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A Modeling Approach to Understanding Glyphosate Transport in the Belize River Watershed

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A MODELING APPROACH TO UNDERSTANDING GLYPHOSATE TRANSPORT IN THE BELIZE RIVER WATERSHED

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Civil Engineering
in the College of Engineering
at the University of Kentucky

By

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2020

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ABSTRACT OF THESIS

A MODELING APPROACH TO UNDERSTANDING GLYPHOSATE TRANSPORT IN THE BELIZE RIVER WATERSHED

Glyphosate is the most widely used herbicide worldwide and is often transported from application areas to surface water when solubilized in runoff or sorbed to eroded sediment. There is evidence that suggests both glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) may pose a risk to human health, as well as cause adverse effects in the environment. However, consistent monitoring data is still limited, especially in developing countries. Belize is a developing nation with agriculture being a major sector of its economy and is heavily reliant on glyphosate. The widespread use of glyphosate in Belize may be resulting in glyphosate transport to drinking water resources. Samples were collected from two rural communities that rely on the Belize River for their drinking water systems, Bullet Tree and Spanish Lookout, at points upstream of the abstraction site, at the abstraction site, and at the site of drinking water distribution. Samples were analyzed using HPLC, ELISA kits, and LC-MS/MS. From these analyses, it was concluded that glyphosate was not present in any water samples at a detectable concentration. The Soil and Water Assessment Tool (SWAT) was used to develop a model of the Belize River Watershed. The model was calibrated and validated for observed flow rates using the SWAT Calibration and Uncertainty Program (SWAT-CUP), which revealed acceptable model performance for simulating flow. Model results indicate that glyphosate transport to the Belize River is occurring, with contributions from glyphosate sorbed to eroded sediment being significantly greater than soluble glyphosate in surface runoff (p-values <0.0). Average simulated concentrations of soluble glyphosate in both wet and dry seasons are below the European Union (EU) standard of 0.1 ppb across the watershed. However, subbasins 2, 3, and 28 were identified as higher risk areas, due to having the highest percentages of days exceeding the EU standard. Subbasin 28, located just downstream of the Spanish Lookout drinking water system, was the most significant contributor of soluble glyphosate to the river, as compared to soluble glyphosate concentrations in subbasins 2 (p-values <0.0) and 3 (p-values <0.0). Soluble glyphosate concentrations in subbasin 28 inflow and outflow exceeded the EU standard 12.53% and 11.65% of the time, respectively. This work demonstrates a framework for applying SWAT for pesticide transport modeling in developing countries, and has the potential to be a powerful and accessible tool for watershed management when monitoring data is unavailable.

KEYWORDS: Watershed Modeling, Pesticide Transport, Glyphosate, AMPA, SWAT, Belize

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A MODELING APPROACH TO UNDERSTANDING GLYPHOSATE TRANSPORT IN THE BELIZE RIVER WATERSHED

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DEDICATION

To my Grandma, who left us the day after I graduated with my bachelor's degree. I will never forget that final day and how happy we were to all be together. I wish she could be at this graduation as well, but I will save a small space in this thesis and a huge space in my heart for her always.

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LIST OF ACRONYMS

SWAT-Soil and Water Assessment Tool

RWS – Rudimentary Water System

BMP-Best Management Practice

EPA - Environmental Protection Agency

WHO – World Health Organization

IARC – International Agency for Research on Cancer

EU – European Union

AMPA - Aminomethylphosphonic Acid

ppb – Parts per Billion

HPLC – High Performance Liquid Chromatography

ELISA – Enzyme-linked Immunosorbent Assay

LC-MS/MS – Liquid Chromatography with Tandem Mass Spectrometry

HRUs - Hydrologic Response Units

FAO – Food and Agriculture Organization of the United Nations

USDA – United States Department of Agriculture

MWSWAT – MapWindow Soil and Water Assessment Tool

WETN - Non-forested Wetlands

WETL - Mixed Forested and Non-forested Wetlands

WETF - Forested Wetlands

RNGE - Range Grasses

FRST - Mixed Forest

FRSE - Evergreen Forest

FRSD - Deciduous Forest

AGRL - Agricultural Land

WGEN – Weather Generator

SWAT-CUP – The Soil and Water Assessment Tool Calibration and Uncertainty Program

SUFI-2 – Sequential Uncertainty Fitting Version 2

95PPU – 95% Prediction Uncertainty

NS – Nash-Sutcliffe

R^2 – Coefficient of Determination

TDS – Total Dissolved Solids

SCS – Soil Conservation Service

CHAPTER 1. INTRODUCTION

1.1 Motivation

The use of pesticides has allowed for increased food production and food security in a world with a rapidly growing population and agricultural demand. Among these pesticides, glyphosate is the most widely applied herbicide worldwide, with its use growing 15-fold since the invention of glyphosate tolerant genetically engineered crops (Benbrook, 2016). With such widespread use, there is increasing concern regarding the implications on human and environmental health.

While the extent of the risk associated with glyphosate exposure is still disputed in literature, many studies have correlated glyphosate exposure to incidences of cancer, kidney damage, neurological disorders, and reproductive problems. (Camacho & Mejía, 2017; De Roos Anneclaire et al., 2005; Fluegge & Fluegge, 2016; Fortes et al., 2016; Swanson, Leu, Abrahamson, & Wallet, 2014). Currently, glyphosate is listed as "probably carcinogenic to humans" by the International Agency for Research on Cancer of the World Health Organization (International Agency for Research on Cancer, 2017). Glyphosate is also known to be able to migrate offsite from application areas into unintended locations. The herbicide is consistently detected in many water bodies around the world, though monitoring data in most regions is still lacking. This may be causing unintended consequences to human health, therefore understanding glyphosate transport and monitoring environmental concentrations is critical to prevent unnecessary exposure.

In the developing world, glyphosate use has surged in recent years, due to increased availability of affordable off-patent glyphosate herbicides (Haggblade, Minten, Pray, Reardon, & Zilberman, 2017). From 2002 to 2014, herbicide use in China increased by 13-fold, and spending on herbicide imports increased by six-fold in Ethiopia (Haggblade et al., 2017). Between 1987 and 1996, herbicide importation into Thailand nearly quintupled (Ecobichon, 2001). Pesticide regulations and oversight are often less stringent in developing areas as compared to developed countries, typically resulting in exacerbated adverse effects on human health and the environment (Ecobichon, 2001). Belize is another example of a developing nation that heavily relies upon glyphosate in agriculture, with glyphosate being 31% of total pesticide imports (Basel Convention Regional Centre for Training and Technology Transfer, 2015). Additionally, Belizeans that live outside of major urban areas rely on rudimentary drinking water systems, or systems that have limited to no water treatment, making them especially vulnerable to contaminants from agricultural runoff (Grau & Rihm, 2013).

The Belize River is an important source of drinking water in the country and serves over one-third of the population, much of which relies on rudimentary drinking water systems (Carrias, Cano, Saqui, Ake, & Boles, 2018). According to a watershed-wide assessment from the University of Belize, the Belize River Watershed has experienced significant degradation due to limited watershed management, deforestation, agriculture, and other anthropogenic activities (Carrias et al., 2018). These stressors contribute to increased runoff and erosion, making it likely that pesticides are being transported to rivers and streams as well. Discussions with regulatory agencies in Belize, including the Department of Environment, Pesticide Control Board, and Ministry of Health, have revealed concern about the potential risks associated with glyphosate and interest in investigating the problem. However, the equipment required for consistent monitoring and accurate quantification of glyphosate concentrations is extremely costly and not currently feasible in Belize. As a result, glyphosate monitoring data is largely nonexistent in Belize, as well as in many other developing countries.

As concerns regarding the safety of glyphosate use continues to grow, regulatory agencies around the world are beginning to respond. Many cities and even entire countries have banned, or begun to phase out, the use of glyphosate; including France banning all sales of the popular glyphosate product Roundup Pro 360, Germany issuing a complete ban on glyphosate by 2023, and Mexico banning glyphosate imports (Casassus, 2019; Resources, 2019; Rinke, Martin, Chamber, & Heavens, 2019). Belize has not yet issued a ban, but has added glyphosate to its national list of Restricted Use Pesticides (Pesticide Control Board, 2019). However, due to the efficacy of glyphosate and the lack of completely safe alternatives, effective ways to manage glyphosate are necessary in order to adapt to changing regulations while meeting agricultural demands. Modeling can be an extremely useful tool to understand glyphosate transport and supplement a lack of data, especially in regions that have limited resources and are unable to conduct robust monitoring studies.

1.2 Research Approach

The motivation of this study was to investigate the risk of glyphosate contamination in drinking water sources in Belize by examining two rudimentary drinking water systems in the Belize River watershed. Results from this work can provide a potential management tool applicable to countries that are often the most vulnerable to glyphosate exposure, but do not have the resources for consistent costly analysis. The objective of this work was to determine whether glyphosate is being transported to the Belize River from agricultural areas, and to demonstrate the use of the Soil and Water Assessment Tool (SWAT) in modeling glyphosate interactions in the Belize River Watershed.

The questions that this work sought to address are:

1. Is glyphosate present in the Belize River?

2. Can SWAT effectively simulate glyphosate fate and transport on a watershed scale?

3. Can SWAT be used to inform watershed management decisions?

Research question 1 was addressed by collecting sediment and water samples in the Belize River watershed, transporting samples back to the United States, and quantifying glyphosate concentrations. Research Question 2 was addressed by using SWAT to develop a model that represents the Belize River Watershed. Glyphosate application was simulated in the watershed and simulated concentrations in the Belize River were compared to concentrations quantified from the experimental portion of the study. Research Question 3 was answered by evaluating model efficiency and performance to determine potential usefulness in place of observed glyphosate data.

The hypotheses of this study were:

1. Glyphosate is transported in the Belize River via agricultural runoff and erosion.
2. SWAT is an effective tool to model the Belize River Watershed and predict glyphosate transport on a watershed-scale.

1.3 Broader Impacts

This work presents a framework for predicting glyphosate transport, risk of drinking water contamination, and informing mitigation strategies. It is ideally applicable for communities limited in resources needed for data collection. The modeling portion was done entirely with free and open source tools and has the potential to be extremely useful in making better-informed watershed management decisions. This research comes at a time when communities around the world are reacting to the growing concern about glyphosate, and stakeholders in Belize are considering the investigation of pesticide transport to their waterways and more stringent pesticide regulations. This work can be shared with stakeholders, such as the Belize Department of Environment and Ministry of Health, to provide them with a tool to aid in their transition to stricter pesticide management, and also provides a framework that can be applied in other developing communities worldwide.

1.4 Structure

This thesis is structured as follows. Chapter 1 introduces the problems associated with growing global glyphosate use and how they relate to developing countries such as Belize. It also introduces research objectives and how they are intended to be met, as well as explains the significance of this work. Chapter 2 presents a review of the literature relevant to this work, including the physiochemical properties, fate and transport, toxicity, prevalence, and management of glyphosate. Literature relevant to the study area is also discussed. The different approaches that have been employed to model glyphosate transport are presented, and a detailed explanation of SWAT and its relevant applications is given. Chapter 3 describes the methodology used to accomplish the research objectives, including study area and sampling location details, sample collection procedures, glyphosate determination and water quality testing methods, and the procedures for model set up, calibration, and validation. Chapter 4 discusses the outcomes of the water quality analysis, glyphosate determination, model performance evaluation, and glyphosate transport simulation. Chapter 5 is a conference proceeding submission to the Institute of Electrical and Electronics Engineers Global Humanitarian Technology Conference 2020, pending review. Finally, chapter 6 summarizes this work and highlights major conclusions.

CHAPTER 2. LITERATURE REVIEW

2.1 Glyphosate Use

Glyphosate, N-(phosphonomethyl)glycine, is a broad-spectrum herbicide best known as the key ingredient in Roundup products. It is the most widely used agricultural chemical on the market, with 6.1 billion kg of glyphosate applied worldwide for agricultural and nonagricultural uses in the last ten years (Benbrook, 2016). Glyphosate has been marketed as an nonhazardous, environmentally friendly, nonselective herbicide, and its use rapidly increased 15-fold with the introduction of crops genetically modified to be resistant to the herbicide (Benbrook, 2016; Van Bruggen et al., 2018). In the last ten years, 72% of the total volume of glyphosate applied globally from 1974-2014 was sprayed (Benbrook, 2016).

2.2 Glyphosate Regulation

The mechanism for glyphosate toxicity is inhibition of an enzyme present in plants and not animals (Sikorski & Gruys, 1997). As a result, glyphosate has long been reported to not to be a risk to human health at the levels detected in the environment. The Environmental Progection Agency (EPA) considers glyphosate to be "not likely carcinogenic to humans," and has a maximum contaminant level for glyphosate in drinking water of 700 ppb (Environmental Protection Agency, 2009). However, the World Health Organization (WHO) now classifies glyphosate as "probably carcinogenic to humans" (Guyton et al., 2015). The maximum residue limit for glyphosate in the European Union is 0.1 ppb (European Comission, 2016). The maximum acceptable concentrations for glyphosate in drinking water is 1000 ppb in Australia, and 280 ppb in Canada (Canada, 1995; Dolan, Howsam, Parsons, & Whelan, 2013).

These discrepancies in classification and management may be because a majority of the literature EPA cited for its classification either focused on technical grade glyphosate alone, were comissioned unpublished regulatory reports, or did not take into account long term chronic exposure (Benbrook, 2019). In contrast, the International Agency for Research on Cancer (IARC), a research arm of WHO, used significantly more studies that were peer reviewed and focused on formulations of glyphosate (Benbrook, 2019). Using glyphosate formulations is a more accurate representation of glyphosate exposure as all glyphosate containing products on the market are sold as mixtures.

Studies have shown that some formulations of glyphosate are more toxic than technical grade glyphosate alone, and that there are likely to be adverse effects to human health for long term exposure to glyphosate forumulations (Benbrook, 2019; Séralini et al., 2014). For example, one study compared four different formulations of glyphosate (Roundup Ultra-Max, Infosato, Glifoglex, and C-K YUYOS FAV) and their effects on tadpoles. A wide variation among the toxicities of these different products was observed, and Roundup Ultra-Max was found to be the most toxic on tadpoles (Lajmanovich, Attademo, Peltzer, Junges, & Cabagna, 2011). Other studies have also demonstrated that formulations containing the surfactant polyoxyethylene amine are more toxic (Tsui & Chu, 2003).

2.3 Human Health and Environmental Effects

2.3.1 *Humans and Terrestrial Species*

Glyphosate, its degradation product aminomethylphosphonic acid (AMPA), and glyphosate formulations have been shown to be able to induce DNA damage, which has the potential to eventually lead to cancer in humans (Kwiatkowska et al., 2017; Woźniak et al., 2018). Incidences of miscarriages, dermatological illness, and respiratory illness in humans have been related to an aerial glyphosate spraying campaign that occurred in a community in Colombia (Camacho & Mejía, 2017). Glyphosate formulations have been shown to have endocrine disrupting effects on human cells (Gasnier et al., 2009). Additionally, exposure of glyphosate to human breast cancer cells caused cell proliferation (Thongprakaisang, Thiantanawat, Rangkadilok, Suriyo, & Satayavivad, 2013). There is evidence suggesting that chronic exposure to ultra low doses may result in kidney and liver damage, based on a study examining the effects of glyphosate exposure on rats (Mesnage et al., 2015). Exposure to Roundup also induced oxidative stress in the livers of rats (El-Shenawy, 2009). A study examining the effects of different concentrations of glyphosate and glyphosate formulations on male piglets concluded that a surfactant in glyphosate formulations and the active ingredient itself caused detrimental effects to the cardiovascular system and in some cases, death (Lee, Kan, Tsai, Liou, & Guo, 2009).

2.3.2 *Aquatic Species*

Glyphosate exposure has also been found to have negative impacts on aquatic ecosystems. Low concentrations of technical grade glyphosate was shown to suppress the enzyme acetylcholinesterase in some species of mussels and fish, which can impair proper neurotransmission (Menéndez-Helman, Ferreyroa, dos Santos Afonso, & Salibián, 2012; Sandrini et al., 2013). Exposing carp to 5 mg/L of glyphosate resulted in hyperplasia and edemas (Nešković, Poleksić, Elezović, Karan, & Budimir, 1996). Signs of oxidative stress were observed in silver catfish at varying concentrations of glyphosate (Murussi et al., 2016). In an experiment conducted on *Jenynsia multidentate*, the LC^{50} was determined to be 19.02 mg/L for a 96-hour test duration. In addition, sexual activity of male *J. multidentate* was reduced at 0.5 mg/L (Hued, Oberhofer, & de los Ángeles Bistoni, 2012).

Glyphosate can have adverse effects on some algae species. For example, a significant decrease in chlorophyll a was observed in one species, *Scenedesmus quadricauda*, when exposed to a 50 mg/L concentration (Sáenz, Di Marzio, Alberdi, & del Carmen Tortorelli, 1997). However, certain species are able to utilize glyphosate as a source of phosphorus and experience increases in growth upon exposure (Qiu et al., 2013). Glyphosate can be degraded in the environment to form phosphorus, resulting in an alteration of the phosphorus cycle and increase in phosphorus concentrations in water bodies containing glyphosate (Sun, Li, & Jaisi, 2019; Vera et al., 2010). This may cause eutrophication, which decreases dissolved oxygen concentrations in water bodies to concentrations that cannot support aquatic life.

