 3.18, which indicate lower mobility potential in the soil (Sabourin et al., 2009). KTF had a slightly higher transport than the other two compounds but, overall, these NSAIDs revealed little transport potential in the sandy loam soil used in this study.

Table 3.2. Mean recovery (100% of initial spiked NSAIDs) of naproxen (NPX), ibuprofen (IBF), and ketoprofen (KTF) measured in leachate across all plots. Data for recovery are presented as Mean  $\pm$  SD (n=9). Means followed by the same letter in a column are not significantly different ( $p < 0.05$ ).

<b>Day</b>	<b>NPX</b>	<b>IBF</b>	<b>KTF</b>
<b>2</b>	0.0072 $\pm$ 0.0015 A	0.0070 $\pm$ 0.0015 A	0.0110 $\pm$ 0.0020 A
<b>8</b>	0.0060 $\pm$ 0.0015 A	0.0037 $\pm$ 0.0007 B	0.0106 $\pm$ 0.0019 A
<b>14</b>	0.0082 $\pm$ 0.0014 A	0.0069 $\pm$ 0.0011 A	0.0116 $\pm$ 0.0015 A
<b>24</b>	0.0066 $\pm$ 0.0016 A	0.0022 $\pm$ 0.0007 BC	0.0059 $\pm$ 0.0010 B
<b>33</b>	0.0035 $\pm$ 0.0012 B	0.0003 $\pm$ 0.0002 C	0.0013 $\pm$ 0.0004 B
Time	0.190	<b>0.000</b>	<b>0.000</b>
ATB amendment	<b>0.009</b>	0.151	0.261
Time x ATB amendment	0.389	0.856	0.664

### **3.3.2 Effect of ATB amendment rates on the transport of NSAIDs in the lysimeter cells**

The effect of the ATB amendment rates on the fate of NSAIDs in the soil varied across the compounds and a two-way interaction of Time x ATB amendment rate was also found to be significant (Table 3.1). NPX residual concentrations measured in the soil on Day 3, at both rates of ATB, were not significantly different from each other. However, the NPX residual concentrations measured in 7 and 28 Mg ATB ha<sup>-1</sup> amended plots were 17.8% and 31.4% higher than the mass measured in the control plots, respectively (Figure 3.2 a). In the present study, the soil OM content in the lysimeter cells were significantly affected by the two-way interaction of soil depth x ATB amendment ( $p < 0.05$ ). On day 3, the lysimeter cells amended with 28 Mg ha<sup>-1</sup> of ATB in the depth of 0-15 cm had highest OM content measured (4.71%), which was 0.91% and 0.71% higher than the control and 7 Mg ATB ha<sup>-1</sup> plots at the same depth, respectively. At a soil depth of 15-30 cm, the ATB amended plots resulted in a significantly higher OM content than the control plots, while no significant differences of OM content were found among three treatments in the 30 cm to 55 cm depth samples. This result indicates that the higher recovery of NPX measured in ATB amended lysimeter cells may be attributed to the increased OM content of soils due to biosolids application. This may have enhanced sorption of the compounds to the OM matrix, decreasing the mobility of NSAIDs, as demonstrated in other arable soil studies (Borgman and Chefetz, 2013; Grossberger et al., 2014; Li et al., 2013; Wu et al., 2009). The fate of NSAIDs in the ATB amended plots may also be affected by the change of soil pH in this study. The lysimeter cells amended with 7 and 28 Mg ha<sup>-1</sup> of ATB neutralized the acidic control soil (pH = 5.10) to a pH of 6.82 and 7.11,

respectively. Based on the pH of soils in this study, all three NSAIDs would be negatively charged in all the lysimeter plots since they have  $pK_a$  values lower than 5. The change in soil pH induced by the amended ATB rates alters the balance of mineral cations present and affects the sorption behavior of NSAIDs (Bui and Choi, 2010). For example, iron (Fe) would mainly be present as  $Fe(OH)_2^+$ , based on the pH, in the ATB amended plots. Higher concentrations of  $Fe(OH)_2^+$  have been reported to offer more simple ligand exchange sites that could enhance the sorption of negatively charged compounds like NPX and IBF (Vulava et al., 2016). Although ATB amendments in the lysimeter plots decreased the mobility of NPX on day 3, the retardation effect due to ATB amendments was temporary and no significant differences of measured NPX residual in the soil were observed between ATB amendment treatments and control plots on day 34 (Figure 3.2 a). For NPX, the leached mass detected in the 7 and 28  $Mg\ ha^{-1}$  of ATB amended plots were not significantly different from each other over time. In contrast, the mass measured in the control plots was 2.5 times lower than both rates of ATB amended treatments. The mass of NPX measured in the leachate samples was very low, ~ a thousand-fold lower than the acute toxicological endpoint of  $4900\ \mu g\ L^{-1}$  reported by (Quinn et al., 2008), which indicates an insignificant risk of acute biological effects as a result of leaching of the NPX in all lysimeter cells.

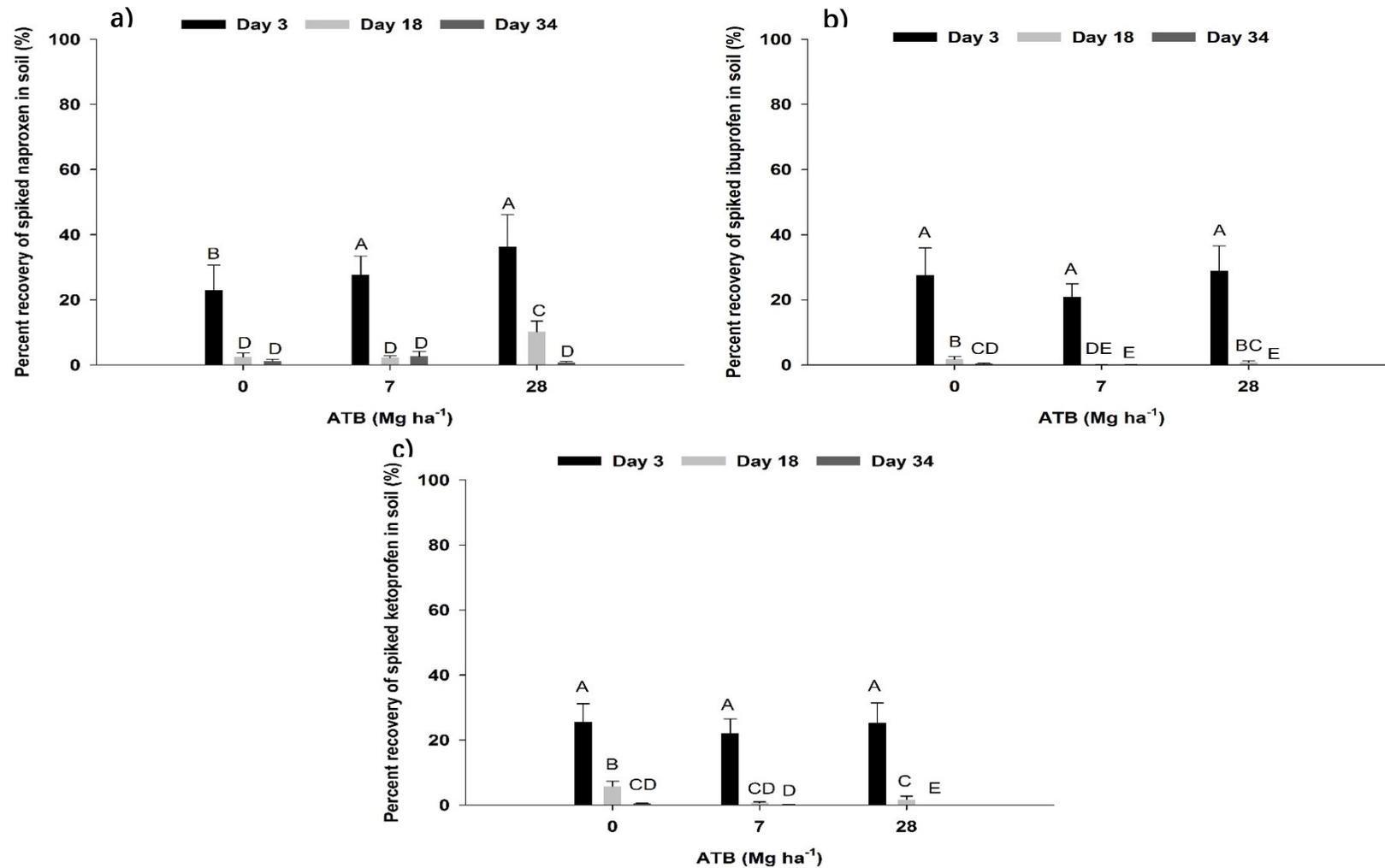


Figure 3.2. Interaction of ATB amendment rate and time on the recovery of a) Naproxen (NPX); b) Ibuprofen (IBF); and c) Ketoprofen (KTF); in the soil from lysimeter cells. Vertical bars represent standard deviation (n=9). Means followed by the same letter are not significantly different ( $p < 0.05$ ).

The recoveries of IBF and KTF were not significantly different among treatments on day 3 (Figure 3.2 b; c). This result was expected since all three NSAIDs were applied as a mixture under field conditions and a previous incubation study conducted by Shu et al. (2020) found ATB amendments had no effect on the dissipation rate of IBF and KTF in a mixture compound environment. On day 34, the lowest recoveries were determined in the 28 Mg ha<sup>-1</sup> ATB amended soils (Figure 3.2 b; c). However, the ATB treatment effect observed was negligible since the mass of IBF and KTF recovered on day 34 were insignificant. Similar to the results observed in the soil, the mass of IBF and KTF leached out of the ATB amended plots were not significantly different from the mass detected in control plots (Table 3.2). Therefore, these results indicated ATB amendment at the rates of 7 and 28 Mg ha<sup>-1</sup> only had a minor effect on the fate and transport of IBF and KTF in the loamy sand textured agricultural soil.

### **3.4 Conclusion**

This study performed the field-based lysimeter tests to evaluate the fate and transport potential of three commonly used NSAIDs (NPX, IBF, and KTF) in a sandy loam textured soil amended with three different rates of ATB. All three NSAIDs in the lysimeter cells showed some vertical migration within the soil profile after spiking, but only very low concentrations of these compounds were recovered in the leachate samples collected from each artificial irrigation event. There were also only small accumulations of target NSAIDs in soil 34 days after spiking. Both the 7 and 28 Mg ha<sup>-1</sup> of ATB application treatments increased the soil OM content and neutralized soil pH in the lysimeter cells, while results from soil and leachate samples indicated that both rates of ATB application had no or minor effects on the mobility of these substances in the soil.

Overall, the field-based leaching study clearly illustrated that these NSAIDs could be characterized as possessing low mobility in loamy sand textured agricultural soil under the environmental exposure and experimental conditions adopted.