2.4 Fate and Transport

Glyphosate is a polar compound made up of carboxyl, amine, and phosphate functional groups (Figure 2-1). It is known to bind strongly to sediment and to be highly water soluble (Maqueda, Undabeytia, Villaverde, & Morillo, 2017). It has a solubility in water of 12 g/L (Maqueda et al., 2017). Glyphosate sorption to sediment is a function of pH, and the adsorption of glyphosate in soil is governed by the soil mineral rather than the soil organic matter (Maqueda et al., 2017). Bed sediment has been shown to serve as a significant sink and release of glyphosate in the water column (Pandey et al., 2019). While sorption is the dominant mechanism for glyphosate transport, glyphosate can also move through water easily once in the aqueous phase due to its high solubility. Glyphosate can be transported from the surface in run-off or soil erosion, or soil pores can be saturated to a point that causes exfiltration of glyphosate to a nearby waterway (Daouk, De Alencastro, & Pfeifer, 2013). The risk of glyphosate leaching into groundwater systems is low as it is most likely inactivated by soil adsorption and degraded relatively quickly except during events of high precipitation.

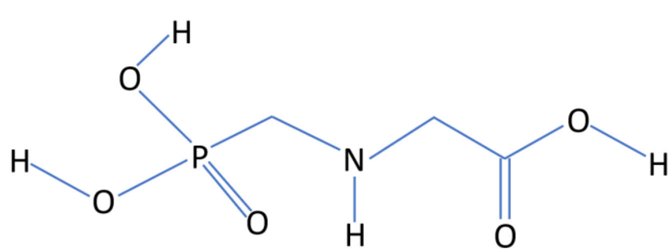

Figure 2-1 Chemical structure of glyphosate

Glyphosate loss from water is due to either adsorption to sediment, microbial degradation, or photodegradation (Maqueda et al., 2017). Its half-life ranges between 2 and 215 days in soil and 2 to 91 days in water (W.A. Battaglin, Meyer, Kuivila, & Dietze, 2014). The half-life significantly varies with soil type as well as microbial communities. One study reported half lives of 4 days in clay loam, 19 days in silt clay loam, and 14.5 days for sandy loam (Al-Rajab & Schiavon, 2010). Another degradation study reported 3 days for silt loam, 27 days for silty loam, and 130 days for sandy loam (Rueppel, Brightwell, Schaefer, & Marvel, 1977). It is suggested to use a half-life in soil of 47 days for estimation purposes (Vencill, 2002). Glyphosate can degrade to form unharmful products sarcosine and inorganic phosphate (Figure 2-2) (Sviridov et al., 2015). However, the carbon-nitrogen bond in glyphosate is more frequently degraded microbially to yield glyoxylic acid and AMPA (Annett, Habibi, & Hontela, 2014; Sviridov et al., 2015). The many pathways glyphosate can take in the environment are illustrated in Figure 2-3.

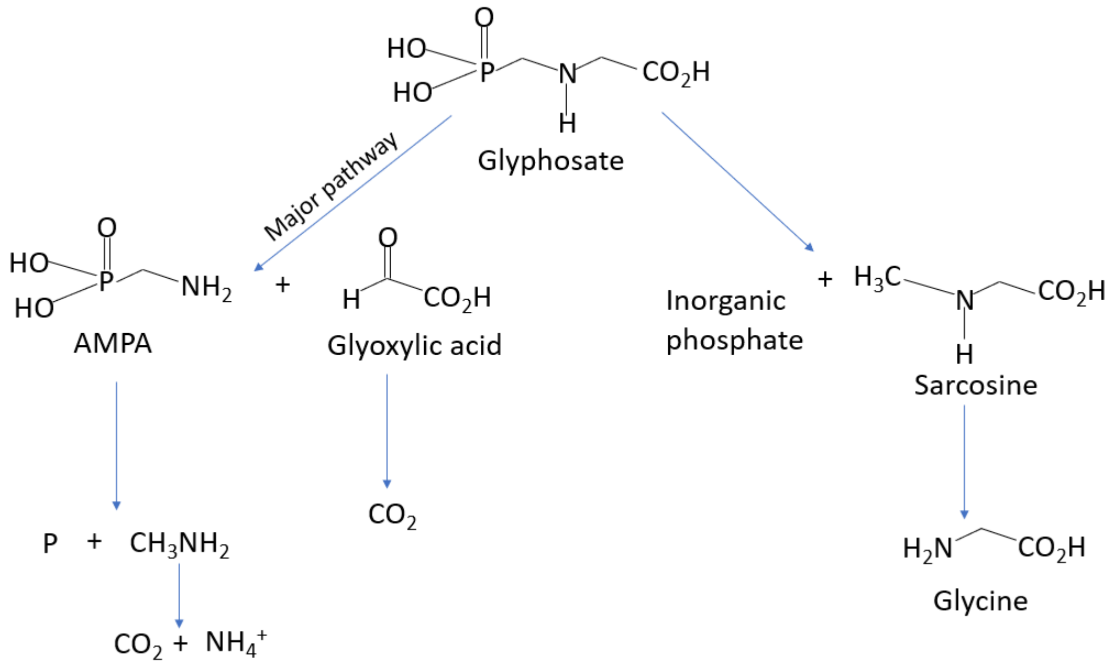

Figure 2-2 Glyphosate degradation pathways

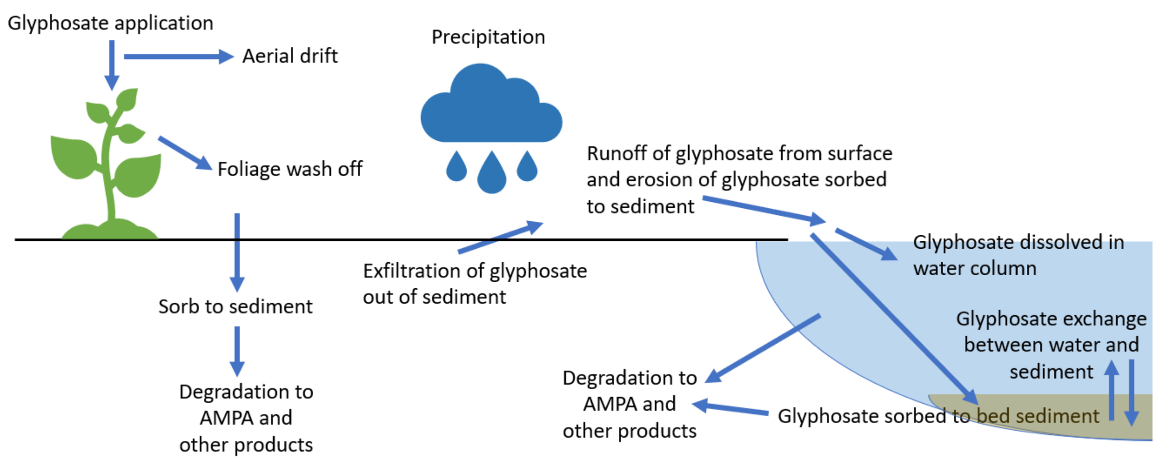

Figure 2-3 Fate and transport of glyphosate in the environment

AMPA is a more persistent compound than glyphosate, with a longer half-life in soil of 76 to 240 days and a half-life in water similar to glyphosate (W.A. Battaglin et al., 2014).

However, in monitoring studies, AMPA is detected more frequently in water than glyphosate (William A. Battaglin, Kolpin, Scribner, Kuivila, & Sandstrom, 2005; Medalie et al., 2020). Glyphosate is broken down by microorganisms such as species of Achromobacter, Arthrobacter, and Pseudomonas (Sviridov et al., 2015). These microorganisms metabolize glyphosate as a source of nitrogen, phosphorus, and carbon (Sviridov et al., 2015).

2.5 Detection

Due to its strong tendency to sorb to sediment, glyphosate was not previously believed to be a water quality issue. However, it is now known that glyphosate and AMPA can be transported in the aqueous phase, especially with heavy precipitation, or in the particulate phase with erosion (Daouk et al., 2013). Additionally, evidence has shown that wastewater treatment effluent is a source of glyphosate and AMPA to streams (Kolpin et al., 2006).

Many monitoring studies all over the world have demonstrated glyphosate and AMPA prevalence in water bodies. In the US, a monitoring study examining streams in the Midwest found glyphosate in 36% of streams tested, in concentrations up to 8.7 µg/L (William A. Battaglin et al., 2005). Another study in the Midwest found glyphosate in 44% of streams tested, in concentrations up to 27.8 µg/L (Mahler et al., 2017). A stream monitoring study in Washington, Maryland, Iowa, and Wyoming found glyphosate in all streams tested, in concentrations up to 328 µg/L (William A. Battaglin et al., 2009). Similarly, most of the streams monitored during a study conducted in Switzerland in 2016 tested positive for glyphosate, in concentrations up to 2.1 µg/L (Poiger et al., 2017). Monitoring in the Netherlands found glyphosate in concentrations ranging up to 0.27 µg/L, with 32% of samples taken from a drinking water intake exceeding the EU drinking water standard. However, AMPA concentrations ranged up to 3 µg/L, with 52% of samples at the drinking water intake being over 1 µg/L (Desmet, Touchant, Seuntjens, Tang, & Bronders, 2016). A study conducted in a protected conservation area of Belize detected concentrations of glyphosate ranging from 0.2-1.7 µg/L in all water samples collected (Kaiser, 2011). A summary of glyphosate detection data can be seen in Table 2-1.

Table 2-1 Summary of major findings from glyphosate monitoring studies in various countries

Country	Date	Glyphosate occurrence	Concentration	Authors
United States (Midwest)	2002	36% of streams tested	up to 8.70 µg/L	(William A. Battaglin et al., 2005)
United States (Midwest)	2013	44% of streams tested	up to 27.8 µg/L	(Mahler et al., 2017)
United States (Washington, Maryland, Iowa, Wyoming)	2005-2006	100% of streams tested	up to 328 µg/L	(William A. Battaglin et al., 2009)
United States	2015-2017	74% of samples tested	up to 8.1 µg/L	(Medalie et al., 2020)
Switzerland	2016	Most streams tested	up to 2.10 µg/L	(Poiger et al., 2017)
Netherlands	2016	Most samples tested (significantly higher concentrations of AMPA)	up to 0.27 µg/L	(Desmet et al., 2016)
Mexico	2013	All samples tested	up to 36.71 µg/L	(Ruiz-Toledo, Castro, Rivero-Pérez, Bello-Mendoza, & Sánchez, 2014)
Argentina	2015-2016	28% of surface water samples	up to 8.2 µg/L	(Okada et al., 2018)
Belize	2006-2007	All samples tested	up to 1.70 µg/L	(Kaiser, 2011)

2.6 Glyphosate in Belize

Glyphosate is the most widely used herbicide in Belize, being 31% of total pesticide imports into the country in 2009 (National Chemical Profile for Chemicals Management Belize 2015, 2015). It is commonly used for many crops in the region; such as sugar cane, corn, grain, beans, citrus and banana (Kaiser, 2011). Currently, there is concern among Belizean Pesticide Control Board and other regulatory agencies regarding the safety of glyphosate use, and it was recently added to the nation's list of priority pesticides of concern and the list of restricted use pesticides (Pesticide Control Board, 2019). Noncompliance with environmental regulations as well as limited watershed and agricultural management in the country has resulted in exacerbated runoff and erosion, meaning glyphosate transport to waterways with the potential of contaminating drinking water is likely. There is very limited reported data on the monitoring of glyphosate concentrations in the environment in Belize. However, one published study conducted from 2006-2007 investigated whether glyphosate was present in the Maya Mountains Protected Area (Kaiser, 2011). Water samples were collected from seven sites during the rainy season of Belize, and it was determined that glyphosate was present in all sites sampled, in average concentrations ranging from 0.2 to 1.7 µg/L. This means that glyphosate had migrated off site from application areas to remote, protected wilderness areas.

Many stakeholders across the country, including the Pesticide Control Board, Belize Water Services Ltd., the Department of Environment, the Ministry of Health, University of Belize, and the Sugar Industry Research and Development Institute, are concerned about the risk glyphosate poses and have expressed interest in furthering understanding of glyphosate transport to drinking water. However, the high cost of complex laboratory equipment and chemical analyses required for glyphosate characterization in environmental samples remain a barrier at this time for consistent monitoring of water bodies for glyphosate.

2.7 Belize River Watershed

2.7.1 Background

The Belize River Water is a transboundary watershed that encompasses 8,389 square kilometers or 3,239 square miles within the countries of Belize and Guatemala. The Belize River is a 180-mile-long river that begins at the confluence of the Mopan and Macal Rivers near San Ignacio, Belize, and empties into the Caribbean Sea near Belize City. The transboundary nature and current territorial disputes between the two countries have made it difficult to come to an agreement on a watershed management plan (Carrias et al., 2018).

Agriculture ranging from small to large in scale is a significant fraction of livelihood and economic activity in the region, being about 22% of the total land use of the watershed within Belize as reported in 2016 (Carrias et al., 2018). Figure 2-4 shows a map of the watershed with agricultural areas highlighted, and the percentages of various land types are given in Table 2.2. Most of the agricultural activity is located within the middle reaches of the watershed. Stakeholders consisting of community leaders, farmers, and individuals from academia, nonprofits, government agencies, and the private sector have identified a number of priority concerns regarding the Belize River, including unsustainable agriculture, rapid expansion of agriculture, degradation of riparian forests and buffers, and pollution of soil and water through runoff (Carrias et al., 2018).

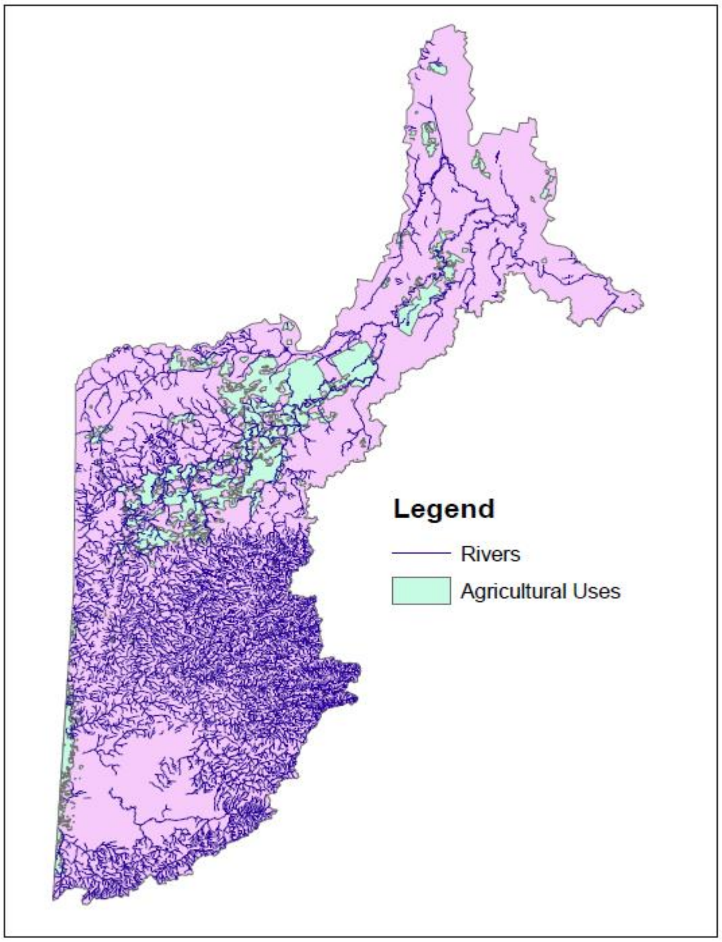

Figure 2-4 Map of the Belize River Watershed showing areas of agricultural land use, and rivers and streams.

Table 2-2 Land use/cover in the Belize River Watershed

Land Use in the Belize River Watershed	
Total Area (ha)	597,500
Land Use	**Fraction of Area**
Urban	2.36%
Agricultural	21.48%
Wooded Vegetation	67.46%
Herbaceous Vegetation	7.00%
Water	1.10%
Wetland	0.49%

2.7.2 Current Conditions of the Belize River Watershed

In 2018, a team from University of Belize conducted a watershed-wide assessment and reported the current conditions of the Belize River watershed (Carrias et al., 2018). The watershed was split into three main regions: the upper reaches, middle reaches, and lower reaches. Their findings for each sector are discussed below.

Much of the land in the upper reaches of the watershed within Belize are well managed, protected areas, though there is some private land being used for purposes that may be putting strain on the environment. These stressors include gold mining, a small amount of farming, and logging. In the upper reaches on the Guatemala side, there is a large population living in both rural and urban settings, a rapid rate of deforestation, and a large amount of agricultural production of corn, beans, and cattle. Farmers in this region have cleared large tracts of forested areas, tilled areas with steep slopes, and cleared pasture areas for cattle to have direct access to the river. This all has resulted in severely degraded riparian zones (Carrias et al., 2018).

The middle reaches are located entirely in Belize and consist of urban and rural populations with a high density of both traditional and intensive agriculture. A farming community called Spanish Lookout, known for producing a significant fraction of Belize's agricultural products and being a major zone of intensive farming, is located in this region (Carrias et al., 2018). Smaller scale, traditional farmers in this region produce grain, vegetables, citrus, and small livestock, while large scale farms are market oriented and produce a large amount of cattle, poultry, grains, corn, beans, and potatoes. This high concentration of agricultural activity and a history of farmers not following environmental regulations have resulted in increasing pressure on the watershed. Riparian zones are severely degraded from deforestation and clearing to allow livestock direct access to water, and a large volume of pesticides are applied to the land (Carrias et al., 2018).