## **Chapter 4 Simulating fate and transport of non-steroidal anti-inflammatory drugs (NSAIDs) in field-based lysimeter cells using Root Zone Water Quality Model 2 (RZWQM2)**

### **4.1 Introduction**

The development of process-based agricultural system models over the last 3 decades offers a time-saving and cost-effective alternative to the traditional field studies in studying the fate and transport of organic contaminants in the environment under different management practices (Ma et al., 2007). Application of these models allow researchers to evaluate complex interactions among physical, chemical, and biological processes in different agricultural systems and to test phenomena that are difficult to be experimentally examined in field studies. Agricultural system models also provide an opportunity for researchers to extend simulation results spatially and temporally (Ma et al., 2007).

The Root Zone Water Quality Model 2 (RZWQM2) is a comprehensive one-dimensional, numerical agricultural systems model developed by the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) that integrates physical processes, soil chemical processes, nutrient process, pesticide processes, plant growth processes, and management processes to simulate the effects of agricultural management on crop production and environmental quality (Ma et al., 2012). The pesticide module of the RZWQM2 was developed to describe various critical processes, such as plant uptake, sorption/desorption, and degradation, for simulating the fate and transport of organic chemicals in the soil profile (Wauchope et al., 2004). Several studies

have shown that RZWQM2 was able to simulate the fate and transport of pesticide and herbicides under different field conditions and management practices with reasonable results (Kumar et al., 2004; Ma et al., 1995, 2004a; Malone et al., 2004a, 2004b). For instance, Ma et al. (1995) reported that RZWQM effectively simulated the water runoff, atrazine runoff, atrazine persistence, and atrazine distribution in the soil profile. Malone et al. (2004b) also suggested that RZWQM (version 1.0.2000.929), correctly calibrated for macropore flow and soil hydrology, could accurately predict metribuzin transport in a silt loam soil using the instantaneous equilibrium-only pesticide model.

Since pesticide and pharmaceuticals undergo a similar registration process and share various commonalities like chemical properties, the pesticide module of the RZWQM2 is expected to be a potential tool to develop a better understand of the fate and transport of pharmaceuticals like NSAIDs in agricultural soils and soils amended with biosolids (Swanton et al., 2011). The objective of the present study was to evaluate the RZWQM2 for simulating the fate of three NSAIDs (naproxen (NPX), ibuprofen (IBF), and ketoprofen (KTF)) in soil and drainage water samples in field-based lysimeter cells receiving three soil amendment treatments (soil, soil + 7 Mg ha<sup>-1</sup> alkaline treated biosolids (ATB), and soil + 28 Mg ha<sup>-1</sup> ATB) over 34 days in 2018. The RZWQM2 was hypothesized to be able to successfully simulate the water seepage and concentration of the target NSAIDs in soil and water samples in the lysimeter cells amended with different rates of ATB.

## **4.2 Materials and Methods**

### **4.2.1 Input data for RZWQM2**

The following section summarizes the major input parameters required by RZWQM2 version 4.2 to simulate the fate and transport of NSAIDs through the soil profiles of three different lysimeter cells, including Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB). Details of the field-based lysimeter cell study were described in Chapter 3. In the present study, one lysimeter cell from each treatment was selected for modeling purposes.

#### **4.2.1.1 Meteorology Data**

The RZWQM2 requires users to input either daily or hourly meteorology data to run the model. This study used hourly meteorology data between January 1<sup>st</sup>, 2015 and December 31<sup>st</sup>, 2018. Hourly data for air temperature, wind speed, shortwave radiation, and relative humidity, were obtained from the Meteorological Service of Canada (Environment and Climate Change Canada, 2022) for the Debert weather station (Nova Scotia, Canada). For the Debert weather station, pan evaporation and photosynthetically active radiation (PAR) were not available and were estimated by the model. The hourly rainfall data were collected from the BEEC where the lysimeter cells are located. However, hourly rainfall data between November 26<sup>th</sup>, 2017 and May 25<sup>th</sup>, 2018 were lost due to an issue with the data logger at the research site where the lysimeters are located. Therefore, data for this period were obtained from the Debert weather station instead. Breakpoint rainfall data for 2015, 2016, 2017, and 2018 were then calculated from hourly rainfall data and the rainfall was organized into 833 individual storm events based on the information on rainfall intensity (minimum intensity was set for 0.1 cm h<sup>-1</sup>,

maximum intensity was set for 10 cm h<sup>-1</sup>) and duration (600 minutes).

#### **4.2.1.2 Soil and water characteristics data**

The soil profile of each lysimeter cell was divided into three horizons (0-15 cm depth, 15 cm-30 cm depth, and 30 cm-55 cm depth) in the RZQWM2. The soil physical properties, such as particle density, bulk density, porosity, fraction sand, fraction silt, and fraction clay, were measured for each lysimeter cell in 2014 and used as input for the model. For each soil horizon, soil hydraulic parameters, such as saturated hydraulic conductivity ( $K_{sat}$ ), 1/3 bar, 15 bar, and residual water content, were measured in 2014 and specified in the model. The RZWQM2 uses Brooks-Corey equations to describe soil water retention curves and the Brooks-Corey parameters for each soil horizon were estimated using measured 1/3 bar and 15 bar water content in the model. All pores in each soil horizon of each selected lysimeter cell were assumed as micropores and no macropore flow was assumed in the model. Chemical properties (pH, major cation, and ions) of rainwater and irrigation water used as artificial rainfall that was applied to the lysimeter cells were also measured for samples collected in 2018 and specified in the model. Data on selected soil physico-chemical characteristics, soil hydraulic parameters, and chemical properties of rainwater and irrigation water are given in Table 4.1 and Table 4.2.

Table 4.1. Soil physico-chemical characteristics (dry weight basis) and chemical properties of rainwater and irrigation water used as input in the RZWQM2 for Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB).

	Depth (cm)	pH (pH Units)	CEC (meq 100g <sup>-1</sup> )	OM (%)	Sand (%)	Silt (%)	Clay (%)	Fraction Exchangeable Ca Ions	Fraction Exchangeable Na Ions	Fraction Exchangeable Mg Ions
Cell 6	0-15	5.28	6.00	3.78	67.67	26.33	6.00			
	15-30	5.22	5.70	3.33	67.67	26.33	6.00	0.52	0.00	0.09
	30-55	5.19	5.10	3.39	72.33	23.67	4.00			
Cell 3	0-15	7.06	21.20	3.89	66.33	26.33	7.33			
	15-30	6.52	16.70	3.32	66.33	26.33	7.33	0.95	0.00	0.02
	30-55	5.90	5.20	3.33	68.33	25.67	6.00			
Cell 4	0-15	7.35	36.80	4.65	67.67	23.00	9.33			
	15-30	7.10	23.40	3.63	67.67	23.00	9.33	0.97	0.00	0.02
	30-55	6.18	15.60	3.51	67.00	25.67	7.33			
		pH (pH Units)	Ca (ppm)	Na (ppm)	Mg (ppm)	Cl (ppm)	HCO <sub>3</sub> (ppm)	SO <sub>4</sub> (ppm)	Al (ppm)	CO <sub>3</sub> (ppm)
<b>Rain Water</b>		5.70	0.56	1.46	0.10	2.15	0.00	1.65	0.00	0.00
<b>Irrigation Water</b>		7.97	41.00	4.28	3.35	3.00	0.00	41.06	0.00	0.00

Table 4.1 (cont.). Soil physico-chemical characteristics (dry weight basis) and chemical properties of rainwater and irrigation water used as input in the RZWQM2 for Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB).

	Depth (cm)	Fraction Exchangeable NH <sub>4</sub> Ions	Fraction Exchangeable Al Ions	Ca (ug g <sup>-1</sup> soil)	Na (ug g <sup>-1</sup> soil)	Mg (ug g <sup>-1</sup> soil)	Cl (ug g <sup>-1</sup> soil)	HCO <sub>3</sub> (ug g <sup>-1</sup> soil)	SO <sub>4</sub> (ug g <sup>-1</sup> soil)	Al (ug g <sup>-1</sup> soil)
Cell 6	0-15									
	15-30	0.00	0.00	623.00	8.00	61.00	0.00	0.00	15.10	829.00
	30-55									
Cell 3	0-15									
	15-30	0.00	0.00	8062.00	20.00	81.00	0.00	0.00	66.00	1533.00
	30-55									
Cell 4	0-15									
	15-30	0.00	0.00	14228.00	30.00	144.00	0.00	0.00	250.00	1446.00
	30-55									
		NH <sub>4</sub> (ppm)	NO <sub>3</sub> (ppm)							
	Rain Water	0.50	1.00							
	Irrigation Water	0.00	0.00							

Table 4.2. Soil physical and hydraulic parameters from field measurement and optimized from manual calibration in the RZWQM2 for Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB) in the lysimeter study.

		Depth (cm)	Bulk density (g cm <sup>3</sup> )	Porosity (g cm <sup>-2</sup> )	Particle density (g cm <sup>-2</sup> )	Ksat (cm h <sup>-1</sup> )	1/3 bar Water Content (cm <sup>3</sup> cm <sup>-3</sup> )	15 bar Water Content (cm <sup>3</sup> cm <sup>-3</sup> )	Residual Water Content (cm <sup>3</sup> cm <sup>-3</sup> )
<b>Soil physical and hydraulic parameters from field measurement</b>	<b>Cell 6</b>	<b>0-15</b>	1.304	0.508	2.650	1.247	0.221	0.064	0.050
		<b>15-30</b>	1.304	0.508	2.650	1.247	0.221	0.064	0.050
		<b>30-55</b>	1.347	0.492	2.650	1.099	0.263	0.061	0.044
	<b>Cell 3</b>	<b>0-15</b>	1.390	0.476	2.650	1.260	0.232	0.070	0.051
		<b>15-30</b>	1.390	0.476	2.650	1.260	0.232	0.070	0.051
		<b>30-55</b>	1.414	0.466	2.650	0.601	0.268	0.061	0.051
	<b>Cell 4</b>	<b>0-15</b>	1.362	0.486	2.650	1.025	0.233	0.077	0.057
		<b>15-30</b>	1.362	0.486	2.650	1.025	0.233	0.077	0.057
		<b>30-55</b>	1.386	0.477	2.650	0.659	0.259	0.061	0.046
<b>Soil physical and hydraulic parameters optimized from manual calibration</b>	<b>Cell 6</b>	<b>0-15</b>	1.440	0.457	2.650	1.350	0.228	0.080	0.055
		<b>15-30</b>	1.450	0.453	2.650	1.351	0.240	0.100	0.050
		<b>30-55</b>	1.470	0.445	2.650	0.750	0.248	0.170	0.048
	<b>Cell 3</b>	<b>0-15</b>	1.390	0.475	2.650	1.340	0.225	0.110	0.051
		<b>15-30</b>	1.400	0.472	2.650	1.280	0.245	0.070	0.051
		<b>30-55</b>	1.460	0.449	2.650	0.800	0.250	0.165	0.051
	<b>Cell 4</b>	<b>0-15</b>	1.360	0.487	2.650	1.285	0.228	0.110	0.057
		<b>15-30</b>	1.400	0.472	2.650	1.225	0.249	0.091	0.057
		<b>30-55</b>	1.440	0.457	2.650	0.900	0.260	0.170	0.046