The lower reaches are primarily urban areas, coastal wetlands, and savannas. There is some agriculture occurring in this region, being mainly small-scale farming apart from one large scale cattle farm. There is significant riparian degradation in this sector as well. Stormwater runoff from canals in urban areas is also an environmental stressor. This region is ecologically important, as it serves as a biological corridor allowing wildlife to travel between the protected areas in southern Belize and northern Belize (Carrias et al., 2018).

2.7.3 The Belize River as a Source of Drinking Water

The Belize River is an important source of drinking water, as it provides drinking water to over one-third of the population of Belize (Carrias et al., 2018). In major urban centers, residents have access to water from the Belize River that has been treated at a municipal drinking water treatment plant. Outside of major cities in Belize, communities rely on rudimentary drinking water systems that often have limited treatment systems, or no treatment at all. Approximately 87% of Belize's rural population relies on these rudimentary systems, and only 38% of these systems employ chlorination (Grau & Rihm, 2013). Some of these water systems extract water directly from the Belize River. For example, the village of Bullet Tree pumps water from the Belize River through a chlorination system before distribution. The community of Spanish Lookout extracts water from the river, pumps the water to a settling pond, and distributes the water throughout Spanish Lookout and to two neighboring villages without any further treatment.

2.8 Glyphosate Management

There are several approaches for the management of both agricultural and urban areas to reduce glyphosate transport to surface water via run-off. One approach is public education and enforcement of responsible herbicide use, such as limiting the bandwidth of spray, restricting application times to avoid storm seasons, or avoiding impervious surfaces. Other approaches utilize natural or engineered designs, known as best management practices (BMPs), to reduce non-point source pollution in flow prior to entering a water body. Constructed wetlands have been shown to have an efficiency of 77-90% glyphosate removal (Lucas, Earl, Babatunde, & Bockelmann-Evans, 2015). Vegetative buffer zones have an efficiency of 14-57% glyphosate removal (Syversen & Bechmann, 2004). Stormwater basins have an efficiency of 85-99% (Bois et al., 2013). Rain gardens have an efficiency of about 99% (Yang, Dick, McCoy, Phelan, & Grewal, 2013) Other approaches involve changing agricultural practices to reduce contaminant transport through erosion. In one experiment, not tilling land resulted in a glyphosate load reduction of 2,520 mg as compared to a plot of tilled land in a single crop year (Shipitalo, Bonta, & Owens, 2012). Filter socks with tilled land had an output/input concentration ratio of 0.48, compared to 0.56 without filter socks (Shipitalo et al., 2012). Filter socks combined with not tilling had an output/input concentration ratio of 0.63 compared to 0.7 without filter socks (Shipitalo et al., 2012). A summary of the glyphosate removal efficiencies for each BMP is shown in Table 2-3.

Table 2-3 Glyphosate removal efficiencies of various BMPs

Management Practice	Glyphosate Reduction	Reference
Constructed Wetlands	77-90% removal	(Lucas et al., 2015)
Vegetative Buffer Zones	14-57% removal	(Syversen & Bechmann, 2004)
Stormwater Basins	85-99% removal	(Bois et al., 2013)
Rain Garden	~99% removal	(Yang et al., 2013)
No Till	2,520 mg less compared to a plot of tilled land in a single crop year	(Shipitalo et al., 2012)
Filter Socks, Tilled Land	output/input concentration ratio of 0.48 with filter socks, compared to 0.56 without filter socks	(Shipitalo et al., 2012)
Filter Socks, No Till	output/input concentration ratio of 0.63 with filter socks, compared to 0.7 without filter socks	(Shipitalo et al., 2012)

2.9 Modeling Glyphosate Transport

2.9.1 Modeling Approaches in Literature

Long term monitoring data of glyphosate in water bodies is scarce in most areas, especially developing regions. Modeling can be a useful tool to supplement a lack of consistent data, as well as for risk assessment. Several modeling approaches for glyphosate fate and transport have been described in literature.

One modeling approach employed a contaminant transport model derived from the governing equation for groundwater flow to model glyphosate transport to drinking water wells (Malaguerra, Albrechtsen, & Binning, 2013). This model was calibrated from data collected from a tracer experiment (Malaguerra et al., 2013). From the model, it was concluded that the wells in the study area were not likely to be contaminated with glyphosate (Malaguerra et al., 2013).

Åkesson et al. used a 2-D groundwater transport model calibrated with tritium and helium-3 data to model glyphosate transport in groundwater, and concluded that the conceptual model was too simplistic to account for the mechanism of glyphosate sorption which is a driving factor of glyphosate transport (Åkesson, Bendz, Carlsson, Sparrenbom, & Kreuger, 2014).

A combined modeling and monitoring approach used historical monitoring data and the River Water Quality Model No. 1 modelling approach from the International Water Association Task Group on River Water Quality Modeling to model a section of the Meuse River in the Netherlands and characterize the sources of pesticide loads to the river (Desmet et al., 2016; Shanahan et al., 2001). This model did not consider sorption or desorption. Simulated concentrations were compared to observed concentrations from historical monitoring data. From the model, the authors stated that an upstream influx and wastewater treatment plants were responsible for greater than 50% and 29% of glyphosate loads in the river, respectively (Desmet et al., 2016).

Aravinna et al. calculated Attenuation Factor and Pesticide Impact Rating indices to assess mobility to surface water bodies (Aravinna, Priyantha, Pitawala, & Yatigammana, 2017). This model was used to predict glyphosate concentrations in reservoirs near paddy lands in Sri Lanka, and predicted concentrations ranged from 25.75-265.45 µg/L in the reservoirs of study (Aravinna et al., 2017).

A risk assessment modeling approach to identify regions at risk of glyphosate contamination synthesized monitoring data across a region and used ArcGIS to show a spatial analysis of water bodies at risk and in need of mitigation actions (Di Guardo & Finizio, 2018).

Lastly, in an attempt to evaluate the potential effects of large scale bioenergy crop production in four large watersheds in Michigan, the Soil and Water Assessment Tool (SWAT) was employed to simulate the transport of eight pesticides and herbicides, including glyphosate (Love, Einheuser, & Nejadhashemi, 2011). This study predicted significant concentrations of glyphosate entering streams resulting from continuous corn rotation, that continuous corn rotation would cause the impairment of 541,152 kilometers of streams, and that the production of traditional intensive row crops potentially pose a risk to aquatic life and drinking water quality (Love et al., 2011). Additionally, an alternative scenario was modeled to simulate the production of less intensive bioenergy crops, and a corresponding 171,667 km reduction in impaired stream length was predicted (Love et al., 2011).

The current literature on methods for modeling glyphosate vary in scale, complexity, and accuracy. While these modelling approaches offer useful insights on glyphosate transport through the environment, there has yet to be a documented attempt to model glyphosate transport to surface water on a watershed-scale outside of the United States.

2.9.2 Soil and Water Assessment Tool (SWAT)

The Soil and Water Assessment Tool (SWAT) is a reliable hydrodynamic model developed by the USDA Agricultural Research Service, and is widely used for watershed simulations.

There are about 3,000 published studies utilizing SWAT for watershed modeling, for a variety of applications such as evaluating BMP impacts, simulating climate change effects, and predicting nutrient, sediment, and pesticide loads. However, there are only about 50 studies that have used SWAT for the purpose pesticide transport modeling (R. Wang et al., 2019). A summary of these studies for case studies outside of the US can be found in Table 2-4. The purposes of these pesticide transport models include sensitivity analysis, exposure modeling for fate and transport, mitigation strategy development, algorithm improvement, and advanced implementations (R. Wang et al., 2019).

Table 2-4 Summary of studies using SWAT to model pesticide transport in watersheds outside of the United States. Adapted from (R. Wang et al., 2019).

Case Study Area	Pesticide	Study Area Size (km^2)	Purpose	Authors
Belgium	Atrazine	32	Sensitivity Analysis	(K. Holvoet, van Griensven, Seuntjens, & Vanrolleghem, 2005)
Belgium	Atrazine	32	BMP	(Katrijn Holvoet, Gevaert, van Griensven, Seuntjens, & Vanrolleghem, 2007)
Belgium	Atrazine	32	Algorithm Improvement	(K. Holvoet, van Griensven, Gevaert, Seuntjens, & Vanrolleghem, 2008)
UK	Bentazone	1.42	Exposure Modeling	(Kannan, White, Worrall, & Whelan, 2006)
France	Metolachlor, trifluralin	1100	Exposure Modeling	(Boithias et al., 2011)
France	Metolachlor, aclonifen	1100	BMP	(Boithias, Sauvage, Srinivasan, Leccia, & Sánchez-Pérez, 2014)
France	Alachlor, atrazine, DEA, isoproturon, metolachlor, tebuconazole, trifluralin	1100	Algorithm Improvement	(Boithias, Sauvage, Merlina, et al., 2014)
Germany	Flufenacet, metazachlor	50	Exposure Modeling	(Fohrer, Dietrich, Kolychalow, & Ulrich, 2014)
Thailand	Atrazine, endosulfan, chlorothalonil	77	Exposure Modeling	(Bannwarth et al., 2014)
Thailand	Chlorothalonil, cypermethrin	77	Advanced Application	(Bannwarth et al., 2016)
Japan	Mefenacet	345	Algorithm Improvement	(Boulange et al., 2014)
Phillippines	Malathion	454.45	Algorithm Improvement	(Ligaray et al., 2017)
Northeast China	Atrazine, oxadiazon, isoprothiolane	141.50	Exposure Modeling	(Ouyang et al., 2017)

SWAT is a powerful model for pesticide modeling because of the extent of the physical and chemical processes it considers to simulate pesticide transport. The following mechanisms are mathematically represented within SWAT. Wash-off, degradation, and leaching are modeled for pesticide application. Surface runoff of both soluble and sorbed pesticide, lateral flow of soluble pesticide, and percolation of soluble pesticide are modeled for the transport phase. Solid-liquid partitioning, degradation, resuspension, diffusion, and burial are modeled for pesticide fate in sediment. Lastly, solid-liquid partitioning, degradation, volatilization, settling, and outflow are modeled for pesticide behavior in water (S. L. Neitsch, 2009). SWAT also incorporates the routing of a pesticide throughout the stream network by using a mass balance approach to quantify the pesticide within a stream segment, considering inflow from upstream, resuspension, and diffusion of pesticide from bed sediment (Love et al., 2011).

To calibrate SWAT to accurately depict a watershed of interest, the watershed is first delineated. Next, a stream network is created, the watershed is divided into subbasins, and outlet points are created (Winchell, Srinivasan, Di Luzio, & Arnold, 2013). Hydrological response units (HRUs) are then created based on the region's land use, soil and slope. HRUS are areas that are hydrologically homogenous according to slope, soil, and land use types and will thus respond similarly hydrologically (Winchell et al., 2013). SWAT has an extensive built in database for soil data within the United States. However, users that are applying SWAT outside the United States will have to create their own database with soil types and characteristics (Winchell et al., 2013). Weather station data for temperature, precipitation, solar radiation, and wind speed is then imported. SWAT also has extensive data for United States weather stations, and international users will need to obtain their own weather station data as well as calculate statistics for each weather parameter (Winchell et al., 2013). Input files are then written by SWAT based on all the user inputs thus far. Users can then edit input files, and editing the management file will allow for manipulation of crop schedules and pesticide application (Winchell et al., 2013). Once the simulation is run, the model should be calibrated for parameters such as flow, sediment, nutrients, or pesticide concentrations using any available monitoring data. Data from a period of at least 3-5 years should be used to provide as accurate representation as possible of streamflow and water quality conditions (Moriasi et al., 2007). Once the model is effectively calibrated, the user can interpret data given for the entire watershed to understand how a pesticide is transported through the watershed.

There are a few limitations to SWAT applications for pesticide transport modeling. One limitation is that only one pesticide can be effectively modeled at a time during each simulation. Therefore, if the user wishes to model the impacts of using multiple pesticides, a separate simulation will need to be run for each pesticide of interest (Love et al., 2011). Additionally, several of the input steps can be especially challenging for application of SWAT outside of the United States. There may be challenges in converting the soil classification systems of different countries or obtaining all the necessary characteristics for each soil type to be inputted into a newly created soil database. There may also be less extensive weather data available for the country of interest, and a learning curve in calculating all the necessary statistics for weather simulation within SWAT. These challenges are likely the cause for significantly less documented applications of SWAT for pesticide modeling outside of the US. Of the 50 published pesticide transport studies, the majority of them were conducted within the United States, with only a few in Europe and

Asia (R. Wang et al., 2019). Only one published study within the United States modeled glyphosate along with several other pesticides used for corn production (Love et al., 2011). There has been no published attempt to apply SWAT in Belize.

2.10 Combined Modeling and Management Approach

As previously mentioned, SWAT can also be used to simulate watershed management decisions. SWAT has built in options to simulate two types of best management practices (BMPs); filter strips and tailwater ponds (Luo & Zhang, 2009). Other BMPs can be simulated by manipulating input parameters (Luo & Zhang, 2009). Arabi et al. has outlined a framework for modeling ten BMPS in SWAT: cover crops, conservation crop rotation, field borders, residue management, parallel terraces, filter strips, grassed waterways, lined waterways/channel stabilization, grade stabilization structures, strip cropping, and contour farming (Arabi, Frankenberger, Engel, & Arnold, 2008).

One study used SWAT to evaluate the fate and transport of two organophosphate pesticides and the impacts of implementing BMPs in an agricultural watershed in California. A management-oriented parameter sensitivity analysis was incorporated to determine the input parameters most influential in model predicted pesticide loads (Luo & Zhang, 2009). For each input parameter, 50 random values were sampled and the change in model prediction was measured (Luo & Zhang, 2009). The most influential parameters give an idea of what the dominating processes for transport are and thus what should be targeted for management. It was concluded that the curve number was the most influential factor for pesticide yield by impacting runoff generation and soil erosion. Universal Soil Loss Equation parameters were also found to significantly impact yields of pesticides sorbed to sediment (Luo & Zhang, 2009). This information can be especially useful for determining which parameters should be prioritized in the selection of BMPs.

Another study conducted a cost effectiveness analysis of best management practices by developing a new BMP cost tool that can be integrated into SWAT using Matlab code (Liu et al., 2019). With the integration of this tool, the efficiencies and cost effectiveness were evaluated for blind inlets, wetlands, grade stabilization structures, filter strips, grassed waterways, cover crops, no-till, and nutrient management. Using this approach, the optimized selections and placements of BMPs within the watershed able to meet water quality goals were obtained.

Using SWAT for a combined modeling and management approach allows for predictive transport modeling as well as an opportunity to evaluate the various environmental and economic impacts of different investments in BMPs. This type of work can provide more meaningful information to regulatory agencies, landowners, and farmers in making informed water quality management decisions, finding appropriate conservation practices, and choosing more cost-effective investments.

CHAPTER 3. METHODOLOGY

3.1 Location and Characteristics of Study Sites

3.1.1 The Belize River Watershed

The Belize River Watershed was selected as a study area because of the significance of the Belize River as a drinking water resource. It serves as a source of drinking water to over one-third of the country's population (Carrias et al., 2018). The watershed encompasses 3,239 square miles (8,389 square kilometers) within the countries of Belize and Guatemala (Carrias et al., 2018). The Belize River is a 180-mile-long river that begins at the confluence of the Mopan and Macal Rivers near San Ignacio, Belize, and empties into the Caribbean Sea near Belize City. For the purposes of this study, only the Belizean side of the watershed is considered, though it is important to note a high population density, rapid deforestation, and a large amount of agricultural production occurring in the Guatemalan fraction of the watershed (Carrias et al., 2018). 71.2% of the total watershed falls within Belize's borders, being about 2,306 square miles (Carrias et al., 2018). This fraction of the watershed is shown in Figure 3-1. Rudimentary drinking water systems within the watershed that draw surface water from the Belize River were selected as sites for sample collection. These sites include the communities of Bullet Tree Falls and Spanish Lookout, as shown in Figure 3-2.

Figure 3-1 Map of Belize showing the Belize River Watershed delineated in SWAT, and the sites at which samples were collected.

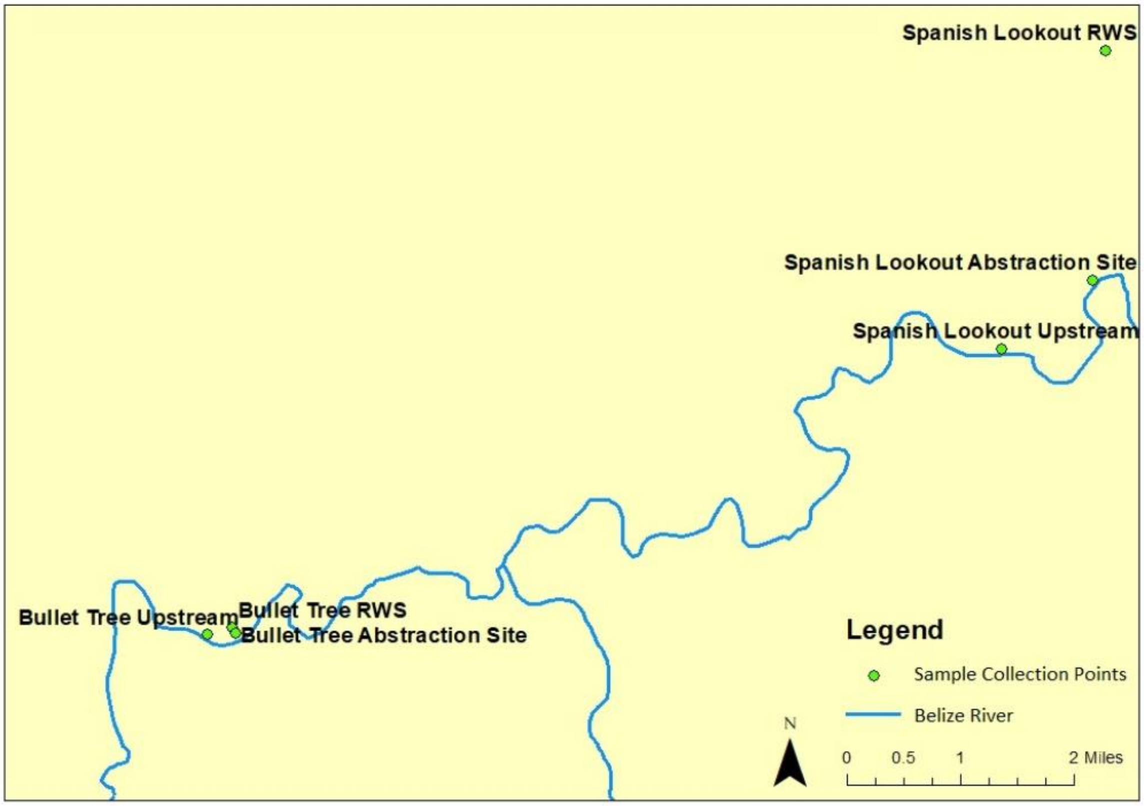

Figure 3-2 Zoomed in map of sample collection sites, showing the three different sampling points in each village. RWS stands for rudimentary water system. Abstraction sites are locations at which drinking water systems pump water from the river.

3.1.2 Sample Collection Site 1: Bullet Tree Falls

Bullet Tree Falls is a village located in the upper reaches of the Belize River Watershed, on the Mopan River. As of 2010, the village had a population of 2,124 residents, and 426 households (The Statistical Institute of Belize, 2013). The village drinking water system pumps surface water from the Belize River to its automatic chlorination system before distribution throughout the village.

3.1.3 Sample Collection Site 2: Spanish Lookout

Spanish Lookout is an agricultural community with a population of 2,253 residents and 482 households (The Statistical Institute of Belize, 2013). The primary drinking water system in the community is located at and managed by a poultry production facility, Quality Poultry Products. This water system draws surface water from the Belize River for use in its production facility and diverts water for drinking water supply to be distributed throughout Spanish Lookout and two neighboring villages. Drinking water is filtered and passes through two settling ponds before distribution. There is no disinfection treatment. Most residents in Spanish Lookout either use private filter systems to further filter water before drinking or rely solely on bottled water. However, it is likely that lower income households in Spanish Lookout consume water without further treatment. It was not disclosed how many residents of neighboring villages consume this water, or if there is any further treatment of the water supply in either village.

3.2 Experimental Work

3.2.1 Sample Collection

A single event of grab sampling occurred for surface water, drinking water, and sediment at each sampling site.

3.2.1.1 Surface Water Samples

For surface water sample collection, preservation, and storage, a method was developed based on the U.S. EPA operating procedure for surface water sampling and Section 8 of U.S. EPA Method 547 for determination of glyphosate in drinking water (U.S. Environmental Protection Agency, 1990, 2013). Surface water samples were collected at two points in each community: upstream of the drinking water intake, and at the drinking water intake. At each sampling point, two 125 mL amber opaque plastic bottles and one 1 L clear plastic bottle were used to collect samples. Plastic amber bottles were used instead of glass as EPA recommends, because glyphosate has been shown to bind to glass (Patsias, Papadopoulou, & Papadopoulou-Mourkidou, 2001). Water samples were collected prior to collecting sediment samples, and care was taken to not disturb sediment while collecting water samples. Depending on the depth of the river at the sampling location, samples were collected either by wading into the middle of the river if shallow enough, or by lowering a Niskin Bottle sampler down to the middle of the water column from an elevated point if the depth was greater than 3 feet.

When using the Niskin Bottle, both stopper ends of the sampler were opened, the sampler was lowered down to roughly the center of the water column, and a weighted messenger was released to shut the two stoppers of the sampler once it was submerged and filled. The sampler was then raised out of the water. Bottles and caps were rinsed three times with sample water before filling for sample collection.

When wading, samples were collected by hand, and bottles were filled facing upstream. Each bottle and cap were rinsed three times with the sample water before collection. Rinse water was emptied away from sampling site.

Collected samples were immediately placed inside a cooler with ice packs, and frozen.

3.2.1.2 Drinking Water Samples

U.S. EPA Method 547 was adapted for drinking water sample collection, preservation, and storage (U.S. Environmental Protection Agency, 1990). At each community drinking water system, two 125 mL amber opaque plastic bottles and one 1 L clear plastic bottle were used to collect samples. At the point of drinking water distribution, bottles and caps were rinsed out three times before being filled with the sample. Bottles were immediately placed inside a cooler with ice packs. Before being frozen at the laboratory, total chlorine concentration was measured for chlorinated water samples (Bullet Tree Falls drinking water samples only). Total chlorine was measured to be 0.678 mg/L. 100 mg/L sodium thiosulfate was added to drinking water samples from Bullet Tree to neutralize chlorine and prevent degradation. Samples were thoroughly mixed and placed in the freezer.

3.2.1.3 Sediment Samples

The sediment sampling method used was based on the U.S. EPA operating procedure for sediment sampling (U.S. Environmental Protection Agency, 2014). Sediment samples were collected at two points in each community: upstream of the drinking water intake and at the intake. Duplicates were collected at each sampling point. Sediment samples were always collected following water sample collection.

For sites that were shallow enough to wade into, samples were collected by wading to the center of the river and scooping sediment along the bottom sediment in the upstream direction. Enough sample was obtained to fill a quart sized Ziploc bag, and was placed in a pan. Care was taken to avoid the loss of fine-grained material.

For sites that were too deep to wade into, a Ponar grab sampler was used. To collect sediment samples, both sides of the sampler were opened, and the sampler was lowered to the bottom sediment. The weighted messenger was released to close the sampler so that it scraped and collected bottom sediment as it closed. The sampler was then raised out of the water, and the captured sediment was emptied into a pan.

In the pan, each sediment sample was quartered to ensure that it was thoroughly homogenized. Samples were then stored in quart sized Ziploc bags, placed in a cooler with ice packs, and frozen as soon as possible.

3.2.1.4 Sample Preservation and Transportation

All samples were kept frozen until the time of shipment. The 125 mL water samples and the sediment samples were packaged in a cooler with icepacks and shipped to Brookside Laboratories in New Bremen, Ohio. The 1 L bottles were packaged in coolers with icepacks and shipped to University of Kentucky.

3.2.2 Water Quality Analysis

In the field, a YSI multiparameter meter was used to collect readings on site coordinates, temperature, conductivity, dissolved oxygen, salinity, total dissolved solids, chloride, and ammonia. Nutrient concentrations and pH were measured for the samples sent to University of Kentucky. Nutrient concentrations were determined using the orthophosphate [method PO-19 (224800) and PO-19A (224801)] and nitrate [method NI-11 (146803)] test kits included in the Hach Surface Water test kit.

For the orthophosphate test, two tubes were each filled with 5 mL of sample. One tube was placed into the left opening of the color comparator box. In the second tube, one of the included PhosVer3 Phosphate Reagent Powder Pillow was added. The second tube was then swirled to mix until a blue color developed and set aside for one minute. Within five minutes, the second tube was also placed in the color comparator box. The box was held in front of a light source, and the color disc was turned until a color match was identified. The given value in the scale window was divided by 10 to obtain orthophosphate concentration in mg/L.

For the nitrate test, two test tubes were each filled with 5 mL of sample. One of the tubes was placed into the left opening of the color comparator box. In the second tube, one of the included NitraVer 5 Nitrate Reagent pillows was added. The second tube was capped and shaken vigorously to mix for one minute, then set aside for one minute. The second tube was then also placed in the color comparator box. The box was held in front of a light source, and the color disc on the box was turned until a match was identified. The associated value gave nitrate concentration in mg/L.

The Mettler Toledo Benchtop FP20 pH/mV Meter was used to measure pH of water samples. The meter was properly calibrated before testing samples, and the probe was rinsed with deionized water and patted dry before each reading. To measure pH, the probe was lowered into the sample, the "read" button was pressed, and a reading was taken once the signal had stabilized.

3.2.3 Glyphosate Determination

3.2.3.1 High Performance Liquid Chromatography (HPLC)

Water and sediment samples were analyzed at Brookside Laboratories using High Performance Liquid Chromatography according to EPA method 547 (U.S. Environmental Protection Agency, 1990). The detection limit for this method is 25 ppb.

3.2.3.2 Enzyme-Linked Immunosorbent Assay (ELISA) Kits

For the larger 1 L water samples sent to University of Kentucky, enzyme-linked immunosorbent assay (ELISA) kits were used to determine glyphosate concentrations. Glyphosate Microtiter Plate kits purchased from Abraxis were used for this analysis, and the included procedure was followed. Contents of the kit were stored in a refrigerator until time of analysis. Sample bottles were removed from the freezer, each individually placed in a sealed plastic bag, and set in a water bath until completely melted. The contents of the kit were allowed to reach room temperature before beginning analysis.

Once all the samples and contents were at an appropriate temperature, the included Wash Buffer was first diluted at a ratio of 1:5. The Derivatization Reagent was diluted with 3.5 mL of Derivatization Reagent Diluent and thoroughly mixed with a vortex mixer. A disposable glass test tube was labeled for each standard, control, and sample. There were six standards, a positive control, negative controls with deionized water and tap water, and the six water samples. Concentrations for each standard and control and shown in Table 3-1. Triplicates of each substrate were prepared. 250 µL of each substrate was pipetted into the appropriate labeled test tube. 1 mL of the Assay Buffer was added to each test tube, and vortexed to mix. 100 µL of the diluted Derivatization Reagent was added to each test tube, and each tube was vortexed immediately after until no swirling lines were present. Test tubes were left at room temperature for 10 minutes. After 10 minutes, each tube was vortexed again, and 50 µL of each substrate was pipetted into individual wells of the microtiter plate. 50 µL of the Antibody Solution was then added to each well using a multi-channel pipette. Wells were covered with parafilm and carefully swirled in a circular motion on the benchtop for 60 seconds to mix contents of wells. The plate was left at room temperature for 30 minutes. After 30 minutes, 50 µL of the Enzyme Conjugate Solution was added to each well using a multi-channel pipette. Once again, the plate was covered with parafilm, and swirled on the benchtop to mix for 60 seconds. The plate was left at room temperature for 60 minutes. After 60 minutes, the contents were then decanted into a sink, and inverted and blotted on a paper towel. The plate was then washed three times with the diluted Wash Buffer, each time with a volume of at least 250 µL in each well.

After each wash, the plate was decanted and blotted on a paper towel. After the last wash, all wash buffer was removed. 150 µL of the Color Solution was added to each well using a multi-channel pipette. The plate was covered with parafilm, swirled on the benchtop for 30 seconds, and left at room temperature for 20-30 minutes. After 20-30 minutes, 100 µL of the Stop Solution was added to each well using a multi-channel pipette. Within 15 minutes of this last step, the absorbance was read at 450 nm using an Abraxis microtiter plate ELISA photometer. Three readings were taken, and the average absorbance of the three runs was calculated for each triplicate.

Table 3-1 Known concentrations of ELISA kit standards and positive control

ELISA Kit Glyphosate Standard/Control Concentrations	
Standard/Control	**Concentration (ppb)**
Standard 0	0
Standard 1	0.075
Standard 2	0.20
Standard 3	0.50
Standard 4	1
Standard 5	4
Positive Control	0.75 ± 0.2

For each substrate, the average absorbance and standard deviation of the three triplicates were calculated. To determine glyphosate concentrations, the mean absorbance for standards 1-5 was divided by the absorbance for the zero standard to yield $\%B/B_0$. The log of each known concentration associated with standards 1-5 was calculated. $\%B/B_0$ was plotted on the vertical axis and the respective log glyphosate concentration was plotted on the horizontal axis. A trendline was determined, and from the trendline equation, the concentration of each sample could be determined (Figure 3-3).

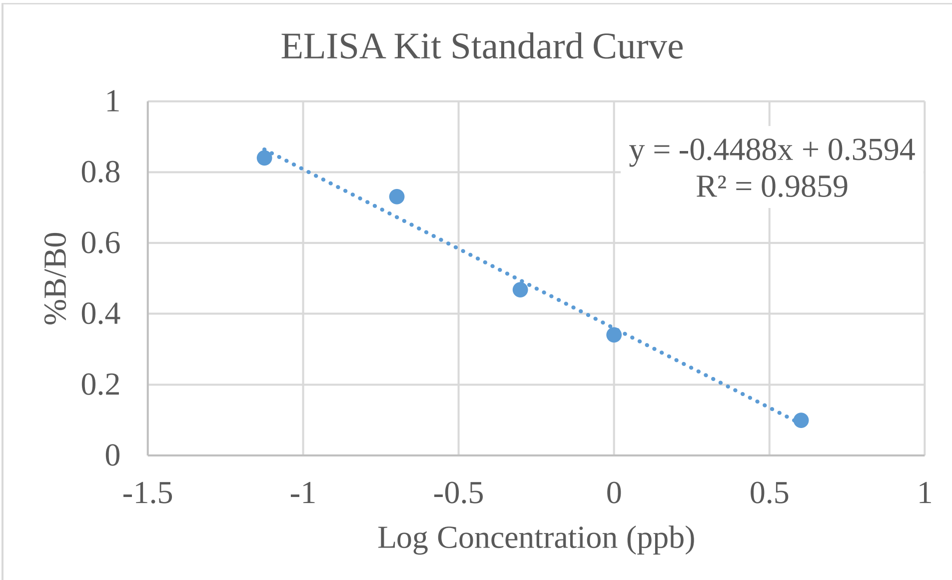

Figure 3-3 Standard curve generated from absorbances of standards, used to calculate glyphosate concentrations in samples.

3.2.3.3 Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS)

Following ELISA kit analysis, water samples were also sent to a Department of Pharmaceutical Sciences laboratory at the University of Kentucky with the capacity to conduct Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). Prior to delivering samples to lab, samples were filtered using vacuum filtration with 0.7 µm pore size glass fiber filter papers. Filters were leached with about 200 mL of sample before sample was collected. The filtered samples were stored in 125 mL amber opaque plastic bottles and delivered to the lab in a cooler. Samples were frozen promptly upon delivery. The lab developed a method based on the USGS method 5-A10 for determination of glyphosate and its degradation products aminomethylphosphonic acid and glufosinate by isoptope dilution, online solid-phase extraction, and LC-MS/MS (Meyer, 2009). However, the lab deviated from the USGS method by forgoing the solid-phase extraction step, resulting in the occurrence of matrix interference which raised the expected method detection limit from 0.02 ppb to 0.19 ppb.

3.3 Modeling Approach

3.3.1.1 Overview of Methodology

The overall modeling approach employed in this study was to first use SWAT to develop a watershed model for the Belize River Watershed and simulate the application of glyphosate for agricultural purposes in this region (Figure 3-4). Model performance was then determined by calibrating the model for observed flow rate data, and validating the model using a flow rate dataset independent from calibration data. Once calibration and validation were performed, a simulation was run. Following the simulation, simulated sediment loads were compared to limited observed data for suspended sediment, and simulated glyphosate loads were compared to the glyphosate concentrations determined from the field work portion of this study. From these comparisons, preliminary conclusions were made on the current state of glyphosate transport in the Belize River Watershed and whether further work is justified.