#### **4.2.1.3 Initial system state**

The RZWQM2 requires users to input initial soil moisture content and soil temperature, soil chemistry state, and pesticide state for each soil horizon defined. For the lysimeter experiment site, initial volumetric water content (0.332) and soil temperature (0 °C) at the beginning of the simulation were estimated based on the temperature and precipitation data available on January 1<sup>st</sup>, 2015 and assumed the same for all three soil layers of each lysimeter cell. The input of the initial soil equilibrium chemistry, such as soil pH and cation exchange capacity (CEC), and concentrations of major cations and anions ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{=}$ ,  $\text{Al}^{3+}$ , and  $\text{CO}_3^{=}$ ) for each soil horizon were based on measurements of each soil horizon in each individual lysimeter cells sampled in 2018. (Table 4.1). Soil test results for soil sampled from each individual lysimeter cell indicated that no NSAIDs residuals were measured at the beginning of the field experiment in 2018. Therefore, the initial pesticide state was assigned as 0 in the RZWQM2 in this study. The initial residue profile in the soil was estimated using the initialization wizard function built in the RZWQM2. Input of the measured values of soil organic matter (OM) content in each soil horizon in the lysimeter cell sampled from 2018 was used to calculate the soil organic carbon (OC) content based on the model default conversion factor for OM to OC of 0.58 (Table 4.1). The total organic carbon (TOC) amount in each soil layer was assumed to split into 10% of fast humus pools, 10% of intermediate humus pool, and 80% of slow humus pool based on the manual suggestion.

#### **4.2.1.4 Management practices**

The physical and chemical characteristics of ATB applied to soils in the field-based lysimeter cell study were described in the RZWQM2 using the Manure option under

Management Options (Table 3.2). The ATB was surface broadcasted on May 29<sup>th</sup>, 2018 and described as organic waste with 61.84% water content, 12.5% carbon (C), and a C:nitrogen (N) ratio of 18.12. Inputs of the amount of ATB applied to cell 6, cell 3, and cell 4 in the model were 0 kg ha<sup>-1</sup>, 7 kg ha<sup>-1</sup>, and 28 kg ha<sup>-1</sup>, respectively. To simulate the fate and transport of NSAIDs in the RZWQM2, the compounds were defined as the user-specified pesticide in the pesticide sub-model with inputs of molecular weight, Henry's law constant, degradation half-lives, and sorption and desorption parameters. Initial inputs of soil subsurface aerobic and abiotic half-lives of each NSAID were obtained from a laboratory-based incubation study described in Chapter 2. The sorption constant for soil organic matter ( $K_{oc}$ ) value of each compound was calculated based on the  $K_d$  value measured by Zhang et al. (2017) divided by the average soil OC content of each lysimeter cell. Data on selected chemical properties of target NSAIDs are listed in Table 4.3.

Table 4.3. Chemical parameters of the NSAIDs used in the pesticide submodel of the RZWQM2 from lab-based experiment and literature and optimized from manual calibration for Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB).

Compound	Cells	Molecular Weight (g mol <sup>-1</sup> )	Water solubility (mg L <sup>-1</sup> )	Vapor Pressure (mm Hg at 25 °C)	Henry's law constant (atm-m <sup>3</sup> /mole at 25 °C)	Degradation		
						Soil Subsurface Aerobic Half-life (d)	Soil Subsurface Abiotic Half-life (d)	
NSAIDs parameters estimated from lab-based experiment and literature	Naproxen	Cell 6					13.4	105
		Cell 3	230.263	15.9	1.89x10 <sup>-6</sup>	3.39x10 <sup>-10</sup>	14.1	216.6
		Cell 4					14.1	216.6
	Ibuprofen	Cell 6					6.1	73.7
		Cell 3	206.285	21	4.74x10 <sup>-5</sup>	1.5x10 <sup>-7</sup>	6	138.6
		Cell 4					6	138.6
	Ketoprofen	Cell 6					35.4	119.5
		Cell 3	254.285	51	3.72x10 <sup>-7</sup>	2.12x10 <sup>-11</sup>	32.4	169.1
		Cell 4					32.4	169.1
NSAIDs parameters optimized from manual calibration	Naproxen	Cell 6					4.6	87.7
		Cell 3	230.263	15.9	1.89x10 <sup>-6</sup>	3.39x10 <sup>-10</sup>	5.2	87.7
		Cell 4					5.6	87.7
	Ibuprofen	Cell 6					2.6	95.7
		Cell 3	206.285	21	4.74x10 <sup>-5</sup>	1.5x10 <sup>-7</sup>	2	95.7
		Cell 4					2.6	95.7
	Ketoprofen	Cell 6					3.3	130
		Cell 3	254.285	51	3.72x10 <sup>-7</sup>	2.12x10 <sup>-11</sup>	3	130
		Cell 4					3.6	130

Table 4.3 (cont.). Chemical parameters of the NSAIDs used in the pesticide submodel of the RZWQM2 from lab-based experiment and literature and optimized from manual calibration for Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB).

	Compound	Cells	Sorption		
			pK <sub>a</sub>	Log K <sub>ow</sub>	Koc (cc g <sup>-1</sup> )
NSAIDs parameters estimated from lab- based experiment and literature	Naproxen	Cell 6	4.15	3.18	3.19
		Cell 3	4.15	3.18	3.18
		Cell 4	4.15	3.18	2.84
	Ibuprofen	Cell 6	4.91	3.97	1.67
		Cell 3	4.91	3.97	1.67
		Cell 4	4.91	3.97	1.49
	Ketoprofen	Cell 6	4.45	3.12	2.36
		Cell 3	4.45	3.12	2.35
		Cell 4	4.45	3.12	2.10
NSAIDs parameters optimized from manual calibration	Naproxen	Cell 6	4.15	3.18	3.30
		Cell 3	4.15	3.18	3.30
		Cell 4	4.15	3.18	3.10
	Ibuprofen	Cell 6	4.91	3.97	1.67
		Cell 3	4.91	3.97	1.67
		Cell 4	4.91	3.97	1.49
	Ketoprofen	Cell 6	4.45	3.12	2.36
		Cell 3	4.45	3.12	2.10
		Cell 4	4.45	3.12	2.30

#### **4.2.1.5 RZWQM2 calibration, validation, and evaluation**

The RZWQM2 of each lysimeter cell was initially parameterized with inputs described in section 4.2.1 then optimized through manual calibration and evaluated by comparing model simulated and experimental daily water seepage out of the soil profiles data collected in 2017 and 2018 from three different lysimeter cells with three different ATB amendments. The model simulation was started on January 1<sup>st</sup>, 2015 before the actual calibration began on June 15<sup>th</sup>, 2017 to have a warm-up period to stabilize the initial C and N pools (Abrahamson et al., 2005; Hanson et al., 1999). The daily water seepage out of the soil profile data collected between June 15<sup>th</sup>, 2017 and October 29<sup>th</sup>, 2017 was used as the calibration dataset and the data measured between June 1<sup>st</sup>, 2018 and September 26<sup>th</sup>, 2018 was used as the validation dataset. Sensitivity analysis was conducted before the model calibration on selected soil hydraulic and physical parameters based on 10% variation of the base value for the soil water balance module (Ma et al., 2004c). The 1/3 bar water content in the 0-15 cm layer, 15 bar water content in the 30-55 cm layer, and soil bulk density were determined to be the most sensitive parameters in the simulation of daily water seepage out of the soil profiles in this study. Therefore, manual model calibration was focused mainly on the most sensitive parameters of interest for all three lysimeter cells simulated in the model using the trial-and-error method. For model validation, calibrated parameters for each lysimeter cell in 2017 were kept the same and used to simulate the daily water seepage out of the soil profile in 2018 and compared with the validation dataset.

The performance of the calibrated and validated model to simulate daily water seepage out of the soil profile in this study was evaluated based on the statistic of Nash-Sutcliffe model efficiency (NSE), the linear regression coefficient of determination ( $R^2$ ),

and percentage bias (PBIAS) as suggested by (Ma et al., 2012). In this study, the performance of the simulation model was considered acceptable with  $NSE > 0.7$ ,  $R^2 > 0.8$ , and  $-15\% < PBIAS < 15\%$  (Ma et al., 2012).

The NSE was calculated with:

$$NSE = 1.0 - \frac{\sum_{i=1}^N (O_i - P_i)^2}{\sum_{i=1}^N (O_i - \bar{O})^2} \quad (1)$$

where  $O_i$  and  $P_i$  are the observed and the model-simulated data, respectively,  $\bar{O}$  is the averaged observed value, and  $N$  is the number of observations. The  $R^2$  was calculated with:

$$r^2 = \frac{[\sum_{i=1}^N (O_i - \bar{O})(P_i - \bar{P})]^2}{\sum_{i=1}^N (O_i - \bar{O})^2 \sum_{i=1}^N (P_i - \bar{P})^2} \quad (2)$$

where  $\bar{P}$  is the averaged simulated value. The PBIAS was calculated with:

$$PBIAS = \frac{\sum_{i=1}^N (P_i - O_i) \times 100}{\sum_{i=1}^N (O_i)} \quad (3)$$

Once the models were calibrated and validated for the soil-water balance in each lysimeter cell, the calibrated models were applied to simulate the fate and transport of target NSAIDs in the soil profile and compared with the measured recovery of spiked NSAIDs in soil and water samples collected in 2018 as described in chapter 3. The pesticide module was calibrated mainly for soil subsurface half-life of the target NSAIDs based on the sensitivity analysis. The model simulated recovery of spiked NSAIDs in soil and water samples were compared with observed data and evaluated using PBIAS and the two-sample t-test at 95% of confidence interval in SAS v.9.4 (SAS Institute, Cary, NC).