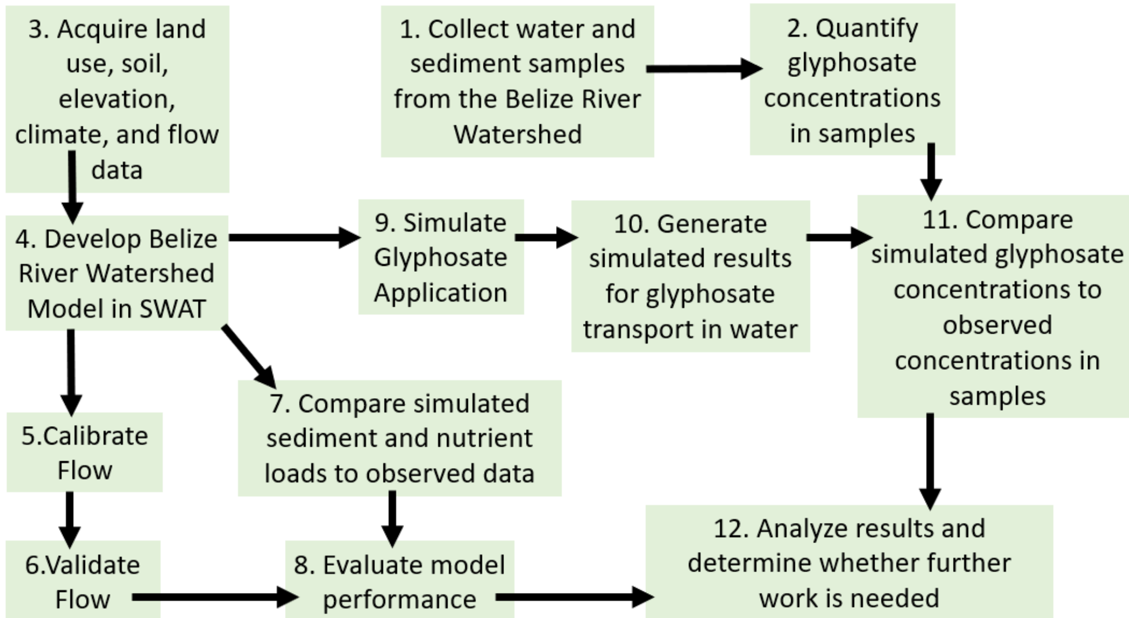

Figure 3-4 Flow diagram of study methodology

3.3.1.2 Data Acquisition

Spatial datasets required for watershed model setup were obtained from public databases. A digital elevation model with 30 m spatial resolution was obtained from the World Bank Data Catalog (World Bank -European Space Agency Partnership, 2018). Stream network data was retrieved from the Biodiversity and Environmental Resource Data System of Belize (Meerman, 2017). Belize land use data was extracted from a land use dataset for Central America with 1 km resolution created by Central American Commission on Environment and Development, U.S. Agency for International Development, International Resources Group Ltd., The Nature Conservancy, and Winrock International, and published by the NASA Socioeconomic Data and Applications Center (Central American Commission on et al., 1998). Soil data were extracted from a 1:5,000,000 scale soil map of the world provided by the Food and Agricultural Organization of the United Nations (FAO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) (FAO/UNESCO, 2020). Higher resolution soil data were available; however it was not in a soil classification system that could be readily applied in SWAT. Therefore, the FAO-UNESCO soil dataset was used for the purposes of this study. Historical weather data was obtained from the National Meteorological Service of Belize from three weather stations within the watershed, located in Ladyville, Belmopan, and Spanish Lookout. Daily precipitation, maximum temperature, and minimum temperature was provided upon request for Ladyville and Belmopan weather stations, from January 1, 1999 to September 30, 2019. Daily precipitation was provided for Spanish Lookout, from January 1, 1999 to July 31, 2019. Daily discharge data was provided upon request by the National Hydrological Service of the Ministry of Natural Resources in Belize. Data was measured at two monitoring locations in the watershed: Big Falls Ranch and Double Run. Data from Big Falls Ranch spanned from August 1, 1981 to October 31, 2005. Data from Double Run spanned from February 9, 1981 to December 31, 2013.

3.3.1.3 Model Set up

3.3.1.3.1 Watershed Delineation

SWAT Version 2012 and the ArcSWAT interface were chosen to set up the watershed model (Winchell et al., 2013). All data was projected to WGS_1984_UTM_ZONE_16N. To delineate the watershed, the digital elevation model was uploaded, and a stream network was imported. Streams and outlet points were defined, and additional outlet points were added for the two sites at which observed flow rate data exists. The study area was delineated into 53 subbasins (Figures 3-5 and 3-6).

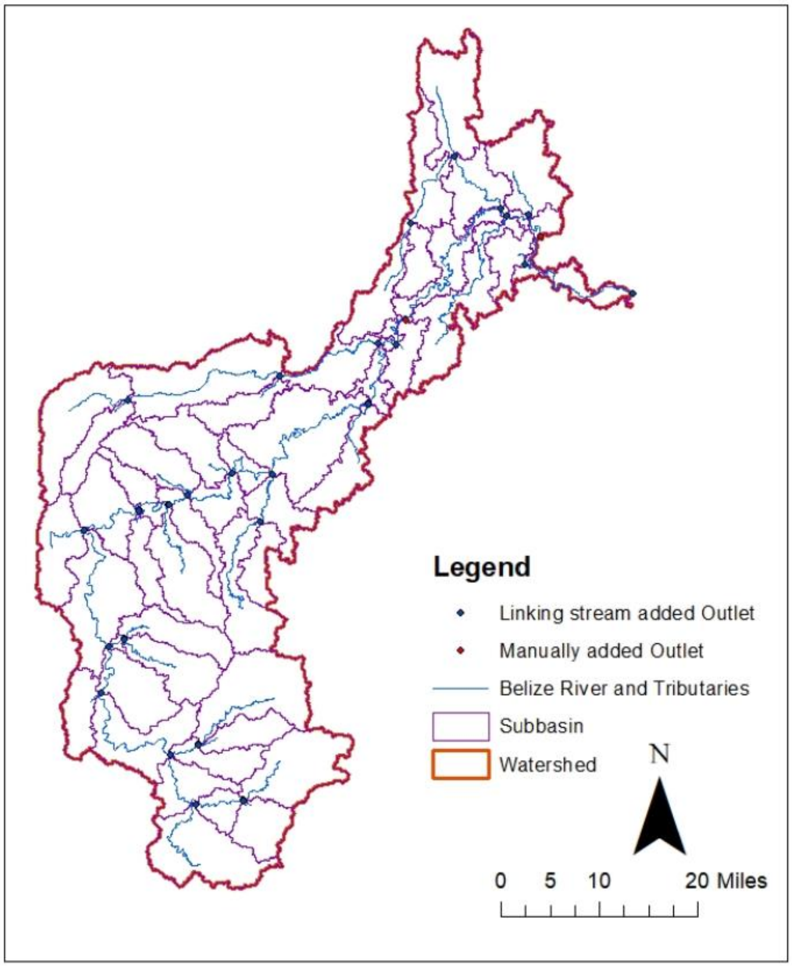

Figure 3-5 Map of the Belize River Watershed delineated in SWAT

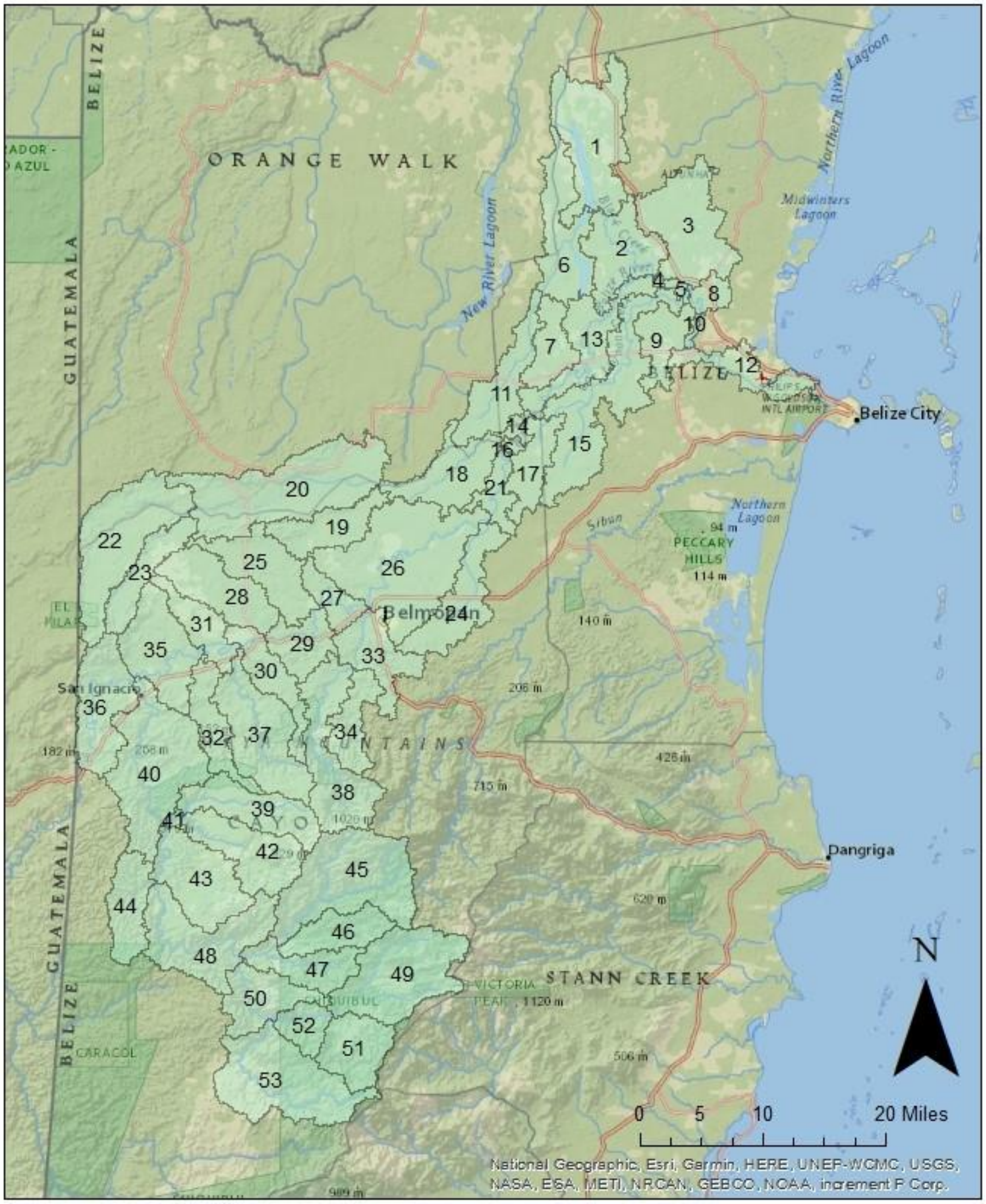

Figure 3-6 Subbasin number key

3.3.1.3.2 Creation of Hydrologic Response Units

Hydrologic response units (HRUs) were created in SWAT to represent regions of the watershed that were homogenous in soil type, land use, and slope, and were therefore assumed to respond similarly to various hydrological conditions (Winchell et al., 2013). Land use, soil, and slope data were required to create HRUs. Before land use data could be used in SWAT, it first had to be converted to land use types in the SWAT database. A lookup table was created to reclassify to the respective SWAT land use code (Figure 3-7, Table 3-2). Because available soil data used FAO soil classification, the user soil table in the SWAT 2012 database using the USDA soil taxonomy system needed to be replaced. MWSWAT 2009, an older version of SWAT for a different user interface, was installed. Within the MWSWAT 2009 database, a soil database using FAO classification with all the required soil data could be found. This table was imported into the SWAT 2012 database. A look up table was created to reclassify the soil ID with the respective soil name now listed in the SWAT 2012 user soil database. The soil layer and respective soil classes are shown in Figure 3-8 and Table 3-3. The slope geoprocessing tool in ArcMap was used to determine the ranges to be used for the slope classification step of HRU analysis, based on the digital elevation model. The number of slope classes selected was 3, and ranges were determined to be 0-14%, 14-32%, and 32% and up (Figure 3-9). In HRU analysis, land use and soil data were uploaded and reclassified, and slope classification was specified. These layers were overlaid, and an HRU feature class was created. To define HRUs, a threshold of 20% land use, 10% soil, and 20% slope was indicated. These thresholds were used because they have been shown to be adequate for most applications (Winchell et al., 2013). Land use classification was further refined to split agricultural land use into four crops; corn, sugarcane, soybean, and beans (represented in SWAT as kidney beans). These crops were selected based on local knowledge and by recommendation of the Pesticide Control Board of Belize. It was assumed that there was an equal distribution of these four crop types. HRUs could then be created, which resulted in 181 HRUs in the watershed.

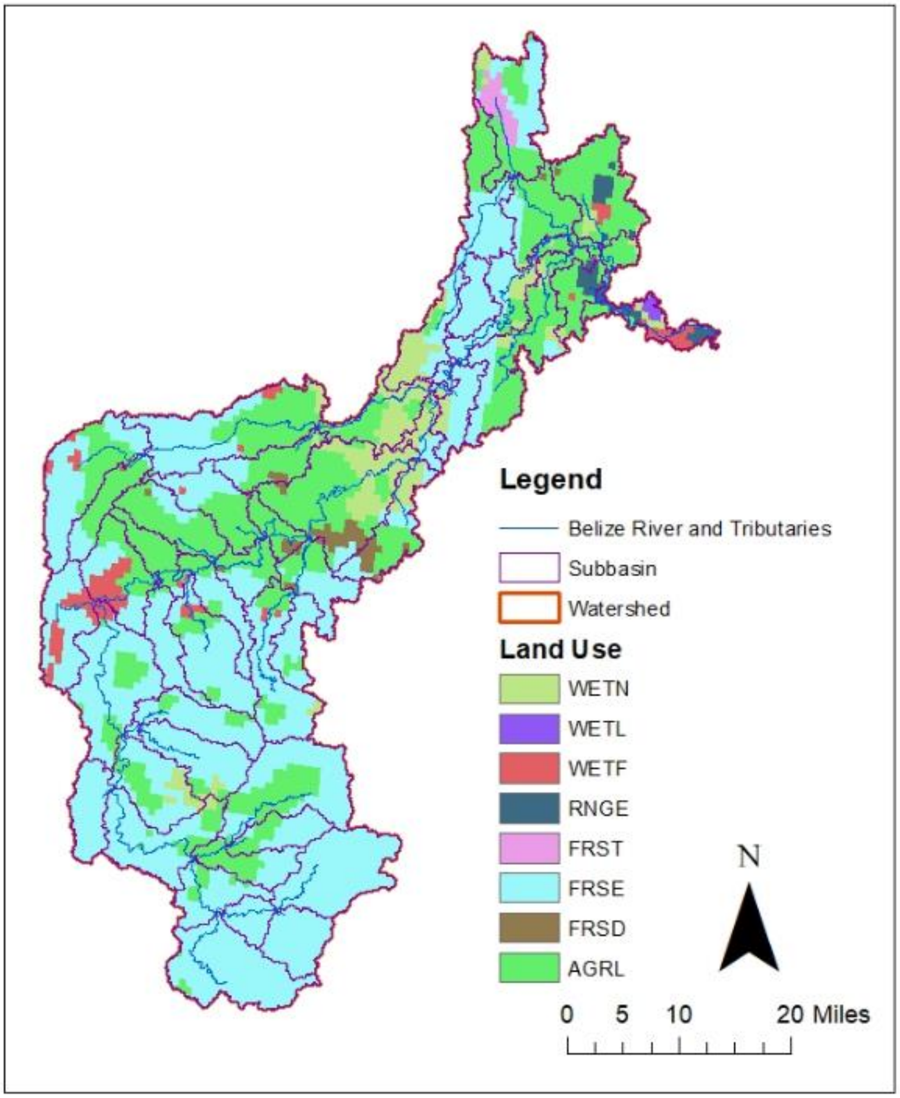

Figure 3-7 Land use layer. WETN is non-forested wetlands, WETL is mixed forested and non-forested wetlands, WETF is forested wetlands, RNGE is range grasses, FRST is mixed forest, FRSE is evergreen forest, FRSD is deciduous forest, and AGRL is agricultural land.

Table 3-2 Belize Land Use Classification Table

Original Dataset Land Cover Type	Reclassified SWAT Code	SWAT Land Cover Name
Tropical Needleleaf Evergreen Forest	FRSE	Forest-Evergreen
Tropical Broadleaf Evergreen Forest	FRSE	Forest-Evergreen
Tropical Broadleaf/Needleleaf Evergreen Forest	FRSE	Forest-Evergreen
Tropical Broadleaf Deciduous Forest	FRSD	Forest-Deciduous
Tropical Swamp Forest	WETF	Wetlands-Forested
Palm Forest	FRSE	Forest-Evergreen
Mangroves	WETF	Wetlands-Forested
Tropical Needleleaf Evergreen Woodland	FRSE	Forest-Evergreen
Tropical Broadleaf Evergreen Woodland	FRSE	Forest-Evergreen
Tropical Broadleaf Deciduous Woodland	FRSD	Forest-Deciduous
Tropical Broadleaf/Needleleaf Woodland	FRST	Forest-Mixed
Tropical Broadleaf Evergreen Savanna	FRSE	Forest-Evergreen
Tropical Needleleaf Evergreen Savanna	FRSE	Forest-Evergreen
Tropical Broadleaf Evergreen Scrub/Shrub	FRSE	Forest-Evergreen
Tropical Cactus/Thorn Shrub	RNGB	Range-Brush
Tropical Swamp Scrub/Shrub	WETN	Wetlands-Nonforested
Tropical Perennial Gramminoid Grassland	RNGE	Range-Grasses
Tropical Herbaceous Wetland	WETL	Wetlands-Mixed
Barron Rock, Sand, and Soil	SWRN	Southwestern US (Arid) Range
Marine	WATR	Water
Inland Water	WATR	Water
Forest-Woodland-Agriculture Complex	AGRL	Agricultural Land-Generic
Urban/Vegetation Complex	URML	Residential-Med/Low Density
Agriculture	AGRL	Agricultural Land-Generic
Urban/Industrial	UIDU	Industrial

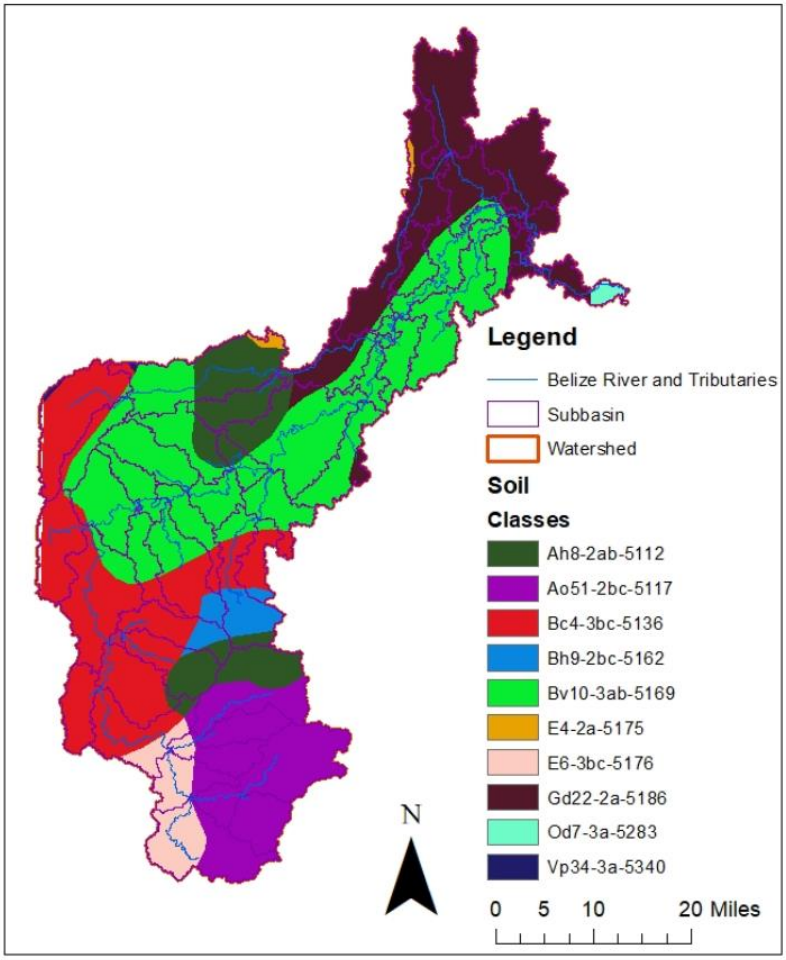

Figure 3-8 Soil Layer.

Table 3-3 Soil Classes in the Belize River Watershed

Soil Type	Hydrologic Soil Group	Texture
Ah8-2ab-5112	C	Loam
Ao51-2bc-5117	C	Loam
Bc4-3bc-5136	C	Clay-Loam
Bh9-2bc-5162	C	Loam
Bv10-3ab-5169	D	Clay
E4-2a-5175	D	Clay-Loam
E6-3bc-5176	D	Clay
Gd22-2a-5186	D	Loam
Od7-3a-5283	C	Clay-Loam
Vp34-3a-5340	C	Clay-Loam

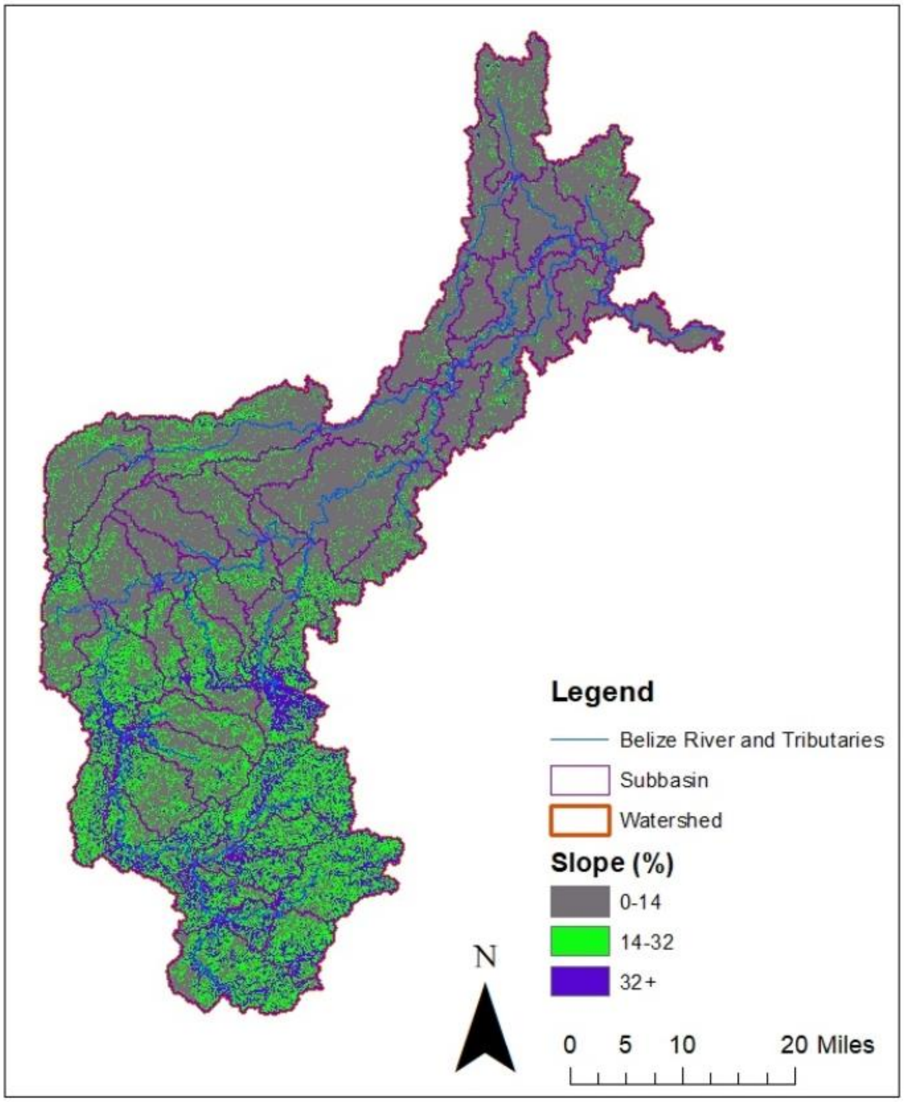

Figure 3-9 Slope layer

3.3.1.3.3 Weather

To model a watershed outside of the United States, the WGEN_user table of the SWAT 2012 database was edited to incorporate weather data from the region. WGEN_user requires climate statistic information to generate weather simulations to fill in missing observed data, model the hydrologic cycle, and predict plant growth. The weather stations in Ladyville and Belmopan were used for this table because their datasets included both temperature and precipitation. The WGNmaker4 excel macro tool was installed and used to calculate temperature and precipitation statistics given the observed data. Information regarding hourly maximum rainfall, solar radiation, wind speed, and dew point are also required for this table, however these data weren't available. The WGEN_US_First_Order table in the SWAT 2012 database contains all of the necessary statistics for weather stations in the United States. A weather station in Key West, Florida was used to supplement the missing data being that it was the closest listed weather station in proximity to Belize and the climate is relatively similar.

Input text files were written for observed daily precipitation data from the Ladyville, Belmopan, and Spanish Lookout stations, and for daily maximum and minimum temperature for the Belmopan and Ladyville stations. Within SWAT, a weather input data was written given observed precipitation and temperature and simulated solar radiation, wind speed, and humidity. All the required input files were then written.

3.3.1.3.4 Glyphosate Application Simulation

The management input file was edited to incorporate the use of glyphosate in the watershed. Table 3-4 lists the selected glyphosate application rate per crop and the reference from which the assumption was based on. It was assumed that "Round-up Ready" crops genetically modified to be resistant to glyphosate are not grown in the region, because while genetically modified crops and products have been imported into the region, the cultivation of these crops is not permitted (Alam, 2019; Jacobs, 2016). Therefore, it was assumed that glyphosate application occurred first in the management schedule, before the planting of crops.

Table 3-4 Glyphosate application rates per crop type

Crop Type	Application Rate (kg/ha)	Reference
Corn	0.87	(Love et al., 2011)
Soybean	0.87	(Love et al., 2011)
Beans	2.36	(University of Kentucky Research and Education Center at Princeton)
Sugarcane	4.93	(Sugar Research Australia, 2017)

Default physiochemical properties of glyphosate from the SWAT pesticide database were applied. These properties can be seen in Figure 3-10, where SKOC is the soil adsorption coefficient normalized for soil organic carbon content in (mg/kg)/(mg/L), WOF is the wash-off fraction, HLIFE_F is the pesticide half-life on foliage, HLIFE_S is the pesticide half-life in soil, AP_EF is the application efficiency, and WSOL is water solubility. The routing pesticide option in the general watershed data input file was edited to allow for the transport of glyphosate through the channel network. The rewrite input files option was then used to account for these changes.

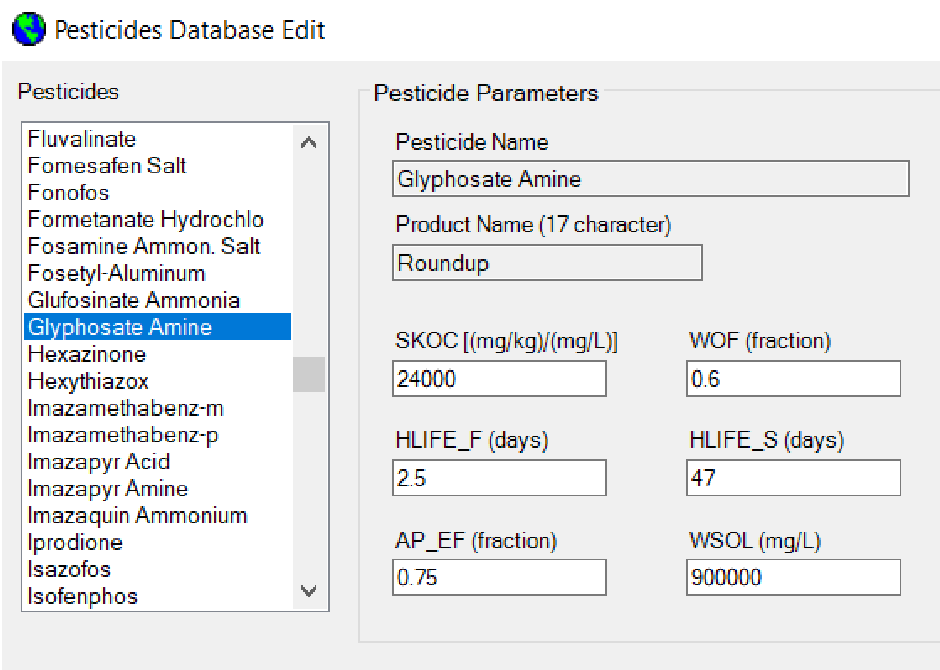

Figure 3-10 Glyphosate chemical properties in SWAT database (adapted from ArcSWAT 2012).

3.3.1.4 Model Calibration

The SWAT Calibration and Uncertainty Program (SWAT-CUP) was selected to be used for calibration of the model. SWAT-CUP is a calibration program designed for use with SWAT and contains five different calibration procedures. Of the five procedures, the Sequential Uncertainty Fitting Version 2 (SUFI-2) procedure was selected based on its repeated use in literature and demonstrated efficiency with large scale models (Abbaspour et al., 2015). SUFI-2 uses Latin Hypercube sampling to obtain a distribution of outputs and creates an uncertainty band called the 95% prediction uncertainty (95PPU), and seeks to contain the largest fraction of observed data within this uncertainty band (known as the P-factor), while minimizing the average thickness of the uncertainty band (known as the R-factor) (Abbaspour, 2015; Khalid et al., 2016).

Because a large dataset of glyphosate monitoring was not available, the watershed model was calibrated for flow to ensure that the model was at least representing hydrological processes in the watershed. Daily observed flow rate data was only available at two locations in the watershed; Double Run Water Treatment Plant located in subbasin 8, and Big Falls Ranch located in subbasin 14. Calibration of flow in just two subbasins to extrapolate to the entire watershed is an imperfect method that gives rise to uncertainty due to the order of magnitude difference in scale. However, the calibration method employed is limited due to the availability of data collected in Belize but serves as a starting point for the calibration of hydrological processes in the Belize River Watershed.

To set up the calibration, calibration input files were created. In a parameterization file, input parameters and their respective ranges were selected. Ranges were determined based on feasible values for each parameter and whether the parameter can be replaced with a new value, or if the parameter will differ spatially with relative changes across the watershed. The initial parameters and ranges selected can be seen in Table 3-5, and were selected based upon recommendations for similar applications in literature (Moriasi et al., 2007). The number of simulations per calibration iteration was specified to be 500, as recommended (Abbaspour, 2015). In an observation file, observed daily flow rate data from Double Run from 2001-2009 and from Big Falls Ranch from 2001-2005 were compiled in the required format. Necessary edits were made to the extraction files to designate the names of the subbasins for which flow rate data was collected, from where to retrieve the respective simulated values, and the duration of simulation time. In the objective function files, the names of variables being calibrated were given, the type of objective function was selected, a solution threshold was given, and the observation data were compiled once again. The Nash-Sutcliffe (NS) function (Equation 1) was specified as the objective function, and a threshold of 0.5 was indicated. Nash-Sutcliffe efficiency is an indicator of the goodness of fit of hydrologic models and is commonly used and recommended in literature for similar applications (ASCE, 1993; Moriasi et al., 2007). NS values range from $-\infty$ to 1, with 1 being representing a perfect fit between simulated and observed data. NS values in the range of 0.5 to 0.65 represent satisfactory model performance (Moriasi et al., 2007). The Coefficient of Determination function (Equation 2) was also considered in evaluating model performance, and also has a minimum of 0.5 for satisfactory performance (Moriasi et al., 2007). The calibration was then able to be executed, and after running 500 simulations, new recommended parameter ranges were given. These new ranges were imported into the initial parameterization file, checked to ensure they were within the absolute feasible ranges, and the calibration was run again. This process was repeated 6 times until the NS efficiency value was within the specified threshold and there were a suitable number of solutions.

Table 3-5 Initial parameter ranges for first iteration of calibration, representing all feasible values of each parameter. The type of changes for parameters were either relative, meaning percent change for all parameter values, or replace, meaning all parameter values were changed uniformly to a new value within the specified range.

Parameter	Type of Change	Minimum	Maximum
Soil Conservation Service curve number	Relative	-0.50	0.20
Baseflow alpha factor (1/days)	Replace	0.10	1
Groundwater delay time (days)	Replace	0	500
Threshold depth of water in the shallow aquifer required for return flow to occur (mm H2O)	Replace	0	5000
Groundwater revap coefficient	Replace	0.02	0.20
Threshold depth of water in the shallow aquifer for revap or percolation to the deep aquifer to occur (mm H2O)	Replace	0	500
Deep aquifer percolation fraction	Replace	0	1
Manning's "n" value for overland flow	Relative	-0.80	2
Soil evaporation compensation factor	Replace	0.01	1
Plant uptake compensation factor	Replace	0.01	1
Available water capacity of soil layer (mm H2O/mm soil)	Relative	-0.90	4.50
Manning's "n" value for main channel	Replace	0.01	0.15
Surface runoff lag coefficient	Replace	1	24

Equation 1. Nash-Sutcliffe Efficiency (NS)

$$NS = 1 - \frac{\sum_i (Q_m - Q_s)_i^{\,2}}{\sum_i \left(Q_{m,i} - \bar{Q}_m\right)^2}$$

Where Q is the variable being calibrated, Q_m is measured data, Q_s is simulated data, $\bar{Q}_m$ is the mean of measured values of Q, and i is the data index. The objective is to maximize NS.

Equation 2. Coefficient of Determination (R^2)

$$R^2 = \frac{\left[\sum_i (Q_{m,i} - \bar{Q}_m)(Q_{s,i} - \bar{Q}_s)\right]^2}{\sum_i (Q_{m,i} - \bar{Q}_m)^2 \sum_i (Q_{s,i} - \bar{Q}_s)^2}$$

Where Q is the variable being calibrated, Q_m is measured data, Q_s is simulated data, $\bar{Q}_m$ is the mean of measured values of Q, $\bar{Q}_s$ is the mean of simulated values, and i is the data index. The objective is to maximize R^2.

3.3.1.5 Model Validation

Model validation was conducted by inputting the parameters that resulted in successful calibration, daily observed flow rate data for subbasin 8 for a period from 2010 to 2013, and running one iteration of 500 simulations to evaluate how well the model performs for data not used in calibration. A NS or R^2 value above the threshold of 0.5 indicates satisfactory model validation.

3.3.1.6 Sensitivity Analysis

Sensitivity analysis was conducted within SWAT-CUP using the Global Sensitivity Analysis tool. The Global Analysis tool estimates in the change in the objective function from the change in each parameter while all parameters are changing, giving the sensitivity of each parameter relative to the other parameters (Abbaspour, 2015). The tool uses a multiple regression analysis and t-test to obtain parameter sensitivity statistics. T-stat and p-value are calculated for each parameter. T-stat is the regression coefficient divided by the standard error, and p-value is used to test the null hypothesis that the coefficient is equal to zero, meaning no significant change in objective function with parameter change. The larger the absolute value of t-stat and smaller the p-value, the greater the sensitivity of the parameter (Abbaspour, 2015).

3.3.1.7 Simulation

Following validation, a watershed simulation was run on a daily time step for the period of January 1, 1999 to September 30, 2019. A warm-up period of 2 years was specified to allow the watershed parameters to come to a reasonable state. A warm-up period of 2-5 years is recommended (Winchell et al., 2013).

3.3.1.8 Analysis of Simulated Results

The length of river or stream within each subbasin in the watershed is referred to as the reach. SWAT reports pesticide loads on units of mg active ingredient during time step, for both simulated soluble glyphosate and glyphosate sorbed to sediment transported with water into and out of each reach. Simulated glyphosate loads were converted to concentrations by first converting average daily streamflows into and out of each reach during each time step to volume of water flowing into and out of each reach during each time step, and then dividing glyphosate load during each time step by volume of water during each time step to yield glyphosate concentrations in water in mg/L. These concentrations were then converted to μg/L.