## 4.3 Results and discussion

### 4.3.1 Simulation results of soil water balance

Figure 4.1 shows the measured and simulated daily water seepage out of the soil profile for calibration datasets in 2017 and validation datasets in 2018 for all three lysimeter cells. The RZWQM2 has built-in snowpack dynamics to account for soil and water balances in winter conditions. However, the model predicted the soil water dynamics poorly in 2017 by including the daily water seepage out of the soil profile simulated for winter with a PBIAS over 70% and NSE less than 0.1 for all three lysimeter cells. By excluding the winter conditions in this study, the calibrated models, based on the calibrated soil hydraulic and physical parameters of each lysimeter cell summarized in Table 4.2, were able to simulate the daily water seepage out of the soil profile for 2017 with greater accuracy. The PBIAS was -0.7%, 0.9%, and -8.7% for Cell 6, Cell 3, and Cell 4, respectively. The daily water seepage out of the soil profile in 2017 for the calibration dataset of Cell 6, Cell 3, and Cell 4 were also simulated with NSE equal to 0.869, 0.694, and 0.706, and an  $R^2$  equal to 0.881, 0.741, and 0.748, respectively (Table 4.4). The same calibrated models adequately predicted the daily water seepage out of the soil profile for 2018 with PBIAS of Cell 6, Cell 3, and Cell 4 equal to 2.4%, 0.5%, and -12.7%, respectively. The NSE and  $R^2$  for all three lysimeter cells were greater than 0.7 when the model simulated data were compared with the measured validation dataset (Table 4.4).

Table 4.4. Statistics for RZWQM2 model calibration in 2017 and validation in 2018 after optimization from manual calibration for simulated seepage for Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB) in the lysimeter study.

		<b>NSE</b>	<b>R<sup>2</sup></b>	<b>PBIAS</b>
<b>2017 Model Calibration</b>	<b>Cell 6</b>	0.869	0.881	-0.07%
	<b>Cell 3</b>	0.694	0.741	0.9%
	<b>Cell 4</b>	0.706	0.748	-8.7%
<b>2018 Model Validation</b>	<b>Cell 6</b>	0.728	0.766	2.4%
	<b>Cell 3</b>	0.769	0.816	0.5%
	<b>Cell 4</b>	0.723	0.793	-12.7%

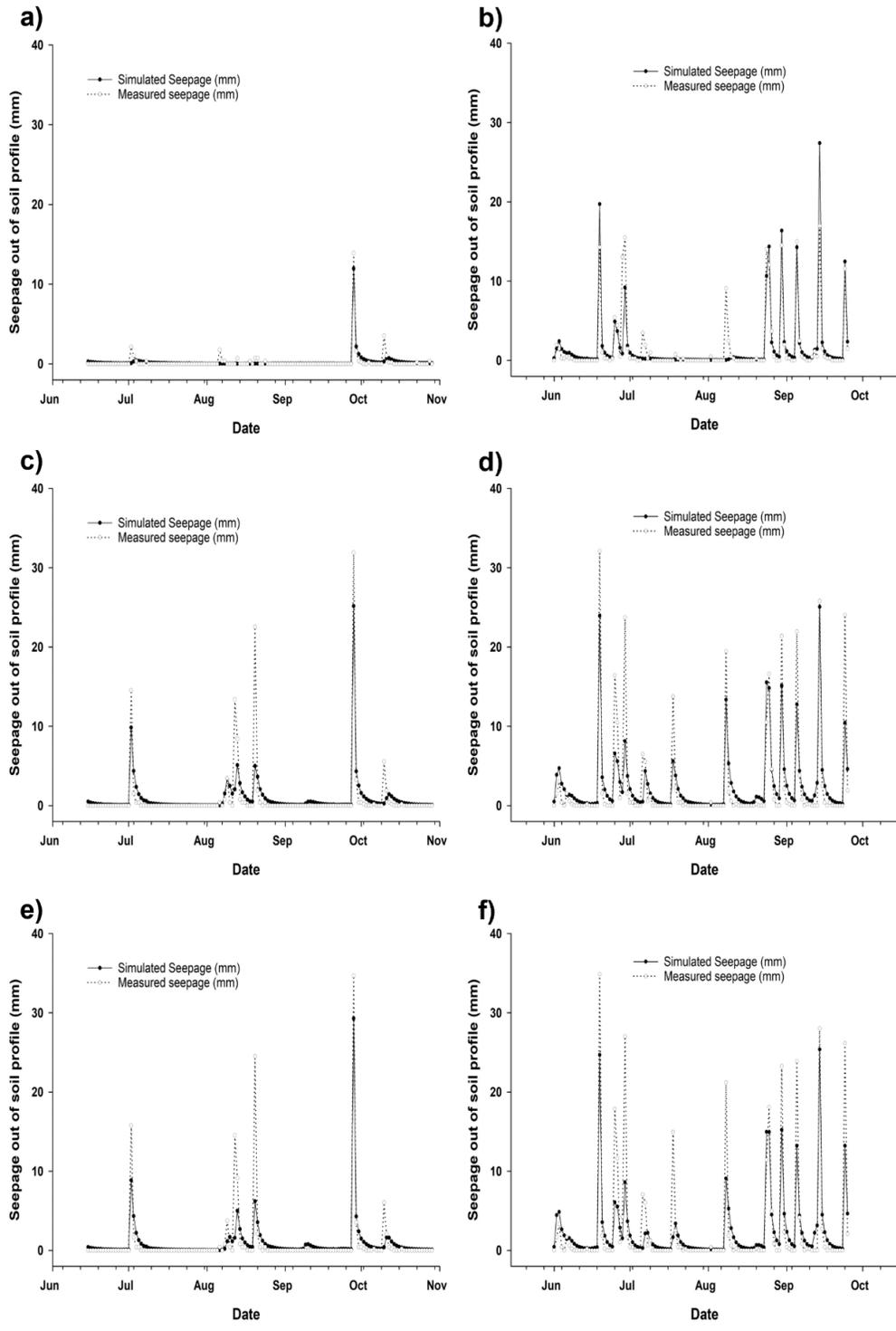


Figure 4.1. Measured vs simulated daily water seepage (mm) out of soil profile of a) Cell 6 2017 calibration model; b) Cell 6 2018 validation model; c) Cell 3 2017 calibration model; d) Cell 3 2018 validation model; e) Cell 4 2017 calibration model; and f) Cell 4 2018 validation model.

Although the overall differences between the measured and simulated data for the calibration dataset were within the 15% range, RZWQM2 underestimated most of the rainfall events in this study (Figure 4.1 a, c, and e). The field measured data showed that no water drained out of the soil profile when there were no rainfall events. The RZWQM2 calculated water redistribution between each rainfall or irrigation event using the Richard's equation and a small amount of water was simulated to seep out of the soil profile daily during the simulation period. This may result in a simulated water seepage lower than the measured data during the rainfall events. For the validation datasets in 2018, the simulated daily water seepage for all five artificial irrigation events conducted between August 26 and September 26 were closely matched to the measured data (Figure 4.1 b, d, and f). For this study, the initial system states of model simulation described in section 4.2.1.3 for three soil horizons were estimated based on a limited available measured dataset. A 2 to 6 years warm-up period for the model simulation has been determined as an important initialization procedure for models with estimated initial conditions that could significantly improve the performance of model simulation in multi-year runs (Hanson et al., 1999). Therefore, the better simulation performance observed during the field-lysimeter cell experiments is attributed to the longer warm-up period (3.5 years) for the 2018 simulation. The overall performance of the model simulation for soil water balance could be further improved with more measured data for the experimental site. For example, evapotranspiration could affect the soil water content simulation and the amount of water available that could drain out of the soil profile (Malone et al., 2004a). However, neither incoming short-wave radiation nor pan evaporation data were available as input in the hourly meteorological data and were, consequently, estimated by the RZWQM2. In this

study, all the lysimeter cells were assumed to not possess macropores, since data on macroporosity in the soil profiles were not available (Ma et al., 2012). In reality, macropores could form through structural cracks and worm or root holes in normal structured soils and act as preferential pathways that would divert some runoff water generated on the soil surface during the rainfall events rapidly down the soil profile (Hanson et al., 1999; Kumar et al., 1999). Simulation accuracy of daily water seepage out of the soil profile in the lysimeter cells may be improved with macropore flow components running in the RZWQM2.

#### **4.3.2 Simulation results of fate and transport of NSAIDs in the lysimeter cells**

Mass balance of the target NSAIDs simulated by RZWQM2 for each lysimeter cell summarized in Table 4.5 showed that 93.83%, 95.54%, and 94.38% of NPX disappeared from Cell 6, Cell 3, and Cell 4 through degradation, respectively, and less than 1.5 % of the compound leached out of the soil profile in all the lysimeter cells. The model also predicted that more than 99% of IBF and 97% of KTF were degraded in the soil during the simulation period with less than 0.35% of IBF and 0.7% of KTF lost due to seepage. These results are in the same range as the measured disappearance of the target NSAIDs in three lysimeter cells and indicate the performance of the model parameterized with calibrated NSAIDs parameters shown in Table 4.3 is adequate in this study. Although the soil adsorption of NSAIDs in the lysimeter cells was not measured, the small percentage of NSAIDs adsorbed to soil predicted by the model was reasonable since biodegradation has been determined as the most important mechanism for the losses of NPX, IBF, and KTF. The soil and field lysimeter study results shown in Chapter 3 indicated that sorption may have had a limited effect on the fate of these target chemicals in this study (Lin and Gan,

2011; Shu et al., 2020). It is worth noting that the soil subsurface aerobic half-life calibrated for both NPX and IBF in all three lysimeter cells was approximately 50% - 60% shorter than the laboratory-measured half-life from the incubation study conducted in Chapter 2 (Table 4.3). The calibrated soil subsurface aerobic half-life for KTF in all the cells was 10 times less than the measured half-life. The initial inputs of soil subsurface aerobic half-life for the target NSAIDs were obtained in the incubator maintained at 20°C with 18% gravimetric moisture content, while chemicals in different field lysimeter cells may be exposed to a more dynamic system that could result in the half-life of NSAIDs varying considerably between soils and with soil depth. The large difference between the calibrated and measured half-life of NSAIDs suggested the importance of developing a better understanding of how field conditions affect half-life to improve the model performance.

Table 4.5. Mass distribution (Percent of initial spiked concentration) of Naproxen (NPX), Ibuprofen (IBF), and Ketoprofen (KTF) in Cell 6 (Soil), Cell 3 (Soil+7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil+28 Mg ha<sup>-1</sup> ATB) during the simulation period using Root Zone Water Quality Model 2 (RZWQM2) for field-based lysimeter study

	Cell 6			Cell 3			Cell 4		
	NPX	IBF	KTF	NPX	IBF	KTF	NPX	IBF	KTF
<b>Total NSAIDs addition at start</b>	100%	100%	100%	100%	100%	100%	100%	100%	100%
<b>Losses:</b>									
Degradation within soil profile <sup>a</sup>	93.83%	99.18%	97.53%	95.54%	99.69%	98.85%	94.38%	99.38%	97.66%
Run off	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Seepage from soil matrix	1.46%	0.31%	0.66%	0.91%	0.26%	0.38%	1.07%	0.33%	0.63%
Total losses	95.29%	99.49%	98.18%	96.45%	99.95%	99.23%	95.45%	99.71%	98.30%
<b>Storage:</b>									
Surface residue layer	0.04%	0.03%	0.04%	0.05%	0.00%	0.05%	0.05%	0.05%	0.05%
Soil layer: solution pool	4.53%	0.46%	1.75%	2.85%	0.04%	0.66%	3.66%	0.25%	1.44%
Soil layer: adsorbed pool	0.16%	0.00%	0.04%	0.70%	0.01%	0.11%	0.88%	0.03%	0.26%
Total storage	4.70%	0.47%	1.79%	3.55%	0.05%	0.77%	4.55%	0.29%	1.70%

<sup>a</sup>The term 'degradation' used here was from RZWQM2 that includes volatilization, photolysis, hydrolysis, anaerobic and aerobic biodegradation of the targeted compounds in the model.