Average glyphosate concentrations in each subbasin were calculated using data from the entire simulation. Because the climate in Belize consists of two seasons; rainy and dry seasons, average concentrations in each subbasin were also calculated for each season. The dry season typically lasts from November to May, with November and May being transition periods. The wet season typically lasts from May to November, with the onset of the wet season ranging from early May in Northern Belize to early June in Southern Belize. For the purposes of determining average concentrations across the watershed for both seasons, the dry season was established as December to April, and the wet season was established as May to November. For comparison of observed nutrient concentrations to standards and simulated nutrient concentrations, measured concentrations of orthophosphate were converted to orthophosphate as phosphorus by multiplying by the conversion factor 0.33 (HACH, 2019b). Nitrate concentrations were converted to nitrate as nitrogen by dividing by the conversion factor 4.43 (HACH, 2019a). Data were analyzed using a single factor ANOVA test with a significance level $\alpha = 0.05$ to determine significant differences based on site, season, or type of glyphosate load.

CHAPTER 4. RESULTS & DISCUSSION

4.1 Water Quality

Table 4-1 presents the results of water quality analyses from multimeter readings from the field for temperature, conductivity, dissolved oxygen, salinity, total dissolved solids, chloride, and ammonia. Belize does not currently have national standards for drinking water quality, or for monitoring river and stream health. Instead, Belize follows the World Health Organization guidelines for drinking water and has set effluent limitations for different industries' wastewater discharges. Therefore, observed data was compared to these standards as well as to EPA guidelines for rivers and streams to consider impacts from non-point source pollution.

The observed dissolved oxygen levels are above the US EPA recommended minimum levels for warm water aquatic life of a 7 day mean of 6 mg/L for early life stages and a 30 day mean of 5.5 mg/L for other life stages (US EPA, 1986). This means dissolved oxygen concentrations in these areas are supportive of aquatic life and not representative of eutrophic activity. The observed levels for ammonia and chloride also are within the ranges recommended for freshwater aquatic life by the EPA (US EPA, 2004, 2013). Total dissolved solids and chloride are within the recommended ranges for the Belize Effluent Limitations, WHO Guidelines for Drinking Water, and the National Secondary Drinking Water Regulations set by the EPA (Belize Department of Environment, 2003; US EPA, 2009; World Health Organization, 2017)

Table 4-1 Water quality parameters of each sample. Dissolved oxygen, total dissolved solids (TDS), chloride, and ammonia concentrations meet standards set by the US EPA.

Sampling Point	Temp-erature (°C)	Conduct-ivity (us/cm)	Dissolved Oxygen (mg/L)	Salinity (ppt)	TDS (mg/L)	Chloride (mg/L)	Ammonia (mg/L)
Bullet Tree Upstream	29.00	430.30	7.93	0.19	259.88	6.10	0.13
Bullet Tree Abstraction Site	28.80	428.90	7.77	0.19	259.91	7.25	0.14
Bullet Tree Drinking Water	30.05	431.75	7.64	0.19	255.92	5.29	0.10
Spanish Lookout Upstream	30.40	350.25	8.40	0.17	233.05	7.55	0.09
Spanish Lookout Abstraction Site	30.60	360.60	13.43	0.17	234.37	7.35	0.10
Spanish Lookout Drinking Water	35.75	414.40	7.15	0.16	223.53	13.01	0.10

Table 4-2 presents results from laboratory analyses for pH, orthophosphate, and nitrate. pH in each sample meet the EPA recommended criteria for aquatic life, as well as the Belize Effluent Limitations and the EPA Secondary Drinking Water Standards (Belize Department of Environment, 2003; US EPA, 2004, 2009). Observed phosphate and nitrate concentrations are all below the Belize Effluent Limitations for phosphate (5 mg/L) and nitrate (3-10 mg/L) (Belize Department of Environment, 2003). EPA standards for total phosphorus and total nitrogen in rivers and streams varies by region and water body type. The criteria for Total Phosphorus in rivers and streams ranges from 10 to 128 µg/L across the United States, and the observed concentrations of orthophosphate reported as phosphorus exceed the criteria in some of these regions (US EPA, 2002). However, when compared to ecoregion XII, the region in the US most similar to the climate of Belize, the observed concentrations fall below the standard of 40 µg/L (US EPA, 2002). It is important to note that orthophosphate as phosphorus does not consider organic forms of phosphorus that may also be present. Measured nitrate was reported as nitrogen concentrations, ranging from 0.45 to 0.90 mg/L. EPA standards for total nitrogen varies across the country from 0.12 mg/L to 2.2 mg/L (US EPA, 2002). In some regions in the US, the observed nitrogen concentrations would exceed EPA standards. When comparing to the standard for total nitrogen in ecoregion XII, concentrations in the samples from Bullet Tree Upstream, Bullet Tree abstraction site, and Spanish Lookout drinking water are equal to the standard of 0.9 mg/L. This means these areas are most likely exceeding the total nitrogen standard when considering nitrite and ammonia concentrations as well. All nitrate concentrations are below the US EPA standard for nitrate in drinking water (10 mg/L) and the WHO guideline for nitrate in drinking water (50 mg/L), which are protections to prevent

Methemoglobinemia often seen in infants ingesting elevated nitrate concentrations in water (US EPA, 2009; World Health Organization, 2017).

Table 4-2 Nutrient concentrations and pH for each sample. For direct comparison to EPA criteria for nutrients in rivers and streams, orthophosphate was converted to phosphorus, and nitrate was converted to nitrogen. While EPA nutrient standards vary across the US, comparison to the closest region's standards showed that observed phosphorus concentration met the standard, while observed nitrogen at Bullet Tree Upstream, Bullet Tree Abstraction Site, and Spanish Lookout drinking water exceeded the standard.

Sampling Point	**Orthophosphate (µg/L)**	**Orthophosphate as Phosphorus (µg/L)**	**Nitrate (mg/L)**	**Nitrate as Nitrogen (mg/L)**	**pH**
Bullet Tree Upstream	80	26.09	4	0.90	6.96
Bullet Tree Abstraction Site	40	13.04	4	0.90	6.93
Bullet Tree Drinking Water	40	13.04	2	0.45	7.77
Spanish Lookout Upstream	40	13.04	2	0.45	7.12
Spanish Lookout Abstraction Site	80	26.09	2	0.45	7.02
Spanish Lookout Drinking Water	40	13.04	4	0.90	7.67

4.2 Glyphosate Determination

4.2.1 HPLC Results

Neither Glyphosate nor AMPA were detected in any of the sediment or water samples analyzed at Brookside Laboratories. However, the detection limit for their method using HPLC was 25 ppb. This is significantly higher than the concentrations reported in the previous monitoring study in Belize, with average glyphosate concentrations ranging from 0.2 to 1.7 ppb (Kaiser, 2011). Additionally, though 2-day shipping was selected to transport samples from Belize, unforeseen difficulties with U.S. Customs prevented the samples from entering the country to be delivered on time. As a result, it took 17 days to deliver the samples to Brookside Laboratories. As the half-life of glyphosate ranges from 2 to 91 days in water , and it is recommended to store samples at 4 °C to analyze within two weeks or to keep frozen if storing for longer than two weeks, it is likely that any glyphosate present would have degraded during shipping time (W.A. Battaglin et al., 2014; U.S. Environmental Protection Agency, 1990). Additionally, expected concentrations were much lower than the detectable limit using HPLC. Though AMPA presence was likely due to its persistence and the period before samples were received that could have allowed for degradation, is likely that AMPA concentrations still would have been below 25 ppb.

4.2.2 ELISA Kit Results

Glyphosate concentrations of each sample were all found to be below the range of quantitation in water (0.075 ppb) as well as the limit of detection (0.05 ppb). The results of this analysis can be seen in Table 4-3

Table 4-3 ELISA Kit Analysis Results.

Sample	Average Concentration
Deionized Water	0.03±0.01*
Tap Water	0.04±0.01*
Bullet Tree Upstream	0.05±0.01*
Bullet Tree Abstraction Site	0.04±0.01*
Bullet Tree Drinking Water	0.04±0.02*
Spanish Lookout Upstream	0.04±0.01*
Spanish Lookout Abstraction Site	0.04±0.01*
Spanish Lookout Drinking Water	0.03±0.01*
Positive Control	0.80±0.09

* Samples at or below the limit of detection.

However, the calculated concentrations of some individual triplicates were at or slightly above the limit of detection. These were triplicates from Bullet Tree Upstream at 0.05 ppb, Bullet Tree Abstraction Site 0.05 ppb, Bullet Tree Drinking Water 0.06 ppb, and Spanish Lookout Upstream 0.05 ppb. Because these values are so close to the limit of detection and none of the average concentrations were above the limit of detection, it is concluded that the concentrations in these samples were all below the detection limit. The concentration for the positive control was measured to be 0.80 ppb, which is within range for the expected concentration, 0.75 ± 0.2 ppb, indicating that the method and analysis were likely to be done correctly.

This analysis was conducted on October 30, 2019, three months after samples were collected. They remained frozen after delivery, apart from being thawed, tested, and refrozen on three occasions for other analyses. According to EPA Method 547, glyphosate has been shown to remain stable in frozen samples for up to 18 months (U.S. Environmental Protection Agency, 1990). However, thawing and refreezing may have impacted the preservation. Additionally, there are some limitations to ELISA kits for glyphosate determination as they have been shown to have the potential for cross-reactivity with other compounds that may be present in environmental samples. Other possible sources for error include inadequate storage conditions of the ELISA kit reagents, pipetting mistakes, or incorrect incubation times, though care was taken to avoid these errors.

4.2.3 LC-MS/MS Results

The same samples tested using the ELISA kit were also analyzed using LC-MS/MS on February 7, 2020. Glyphosate was not detected in any of the samples. This analysis was conducted five months after sample collection and four incidences of thawing and refreezing, so degradation of any originally present glyphosate is likely. AMPA was not measured but may have been detectable at these concentrations. Results from this analysis can be seen in Table 4-4.

Table 4-4 LC-MS/MS Results.

Sample Name	Analyzed Glyphosate Concentration (µg/L)
Bullet Tree Upstream	0.01*
Bullet Tree Abstraction Site	0.00*
Bullet Tree Drinking Water	0.11*
Spanish Lookout Upstream	0.01*
Spanish Lookout Abstraction Site	0.00*
Spanish Lookout Drinking Water	0.00*

* Concentration below the detection limit established by this method (0.19 µg/L)

4.2.4 Summary of Glyphosate Determination Results

After three different methods of analysis, it can be concluded that glyphosate was not present in any of the water samples in concentrations above the lowest detectable limit of LC-MS/MS quantification. Therefore, the hypothesis that glyphosate is present in these locations of the Belize River is rejected. This is unexpected because of the proximity of the two sample locations to agriculture areas, extensive glyphosate application, and results from previous studies reporting widespread glyphosate presence in surface water bodies. Although in a different region of Belize, a published study examining glyphosate presence in surface water in Belize found all samples to be positive for glyphosate ranging from 0.2 to 1.7 ppb (Kaiser, 2011). Another monitoring study conducted in Mexico reported dry season average concentrations ranging from <0.13 to 36.71 ppb, and wet season average concentrations from <0.13 to 1.33 ppb (Ruiz-Toledo et al., 2014). A second study conducted in Mexico examining concentrations in groundwater and drinking water found concentrations ranging from 0.44 to 1.41 ppb in groundwater and 0.35 to 0.65ppb in drinking water (Rendon-von Osten & Dzul-Caamal, 2017).

These results indicate that it is likely if glyphosate was present in the samples, concentrations would have been below the detection limit for HPLC analysis at 25 ppb. While the ELISA and LC-MS/MS analyses would have been able to detect similar concentrations, analyses occurred several months after sample collection, and preservation may have been impacted by thawing and refreezing during that time. Glyphosate half-life in water ranges from 2-91 days, and an experiment investigating glyphosate biodegradation in a water sediment system reported that glyphosate was completely removed from water due to sorption or biodegradation after 40 days (S. Wang et al., 2016). After this point, glyphosate was only detected in sediment. If preservation was compromised, it is very likely that by the time analysis occurred, glyphosate would have been degraded to AMPA or other metabolites, or sorbed to particulate matter in the samples. Because the ELISA and LC-MS/MS analyses did not investigate glyphosate in sediment or AMPA concentrations, and samples were filtered through 0.7 µm filters before LC-MS/MS analysis, it is probable that these methods would not have been able to capture any glyphosate processes that would have been occurring at that time.

4.3 Model Results

4.3.1 Calibration

An acceptable value of 0.56 was achieved for the Nash-Sutcliffe (NS) efficiency of subbasin 8 in the sixth iteration of flow calibration (Figure 4-1). However, subbasin 14 was poorly simulated and not able to meet the threshold, with a NS efficiency of 0.15 (Figure 4-2). R^2 values are also reported, at 0.7 for subbasin 8 and 0.48 for subbasin 14, bringing subbasin 14 to nearly meeting the acceptable threshold for R^2.

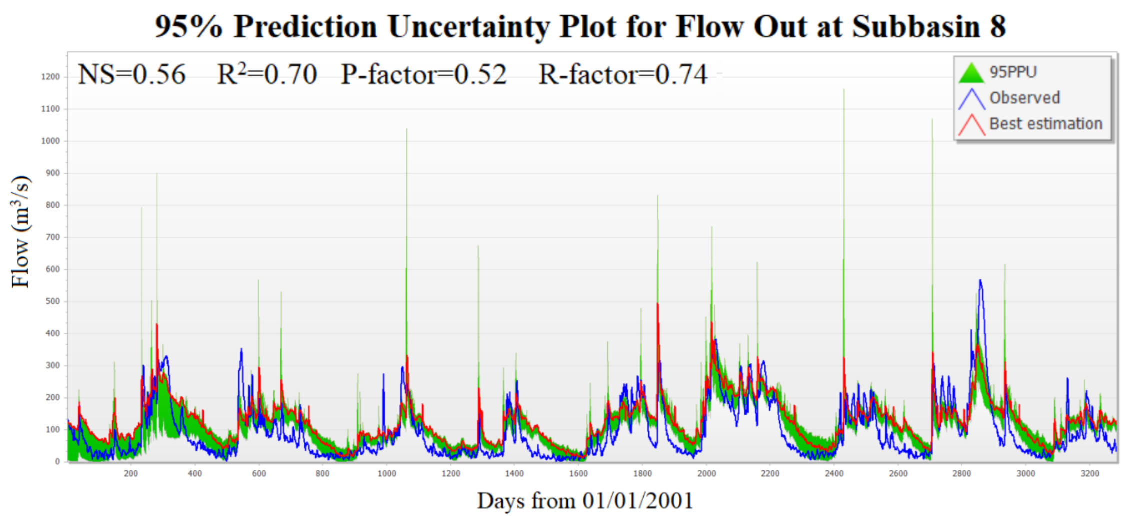

Figure 4-1 Summary of flow calibration at subbasin 8. Both NS efficiency and R^2 meets the threshold for adequate model performance, meaning that the model well represents the flow out of subbasin 8.

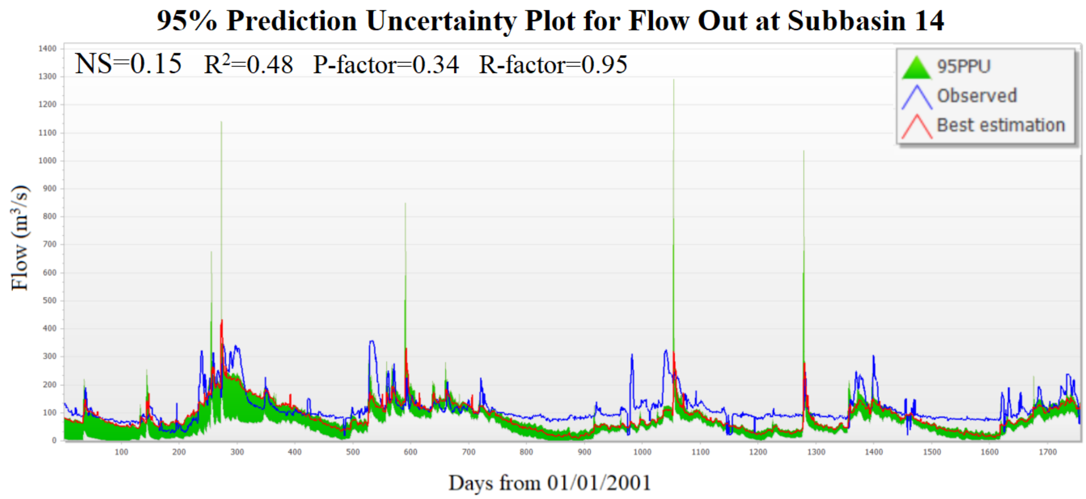

Figure 4-2 Summary of flow calibration at subbasin 14. NS efficiency does not meet the threshold for adequate model performance, while R^2 does meet the threshold. The model is close to being satisfactory for representing flow out of subbasin 14.

Because flow at subbasin 8 was well simulated, and subbasin 14 was far improved from the initial iteration, it was determined to move forward with validation using the parameter ranges from this iteration, shown in Table 4-5. Sensitivity analysis revealed that the following parameters, listed in order of decreasing sensitivity, were most influential to model outcomes for flow: Soil Conservation Service (SCS) curve number, threshold depth of water in the shallow aquifer required for return flow to occur, surface runoff lag coefficient, groundwater delay time, available water capacity of soil layer, Manning's "n" value for the main channel, soil evaporation compensation factor, and groundwater revap coefficient. This means that these parameters were the governing factors for simulating flow rate in the Belize River. SCS curve number was the most sensitive parameter, meaning the modeled flow is most sensitive to runoff. Curve number values were decreased throughout the watershed for calibration. Lower curve number values are representative of increased water retention in soil, while higher curve number values represent increased surface runoff. Because surface runoff in the Belize River watershed was minimized to calibrate simulated flow to observed flow, simulated glyphosate yields associated with runoff would likely be impacted and decreased from initial yields prior to calibration. Another study using SWAT to model pesticide transport reported that SCS curve number was the most influential parameter for governing Diazinon and Chlorpyrifos yields from agricultural areas (Luo & Zhang, 2009).