Comparison between the model simulated and field-measured recovery of initial spiked NPX, IBF, and KTF in the soil at three individual depths for three different lysimeter cells on day 3, day 18, and day 34 over the 34 days of field experiments conducted in 2018 fall are shown in Figure 4.2 and evaluated based on PBIAS in Table 4.6. Although the differences between the simulated total recovery for each NSAID at all soil depths of each sampling day and the measured total recovery were not significant in all three lysimeter cells ( $p < 0.05$ ), visual analysis of Figure 4.2 showed that the model underestimated the recovery of all the target NSAIDs in the 30-55 cm soil horizon on day 3 with the calculated PBIAS  $> -80\%$  (Table 4.6). The measured percent recovery of NSAIDs on day 3 in the 30-55 cm depth ranged between 11.5% and 22.4% for different compounds, which indicated the potential presence of macropores in the lysimeter cells leading to faster vertical movement of NSAIDs in the soil profile through preferential flow in the first artificial rainfall event. Preferential flow in macropores may be critical in describing chemical transport in the soil subsurface and the accuracy of model prediction in NSAIDs concentrations in the soil profiles may be improved by taking macropores into account (Azevedo et al., 1997; Malone et al., 2001). For day 18 and day 34, the simulated recovery of the target NSAIDs was in the same range as the measured recovery, while high PBIAS was observed for some compounds at certain depths. This may be attributed to the low measured percent recovery of NSAIDs ( $< 7.5\%$ ) in the soil profile at the last two sampling days, for which any small differences between the simulated and measured values could result in large percent differences. Figure 4.3 shows the model simulated recovery of initial spiked NPX, IBF, and KTF in water samples of three different lysimeter cells that were closely matched with field measured data within the same order of magnitude on most of

the sampling days. However, the RZWQM2 underestimated the percent recovery of initial spiked NSAIDs for all the compounds in all the cells on day 2, except for IBF in Cell 3. The higher percent recovery of NSAIDs measured on day 2 from the field experiment matched the higher recovery of NSAIDs measured in the 30-55 cm depth on day 3, which also indicated the possible presence of macropores in the lysimeter cells. Statistical analysis showed that the sum of simulated recovery of initial spiked NSAIDs in the water samples of each lysimeter cell were not significantly different from the measured data ( $p < 0.05$ ). The simulated and measured percent recovery of the target NSAIDs in water samples were all less than 0.08%, which could also result in the high calculated PBIAS shown in Table 4.7. Data measured from field experiments typically have errors greater than 10% and considering the difficulty in predicting the concentration of organic compounds in field conditions, the performance of RZWQM2 in simulating the recovery of target NSAIDs in different lysimeter cells at different soil depths and in water samples is considered satisfactory in this study. The simulated NSAIDs in the soil and water samples also showed a similar distribution among the three lysimeter cells with different amounts of ATB application. This was consistent with the results reported in Chapter 3, which suggested that the ATB application had limited effects on the fate and transport of the target NSAIDs in these soils.

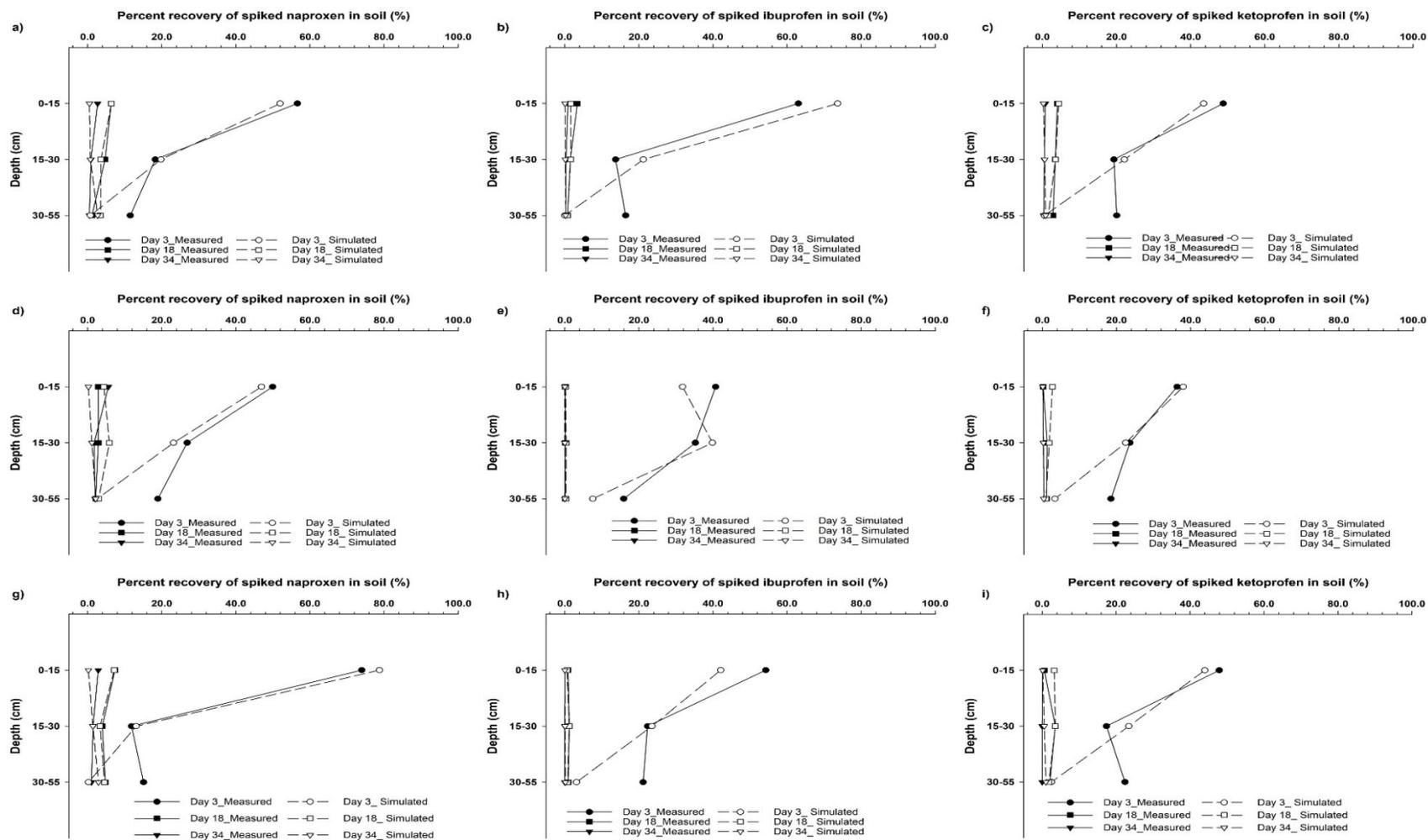


Figure 4.2. Measured vs simulated recovery of a) Cell 6 Naproxen (NPX); b) Cell 6 Ibuprofen (IBF); c) Cell 6 Ketoprofen (KTF); d) Cell 3 NPX; e) Cell 3 IBF; f) Cell 3 KTF; g) Cell 4 NPX; h) Cell 4 IBF; and i) Cell 4 KTF; in the soil from lysimeter cells.

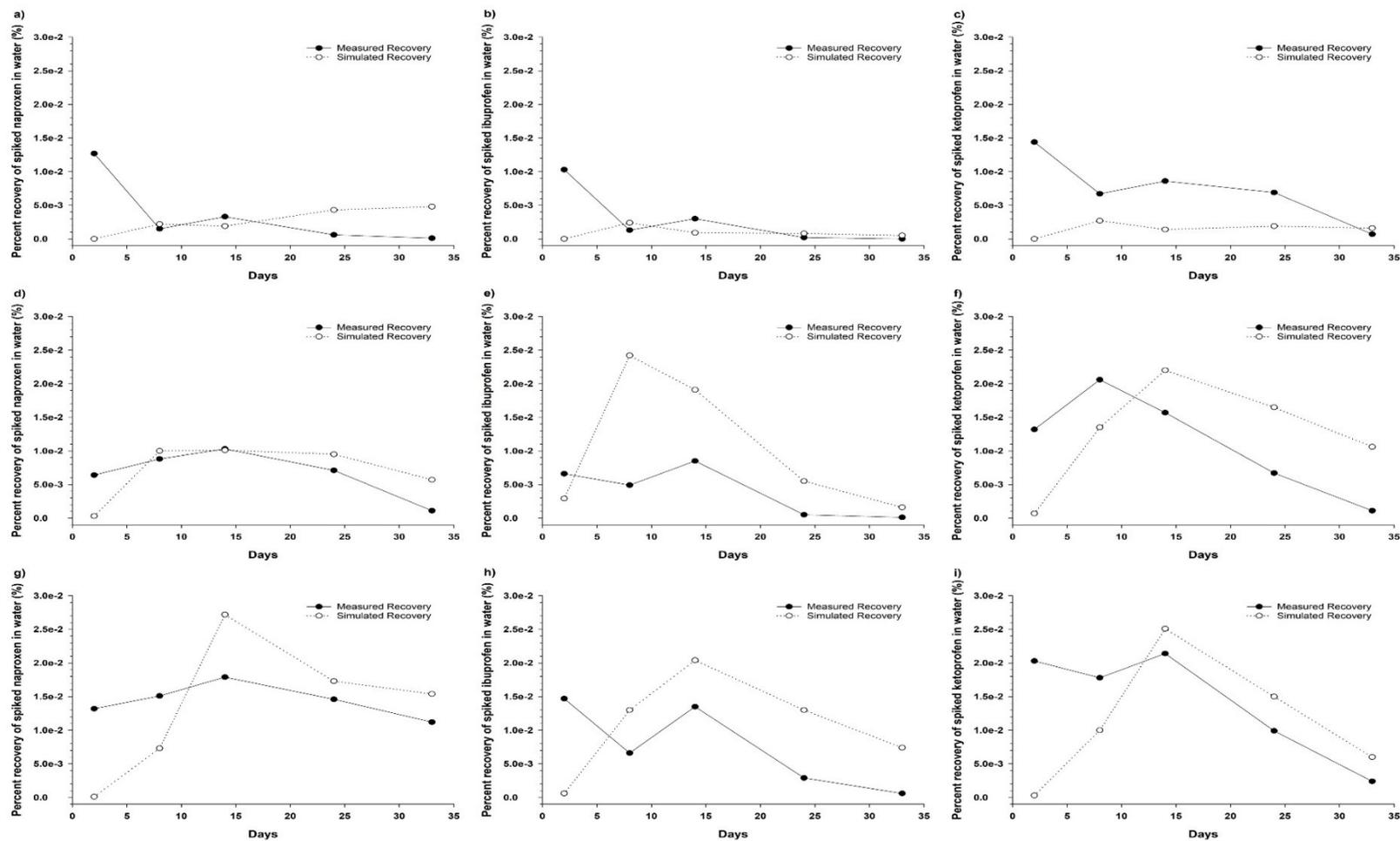


Figure 4.3. Measured vs simulated recovery of a) Cell 6 Naproxen (NPX); b) Cell 6 Ibuprofen (IBF); c) Cell 6 Ketoprofen (KTF); d) Cell 3 NPX; e) Cell 3 IBF; f) Cell 3 KTF; g) Cell 4 NPX; h) Cell 4 IBF; and i) Cell 4 KTF; in leachate from lysimeter cells.

Table 4.6. Evaluation of RZWQM2 model for simulated vs measured recovery of Naproxen (NPX), Ibuprofen (IBF), and Ketoprofen (KTF) in the soil from Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB) in the field-based lysimeter study.

Depth (cm)		NPX			IBF			KTF			
		Day 3	Day 18	Day 34	Day 3	Day 18	Day 34	Day 3	Day 18	Day 34	
Cell 6	0-15	Simulated	52.0%	6.4%	0.5%	73.7%	1.6%	0.1%	43.5%	4.4%	0.3%
		Measured	56.6%	6.4%	2.7%	63.1%	3.4%	0.9%	48.8%	4.0%	0.8%
		PBIAS	-8.2%	0.4%	-81.3%	16.8%	-52.9%	-91.8%	-10.9%	8.6%	-63.8%
	15-30	Simulated	19.8%	3.6%	0.7%	21.2%	1.6%	0.2%	22.1%	3.5%	0.6%
		Measured	18.3%	4.7%	0.9%	13.7%	1.5%	0.3%	19.3%	3.5%	0.5%
		PBIAS	8.5%	-23.8%	-16.4%	54.5%	5.2%	-55.2%	14.6%	1.6%	16.3%
	30-55	Simulated	0.8%	3.5%	2.7%	0.0%	0.7%	0.2%	0.6%	1.6%	0.9%
		Measured	11.5%	1.2%	0.5%	16.4%	0.8%	0.2%	20.1%	2.9%	0.3%
		PBIAS	-93.3%	196.9%	444.8%	-100.0%	-1.4%	-2.9%	-97.1%	-45.5%	156.9%
Cell 3	0-15	Simulated	46.9%	4.4%	0.2%	42.1%	0.6%	0.0%	38.0%	2.7%	0.1%
		Measured	50.0%	2.9%	5.6%	40.7%	0.1%	0.2%	36.3%	0.2%	0.2%
		PBIAS	-6.1%	50.8%	-96.1%	3.3%	654.3%	-87.2%	4.5%	1133.2%	-26.4%
	15-30	Simulated	23.2%	5.9%	1.2%	23.5%	1.2%	0.1%	22.5%	1.8%	0.3%
		Measured	26.9%	2.9%	1.8%	35.3%	0.1%	0.1%	23.6%	1.3%	0.3%
		PBIAS	-13.6%	103.1%	-34.4%	-33.3%	1010.0%	-11.4%	-4.9%	40.4%	5.1%
	30-55	Simulated	2.6%	3.0%	2.2%	3.2%	0.8%	0.2%	3.4%	1.0%	0.4%
		Measured	19.0%	2.2%	2.0%	15.9%	0.1%	0.0%	18.5%	1.1%	0.4%
		PBIAS	-86.2%	36.3%	8.1%	-80.0%	590.6%	2513.7%	-81.7%	-13.3%	-11.9%

Table 4.6 (con't). Evaluation of RZWQM2 model for simulated vs measured recovery of Naproxen (NPX), Ibuprofen (IBF), and Ketoprofen (KTF) in the soil from Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB) in the field-based lysimeter study.

Depth (cm)		NPX			IBF			KTF			
		Day 3	Day 18	Day 34	Day 3	Day 18	Day 34	Day 3	Day 18	Day 34	
Cell 4	0-15	Simulated	78.8%	7.1%	0.2%	42.1%	0.6%	0.0%	44.0%	3.2%	0.1%
		Measured	74.0%	7.4%	2.9%	54.3%	0.9%	0.0%	47.9%	0.6%	0.1%
		PBIAS	6.4%	-2.9%	-92.6%	-22.5%	-30.3%	13.1%	-8.2%	465.3%	6.9%
	15-30	Simulated	13.1%	3.3%	1.4%	23.5%	1.2%	0.1%	23.4%	3.6%	0.5%
		Measured	11.8%	4.0%	1.5%	22.4%	1.2%	0.0%	17.4%	3.5%	0.0%
		PBIAS	10.6%	-15.9%	-3.2%	5.1%	-4.2%	1721.0%	34.8%	0.5%	N/A
	30-55	Simulated	0.3%	4.6%	2.9%	3.2%	0.8%	0.2%	2.5%	1.8%	1.0%
		Measured	15.1%	4.8%	1.0%	21.2%	0.9%	0.0%	22.4%	2.1%	0.0%
		PBIAS	-98.3%	-4.6%	179.9%	-84.9%	-17.9%	2546.7%	-88.7%	-13.4%	N/A

Table 4.7. Evaluation of RZWQM2 model for the sum of simulated vs measured recovery of Naproxen (NPX), Ibuprofen (IBF), and Ketoprofen (KTF) in the water samples over five sampling time from Cell 6 (Soil), Cell 3 (Soil + 7 Mg ha<sup>-1</sup> ATB), and Cell 4 (Soil + 28 Mg ha<sup>-1</sup> ATB) in the field-based lysimeter study.

		<b>Cell 6</b>	<b>Cell 3</b>	<b>Cell 4</b>
<b>NPX</b>	<b>Simulated</b>	0.013%	0.035%	0.067%
	<b>Measured</b>	0.018%	0.034%	0.072%
	<b>PBIAS</b>	-27.8%	5.5%	-6.4%
<b>IBF</b>	<b>Simulated</b>	0.005%	0.053%	0.054%
	<b>Measured</b>	0.015%	0.021%	0.038%
	<b>PBIAS</b>	-68.4%	159.2%	42.3%
<b>KTF</b>	<b>Simulated</b>	0.008%	0.063%	0.056%
	<b>Measured</b>	0.037%	0.057%	0.072%
	<b>PBIAS</b>	-79.5%	10.1%	-21.6%

#### **4.4 Conclusions**

This study evaluated the performance of RZWQM2 in simulating the fate and transport of NPX, IBF, and KTF in sandy loam soil and soil amended with ATB. The calibrated model successfully simulated the daily water seepage out of the soil profile, with less than 15% error compared to measured data, and NSE and  $R^2$  all greater than 0.7 for both calibration and validation datasets. The performance of the calibrated model was also satisfactory in simulating the percent recovery of initial spiked NSAIDs in soil profiles at three different depths and in water samples in each lysimeter cell, where the range of simulated data was within the same order of magnitude as that of measured percent recovery of NSAIDs. The model simulation did not show any clear effect of ATB amendment on simulated recovery of NSAIDs in the lysimeter cells. The model performance in this study may be further improved by i) collecting hourly pan evaporation data from experimental site; ii) developing a better understanding of macropores within the lysimeter cells; and iii) obtaining field-based data on the half-life of the target NSAIDs in different soils and at various depths in the soil profile.

## Chapter 5 Conclusions and Recommendations

### 5.1 Conclusions

This research was designed to conduct laboratory- and field-based empirical studies that could be integrated with process based numerical modeling to simulate the behavior of three NSAIDs, including NPX, IBF, and KTF, in a biosolids-amended agricultural soil. The following section reviews the major discoveries from this research.

Initially, a laboratory soil incubation study (Chapter 2) determined the dissipation rates and half-lives of the target NSAIDs, both as a single compound or as a mixture of three compounds, when added to an acidic loamy sand textured agricultural soil, with or without ATB amendment. The study determined that biodegradation following first-order kinetics was the dominant mechanism leading to the disappearance of the target compounds in soil. IBF and NPX showed low to moderate persistence in soil and ATB amended soil, with half-lives ranging from 4.9 to 14.8 days, while KTF appeared to be highly persistent with an average half-life of 33 days. The order in which the target NSAIDs disappeared in both soil and ATB amended soil was: IBF>NPX>KTF, for both individual and mixture compound treatments. Soils that received the ATB amendment inhibited the dissipation of NPX in both the individual and mixture compound environment, as well as for IBF and KTF in individual compound treatment over the 14-day incubation study. An inhibition effect from the ATB amendment in sterile soil treatments was also observed in this study. In mixture compound treatments, IBF dissipation was inhibited in both soil and ATB amended soil. The dissipation rate of KTF in a mixture compound environment in soil was lower, while the opposite effects were observed in ATB amended soils. For NPX, the dissipation was enhanced in the mixture

compound environment in ATB amended soil, while the same dissipation rate of NPX was calculated in soil.

The bench-scale incubation study was followed by a field-based lysimeter study (Chapter 3) that assessed biodegradation and leaching transport of the target NSAIDs in an acidic sandy loam textured soil amended with three different rates of ATB (0, 7, and 28 Mg ha<sup>-1</sup>) over 34 days in 2018. All three NSAIDs spiked to the soil surfaces of the lysimeter cells irrigated with fixed volumes of water lead to the vertical transport of the compounds into the soil profile. However, the sum totals of all NPX, IBF, and KTF detected in the leachate samples across all treatments were only 0.03%, 0.02%, and 0.04%, respectively, of the initial mass spiked to the surface soil. A mass balance analysis indicated a low accumulation of these compounds in the soil profile by the end of the study period (Day 34) from all treatments, with only 5%, 1%, and 1% of initial spiked NPX, IBF, and KTF, respectively, detected. Both rates of ATB application significantly increased soil pH and OM content of the soils, especially at the top 0-15 cm depth, but this did not impact the retention of the compounds in the soil profile. Overall, all three NSAIDs in the lysimeter study presented low mobility and persistence in the acidic loamy sand textured agricultural soil.

The final study in the thesis used the empirical data from the lab incubation and field studies to parameterize and test the RZWQM2 for simulating NSAID fate (Chapter 4). The model was calibrated for the soil water balance module and pesticide module using the trial-and-error method. The model was capable of predicting water seepage out of the soil profile in 2017 and 2018 within 15% error of the field measured data with calibrated soil water balance module, along with calculated statistics such as NSE and R<sup>2</sup>

all greater than 0.7. The overall percent recovery of initial spiked NSAIDs in both soil and water samples predicted by the model, after further calibration of the pesticide module, were within the same order of magnitude of the measured data. The model significantly underestimated the percent recovery of initial spiked NSAIDs at the 30-55 cm soil depth for all treatments on day 3. The overall performance of RZWQM2 in simulating the soil hydrology and fate and transport of NSAIDs in soil profiles with various rate of ATB amendments were satisfactory.

In summary, the major contributions of this thesis research were to:

- Identify biodegradation as the dominant mechanism that governs the disappearance of the target NSAIDs in a loamy sand textured agricultural soil;
- Calculate abiotic and biodegradation rate constants and half-lives for the target NSAIDs in a Nova Scotia soil with and without ATB amendments, under both individual and mixture compound environments;
- Identify that the relative mobility of the target NSAIDs in a loamy sand textured agricultural soil were low;
- Determine that ATB amendments had a limited effect on the fate and transport of the target NSAIDs in soils;
- Demonstrate, to the best of the author's knowledge, the first attempt to use a numerical agricultural systems computer model to simulate fate and transport of NSAIDs in soil profiles, and calibrate the models to yield satisfactory simulation results.

## 5.2 Future Research Recommendations

The results from this thesis revealed that additional research could be undertaken to develop a better understanding of the fate and transport of ESOCs, including NSAIDs, in agricultural systems. For example, the complexity of the fate and transport of ESOCs in a soil system as a mixture compound treatment are still overlooked based on the available literature. The results from the incubation study showed that the dissipation of NSAIDs in the mixture compound environment varied among different compounds. This study only focused on the compound mixture consisting of NSAIDs from the same class in the soil, while ESOCs are usually exposed to a mixture compound environment with the presence of a more diverse class of compounds. Although RZWQM2 has the capability to simulate the fate and transport of three organic contaminants simultaneously, specifically for pesticides, the interactions among the different compounds were not considered in any numerical agricultural systems model developed to date. Further research needs to be conducted to develop a better understanding of the behavior and mechanisms of compound mixtures in soils.

The ATB treatments have been found to increase both soil pH and OM in the soil profiles, which impacted dissipation of the target NSAIDs in this study. Long-term ATB application in soils has been also found to result in a shift in the microbial community that could affect the biodegradation of the organic compounds (Price et al., 2020). However, microbial community analysis was not conducted in this thesis research, and the effect of the microbial community shift on the fate of ESOCs remains an area requiring further investigation.

Modeling the fate and transport of NSAIDs from the soil solution into the subsurface is dependent on a wide range of biotic and abiotic factors that vary widely, and rapidly, over time. The dissipation rate constants and half-lives of NPX, IBF, and KTF were derived from a bench-scale incubation study. However, the RZWQM2 modeling using the dissipation half-lives derived from bench-scale incubation experiments led to overprediction of concentrations of NSAIDs in the soil profiles. As a result, the dissipation half-lives in the calibrated pesticide module needed to be significantly shortened to achieve a more accurate prediction. Greskowiak et al. (2017) reviewed the first-order biodegradation rate constants for 82 ESOCs in soil and groundwater available in the literature and found huge variability in values for each specific compound, even for the rate constants obtained from similar types of studies. The observed discrepancy between measured and calibrated half-lives highlights the importance of obtaining field measured rate constants and half-lives of organic contaminants for more accurate model simulation.

In this thesis research, model calibration was conducted on the most sensitive parameters for individual lysimeter cells using manual calibration by trial-and-error, which was a time-consuming procedure to calibrate a model to a certain level of satisfaction. The latest update of the RZWQM2 incorporates the Parameter Estimation Software (PEST), which features a sensitivity and uncertainty analysis along with autocalibration for one or more selected model outputs, to enhance the efficiency of the model parameterization process (Ma et al., 2012). Applying PEST for model parametrization is highly recommended for future modeling work.

Overall, the outcomes from this thesis research showed that a well-calibrated

numerical agricultural system model, such as RZWQM2, can provide a robust decision-support tool that helps key decision and policy stakeholders to gain a better insight into the potential impact of ESOCs, including NSAIDs, in soils under actual environmental conditions and to develop the most suitable environmental management strategies.

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## APPENDIX A SUPPLEMENTARY INFORMATION

Table A.1. Experimental units for the first and second incubation studies.

NSAIDs	Sampling times								Total
	1	2	3	4	5	6	7	8	
<b>Naproxen (NPX)</b>	9 <sup>a</sup>	9	9	9	9	9	9	9	<b>72</b>
<b>Ibuprofen (IBF)</b>	9	9	9	9	9	9	9	9	<b>72</b>
<b>Ketoprofen (KTF)</b>	9	9	9	9	9	9	9	9	<b>72</b>
<b>NPX:IBF:KTF mixture</b>	9	9	9	9	9	9	9	9	<b>72</b>
<b>Total</b>	<b>36</b>	<b>36</b>	<b>36</b>	<b>36</b>	<b>36</b>	<b>36</b>	<b>36</b>	<b>36</b>	<b>288<sup>b</sup></b>

<sup>a</sup> The 9 experimental units for each NSAIDs in each sampling time include triplicate samples for each of soil, ATB amended soil (Soil+ATB), and Control.

<sup>b</sup> For the first incubation study, another 96 experimental units were included to make total experimental units 384, with NSAIDs spiked in 0.1% HgCl<sub>2</sub> sterilized soil. The number was calculated as: 3 (reps) x 4 (NSAIDs) x 8 (sampling time) = 96.

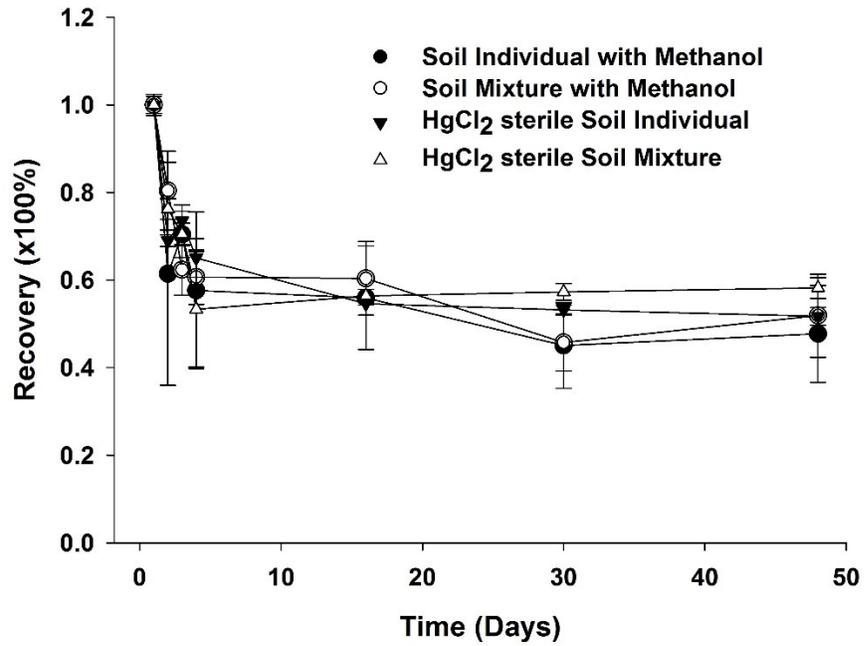


Figure A.1. Example of mean recovery values from first incubation study showing HgCl<sub>2</sub> sterilized soil with naproxen dissolved in methanol and unsterilized soil with naproxen (NPX) dissolved in methanol over a 48 day aerobic soil incubation study.

## **APPENDIX B Closed chamber aerobic respiration incubation study**

A closed chamber incubation study was conducted to test the effect of methanol carrier solvent with NSAIDs and HgCl<sub>2</sub> on sterilizing soil samples. Experimental units were established by weighing 100 g of soil into 1 L Mason jars with the following treatments: 1) blank (empty Mason jar) to determine background CO<sub>2</sub> concentrations; 2) Soil+Deionized Water (DI water); 3) Soil+HgCl<sub>2</sub>; 4) Soil+NSAIDs dissolved in methanol. Samples were prepared for different treatments as presented in Table A.2. The solution was dripped onto the soil surface using a 10 ml pipette and thoroughly mixed by hand through the soil using a spatula. For the Soil+NSAIDs dissolved in methanol treatment, samples were spiked with 10 ml from a 10 ppm NSAIDs mixture stock solution dissolved in methanol to achieve a final concentration of 1 µg NSAIDs g<sup>-1</sup> dry weight soil. The soil was then brought to a gravimetric soil moisture content of 18% using deionized water and the soils were thoroughly mixed by hand using a spatula. After mixing, all the experimental units were placed in the incubation chamber marking the start of the study. Soils receiving NSAIDs dissolved in methanol were not allowed to sit overnight to volatilize the methanol. Experimental units were set up on shelves in a randomized block design (RBD) in an environmentally controlled chamber with three blocks to account for potential vertical differences in temperature. Each treatment was replicated three times and incubated in the environmentally controlled chamber set to 20±2°C. Each Mason jar was wrapped with aluminum foil and sealed with a lid for the duration of each sample interval. Inside each Mason jar, one glass vial containing 10 ml of 1 M sodium hydroxide (NaOH) solution was placed to trap carbon dioxide (CO<sub>2</sub>) (Hopkins 2008). The NaOH traps were collected at the end of each time interval and replaced with new NaOH traps over five time intervals (Day

3, 7, 10, 13, and 16) and then backtitrated with 0.5 M hydrogen chloride (HCl) after the addition of 2 ml of 1M barium chloride (BaCl<sub>2</sub>) and phenolphthalein to determine the amount of CO<sub>2</sub> produced during the incubation using the following equation:

$$\text{CO}_2 \text{ in NaOH trap (mol C)} = 0.5 \times ((V_{\text{NaOH}} \times C_{\text{NaOH}})/1000 - (V_{\text{HCl}} \times C_{\text{HCl}})/1000)$$

Where  $V_{\text{NaOH}} = 10 \text{ ml}$ ,  $C_{\text{NaOH}} = 1 \text{ M}$ ,  $V_{\text{HCl}}$  is the volume of HCl used in the titration (mL), and  $C_{\text{HCl}} = 0.5 \text{ M}$ . The CO<sub>2</sub> in NaOH trap measured in blank samples (background CO<sub>2</sub>) were subtracted from each treatment. The carbon (C) mineralization rate was calculated as:

$$\text{C mineralization rate (umol C g}^{-1} \text{ soil h}^{-1}) = \text{CO}_2 \text{ in trap}/(\text{soil mass (g}^{-1}) \times \text{incubation time (h}^{-1}))$$

The background subtracted cumulative C mineralization rate for each treatment was then calculated by adding up the C mineralization rates over time.

The effect of two factors of interest, including treatment (3 levels: Soil+DI water; Soil+HgCl<sub>2</sub>; Soil+NSAIDs dissolved in methanol) and time (5 sampling time) on background subtracted cumulative C mineralization rates over the incubation study was determined using a repeated measure analysis of variance (ANOVA) in a randomized block design with three blocks using the Mixed Procedure of SAS v.9.4 (SAS Institute, Cary, NC). The model adequacy was tested prior to ANOVA to determine if the assumption of normality and constant variance was violated (Montgomery, 2009). If violated, proper data transformation was conducted on response variables. The variance-covariance matrix of the residuals, selected based on the basis of Akaike's Information Criterion, was variance components (VC; Montgomery, 2009). If effects were significant ( $p < 0.05$ ), means were compared with the Least Square Means test in SAS.

## Results

The background subtracted cumulative C mineralization rates measured over a 16 day incubation for each treatment are shown in Figure A.2. A two-way interaction of Treatment x Time was found to be significant for background subtracted cumulative C mineralization rates ( $p < 0.05$ ). The results showed that cumulative C mineralization rate in Soil+DI water was significantly higher than all the other treatments, and was approximately 7 times higher than the concentrations measured in Soil+NSAIDs dissolved in methanol treatments (Figure A.2). The cumulative C mineralization rate in HgCl<sub>2</sub> sterilized soil was not significantly different than the Soil+NSAIDs dissolved in methanol treatment on day 3. However, microbial respiration increased gradually over time and the cumulative CO<sub>2</sub> trapped was three times higher than the Soil+NSAIDs dissolved in methanol by day 16. The cumulative C mineralization rate in Soil+NSAIDs dissolved in methanol was close to 0 over the whole incubation period. The results of this study show that microbial activity was effectively inhibited over 16 days with the addition of NSAIDs dissolved in methanol that was not allowed to evaporate overnight but the HgCl<sub>2</sub> resulted in a gradual increase in microbial activity.

Table A.2. Treatments in an aerobic soil respiration experiment with Soil+DI water, Soil+HgCl<sub>2</sub> sterilizing agent, Soil+NSAIDs in unevaporated methanol solvent carrier, and an atmospheric Blank.

Treatment	Soil (g)	Water (ml)	0.1% HgCl <sub>2</sub> solution (ml)	NSAIDs in methanol (ml)
Blank	0	0	0	0
Soil + water	100	18	0	0
Soil + HgCl <sub>2</sub>	100	10	8	0
Soil + NSAIDs	100	8	0	10

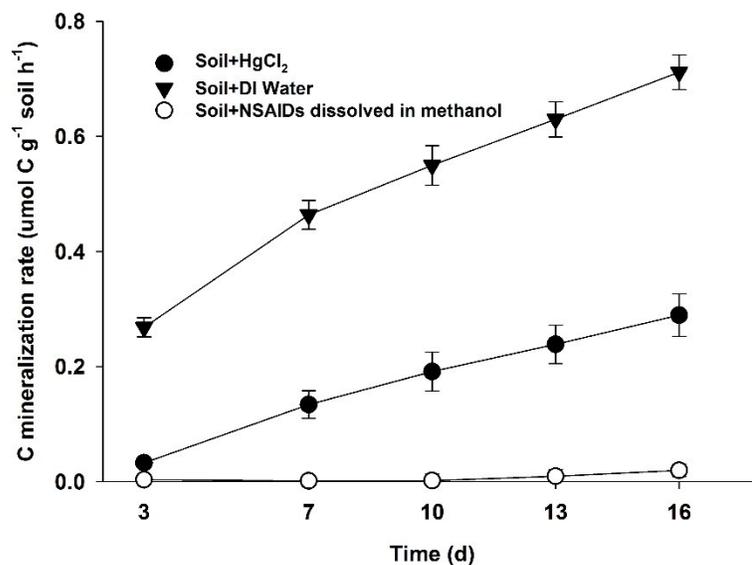


Figure A.2. Background subtracted cumulative carbon (C) mineralization rate measured in a Soil+HgCl<sub>2</sub>, Soil+Deionized Water (DI water), and Soil+NSAIDs dissolved in methanol over a 16 day incubation period. Vertical bars represent standard deviation (n=3).

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## APPENDIX C Lysimeter cell set up

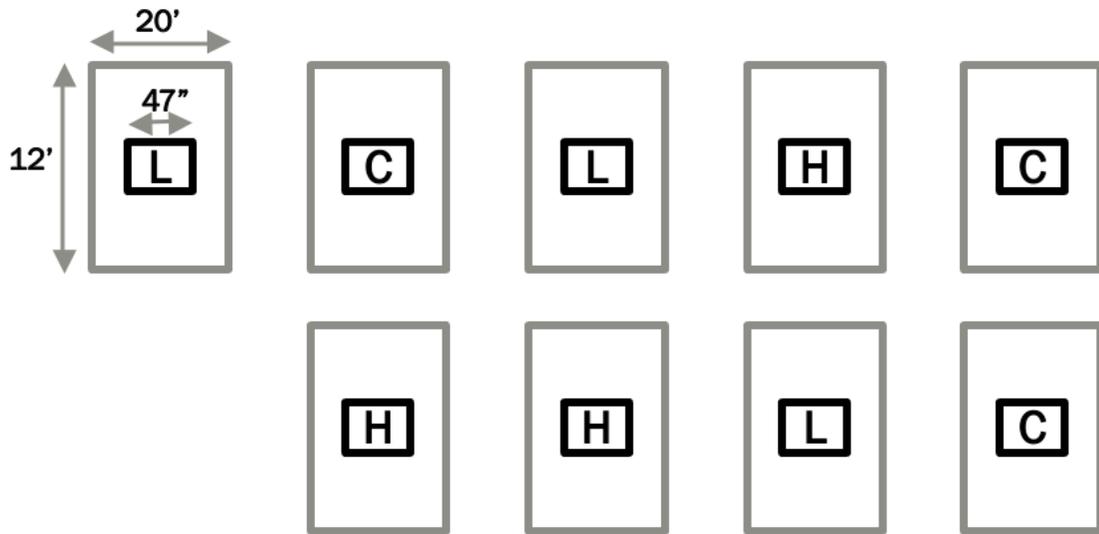


Figure C.1. Layout of lysimeter cells used in this study. The three ATB amendment treatments are labeled as: C (Control, 0 Mg ha<sup>-1</sup>), L (7 Mg ha<sup>-1</sup>), and H (28 Mg ha<sup>-1</sup>).



Figure C.2. Picture of lysimeter cell 5.



Figure C.2. Picture of ISCO 6700 Full-size Portable Sampler (Teledyne Isco, Lincoln, NE, USA) set up for the lysimeter cell study.

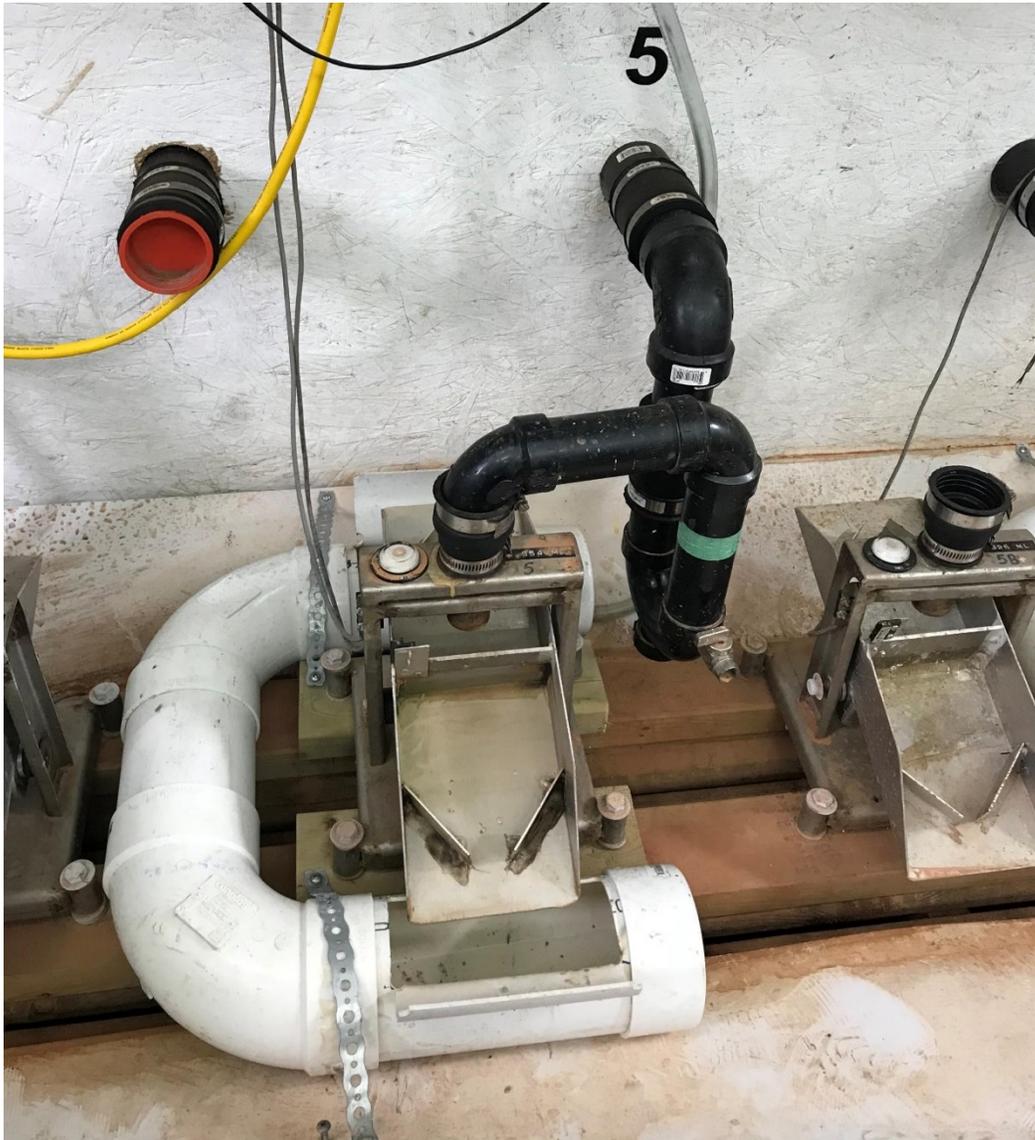


Figure C.3. Picture of drainage and calibrated tipping bucket set up connected to the lysimeter cell 5.

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