The model was found to not be very sensitive to the following parameters: threshold depth of water in the shallow aquifer required for revap or percolation to the deep aquifer to occur, baseflow alpha factor, Manning's "n" value for overland flow, deep aquifer percolation fraction, and plant uptake compensation factor. This means that these parameters did not play a significant role in modifying simulated flow rate. A summary of these statistics is found in Table 4-6.

Table 4-5 Final parameter ranges for model calibrated for flow. The type of changes for parameters were either relative, meaning percent change for all parameter values, or replace, meaning all parameter values were changed uniformly to a new value within the specified range.

Parameter	**Type of Change**	**Minimum**	**Maximum**
Soil Conservation Service curve number	Relative	-0.58	-0.30
Baseflow alpha factor (1/days)	Replace	0.20	0.26
Groundwater delay time (days)	Replace	56.87	172.29
Threshold depth of water in the shallow aquifer required for return flow to occur (mm H_2O)	Replace	2112.45	2875.67
Groundwater revap coefficient	Replace	0.04	0.06
Threshold depth of water in the shallow aquifer for revap or percolation to the deep aquifer to occur (mm H_2O)	Replace	448.28	500.00
Deep aquifer percolation fraction	Replace	0.00	0.06
Manning's "n" value for overland flow	Relative	0.74	1.55
Soil evaporation compensation factor	Replace	0.95	1.00
Plant uptake compensation factor	Replace	0.78	1.00
Available water capacity of soil layer (mm H_2O/mm soil)	Relative	-0.48	-0.12
Manning's "n" value for main channel	Replace	0.08	0.11
Surface runoff lag coefficient	Replace	1.00	11.36

Table 4-6 Summary of sensitivity analysis statistics for all parameters. The large the absolute value of t-Stat and the smaller the p-value, the more sensitive the parameter. The model was most sensitive to SCS Curve Number.

Parameter	**t-Stat**	**P-value**
SCS curve number	-38.76	0.00
Threshold depth of water in the shallow aquifer required for return flow to occur (mm H_2O)	-16.57	0.00
Surface runoff lag coefficient	-13.54	0.00
Groundwater delay time (days)	-12.93	0.00
Available water capacity of soil layer (mm H_2O/mm soil)	-9.03	0.00
Manning's "n" value for main channel	6.88	0.00
Soil evaporation compensation factor	5.25	0.00
Groundwater revap coefficient	-4.30	0.00
Threshold depth of water in the shallow aquifer for revap or percolation to the deep aquifer to occur (mm H_2O)	-1.38	0.17
Baseflow alpha factor (1/days)	-0.22	0.22
Manning's "n" value for overland flow	-0.99	0.32
Deep aquifer percolation fraction	-0.79	0.43
Plant uptake compensation factor	-0.48	0.63

4.3.2 Validation

The model was validated using the remaining available flow rate data for subbasin 8 only, from 2010 to 2013. The resulting 95 PPU plot and statistics can be seen in Figure 4-3. Validation resulted in 76 acceptable solutions, a NS efficiency of 0.64 and a R^2 value of 0.67, meaning that model performance for flow can be considered satisfactory. These parameter ranges were then used to run a new SWAT simulation and simulate glyphosate transport.

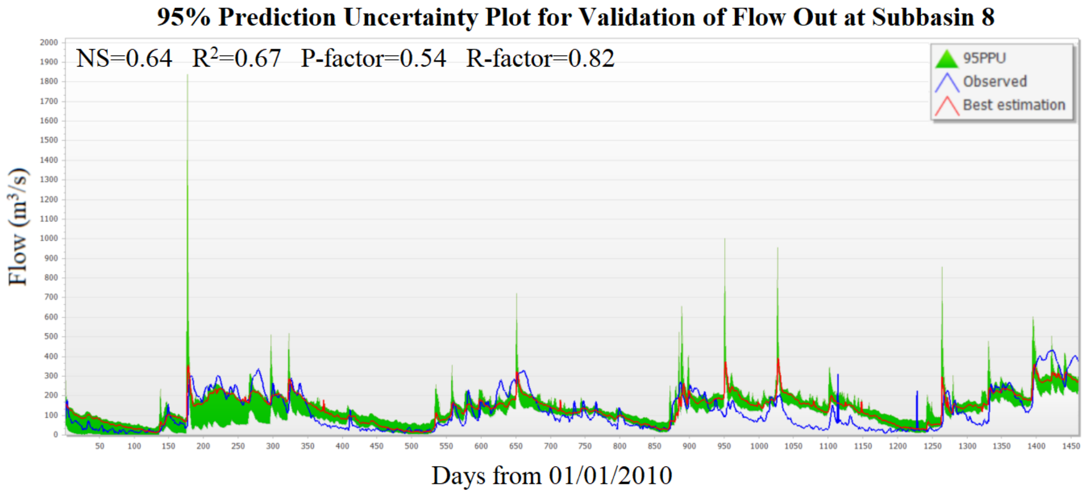

Figure 4-3 Summary of model validation for subbasin 8. Both NS efficiency and R^2 are above the threshold for adequate model performance, meaning that the flow in this subbasin is well modeled.

4.3.3 Glyphosate Transport Simulation

The following sections present simulated results predicted by the model regarding glyphosate transport through the watershed. Please note that these modeling predictions were generated from assumed values of glyphosate application and lacking glyphosate transport calibration, and therefore are presented herein to support future work.

4.3.3.1 Evaluating Model Performance and Results at Calibrated Subbasin

The model simulation was run from January 1, 2001 to September 30, 2019, encompassing the time periods used for calibration and validation, and continues on past the period for which observed flow data is available. Observed flow compared to simulated flow is shown in Figure 4-4. Simulated flow seems to match observed flow quite well, however, the model still has a tendency to overestimate peak flows.

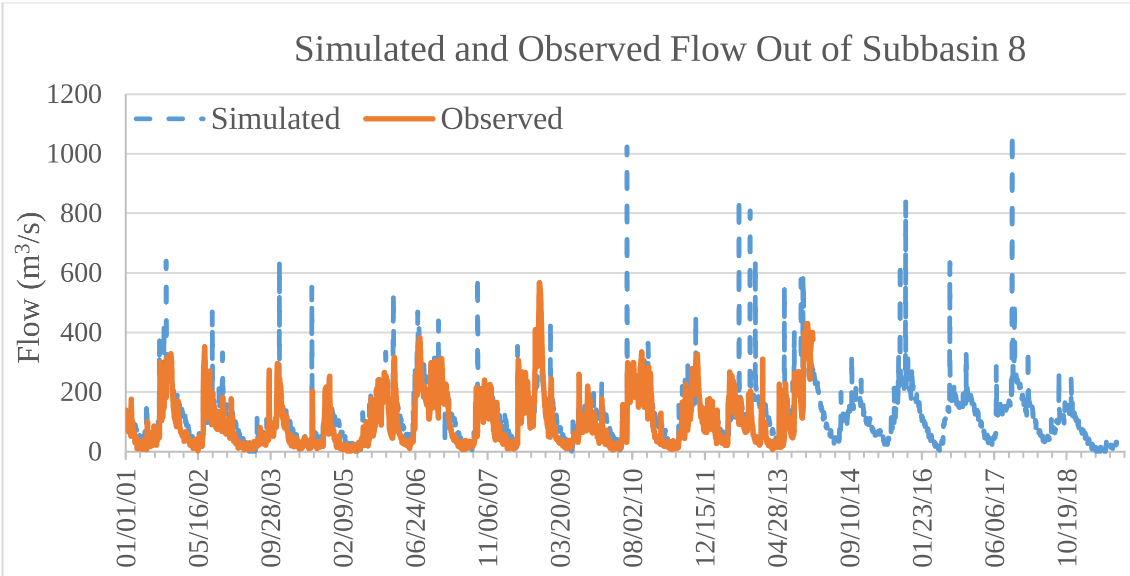

Figure 4-4 Comparison of simulated and observed flow out of subbasin 8. Simulated flow rate performs well at modeling actual flow out of the subbasin, apart from the tendency to overestimate peak flows.

Simulated daily soluble and sorbed glyphosate concentrations in the flow into and out of subbasin 8 for the duration of the simulation are shown in Figures 4-5 and 4-6. Simulated concentrations of glyphosate sorbed to sediment were significantly greater than soluble glyphosate concentrations (*p*-values<0.0 for both inflow and outflow). Additionally, both sorbed and solubles simulated glyphosate concentrations in the inflow are greater than concentrations in the outflow (*p*-values<0.0 for both soluble and sorbed). Simulated concentrations occasionally exceeded the European Union standard for glyphosate of 0.1 ppb, 0.25% and 0.04% of the time for soluble concentrations in the inflow and outflow respectively ("Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption," 1998; Dolan et al., 2013). Simulated sorbed concentrations in the inflow and outflow exceeded the EU standard 3.80% and 2.61% of the time, respectively.

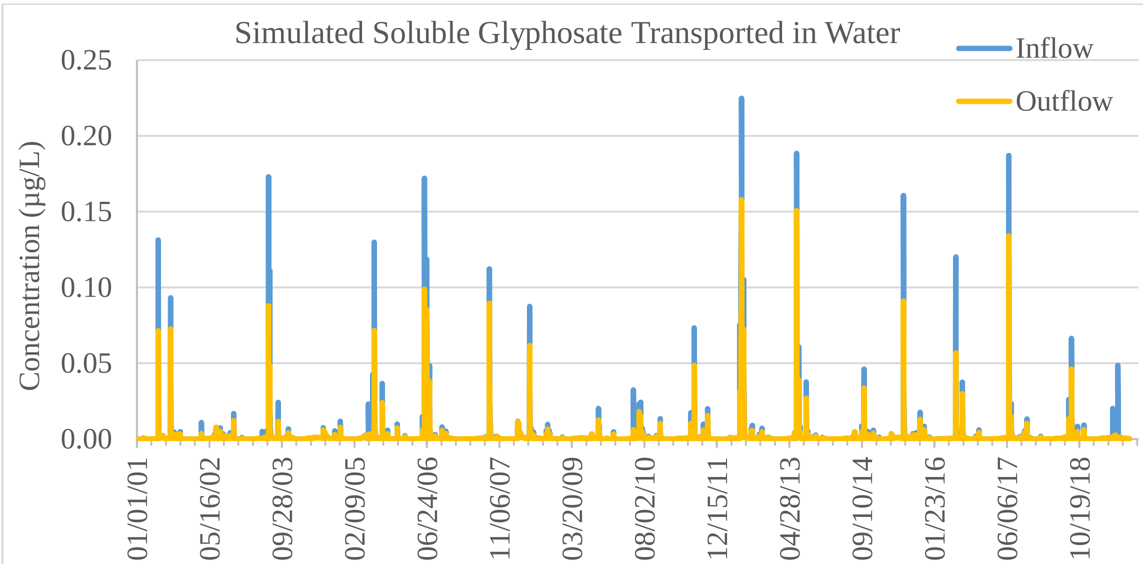

Figure 4-5 Simulation results for soluble glyphosate transported into and out of subbasin 8. Concentrations in the inflow are typically greater than concentrations in outflow. Inflow concentrations exceed the EU standard 0.25% of the time. Outflow concentrations exceed the EU standard 0.04% of the time.

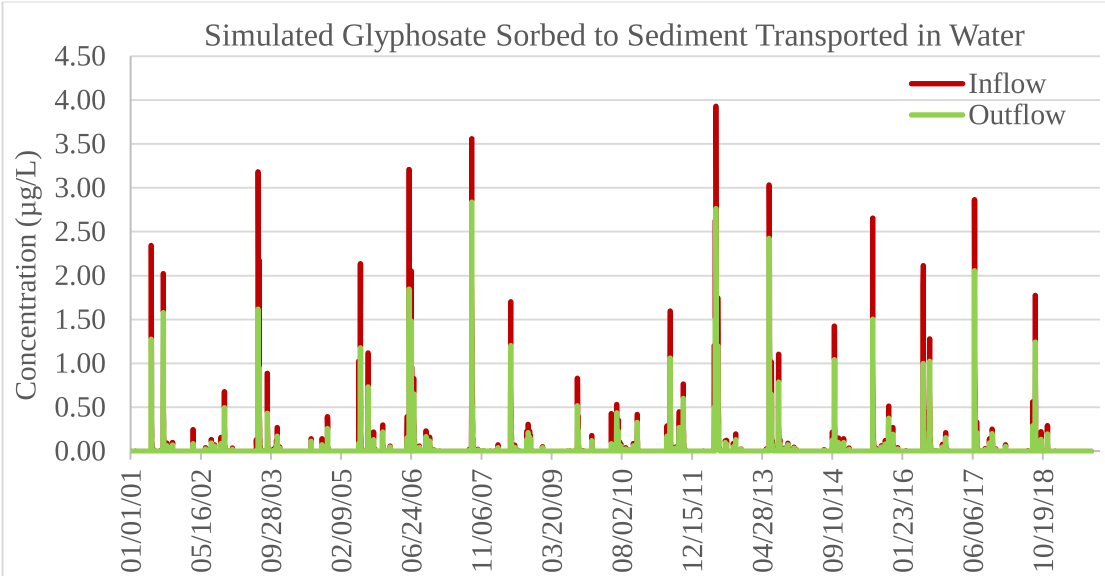

Figure 4-6 Simulated results for glyphosate sorbed to sediment transported into and out of subbasin 8. Concentrations in the inflow are typically greater than concentrations in outflow and are significantly greater than soluble concentrations. Inflow concentrations exceed the EU standard 3.80% of the time. Outflow concentrations exceed the EU standard 2.61% of the time.

Glyphosate has been shown to be able to be re-released into the water column once deposited in bed sediment (Pandey et al., 2019). Therefore, it is important to consider the possibility of bed sediment serving as a source of glyphosate to the water column. SWAT accounts for this with its diffusion function, and an example of this is given for subbasin 8 in Figure 4-7. Figure 4-7 shows the predicted diffusion of simulated glyphosate concentrations between the dissolved and sorbed phases, with positive values representing transfer from bed sediment to water, and negative values representing transfer from water to the sediment. As shown, diffusion in this region is predicted by the model to be dominated by transfer of glyphosate from water to sediment.

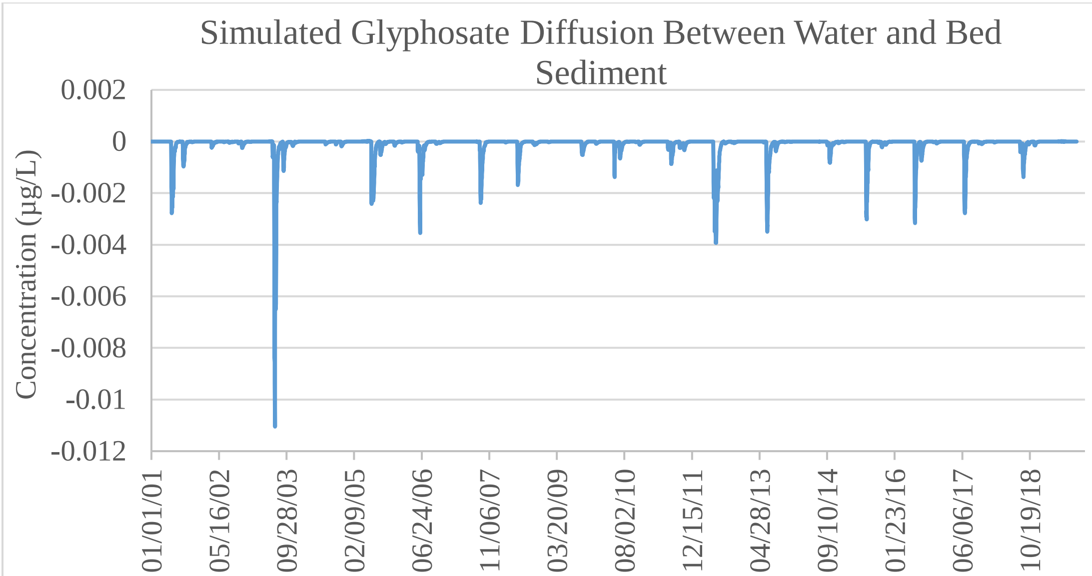

Figure 4-7 Simulated glyphosate transfer from water to sediment (negative) and sediment to water (positive). Diffusion in the system is dominated by transfer from water to sediment, so re-release into the water column is neglible in this subbasin.

Another important factor when trying to model pesticide fate and transport is to consider the loss of glyphosate due to degradation. While glyphosate half-life varies from 2 and 215 days in soil and 2 to 91 days in water, it is degraded most readily to AMPA. Figure 4-8 shows the amount of glyphosate in the subbasin that is predicted to be degraded daily. Because AMPA is the primary degradation product of glyphosate, it can be assumed that a considerable fraction of this loss is conversion to AMPA. Previous work using stable isotope labeling to trace the degradation process of glyphosate in a sediment water system determined that the ^{15}N-AMPA present in the system represented 79% of initial ^{15}N-glyphosate concentration (S. Wang et al., 2016). Another stable isotope labeling study determined that AMPA accounted for 48-68% of glyphosate degradation (Sun et al., 2019). Using these findings, it can be estimated that roughly 48-79% of the simulated glyphosate loss due to degradation will result in AMPA production, yielding AMPA concentrations up to 0.02 ppb being added to the system during each time step. It was also reported by Wang et al. that AMPA degraded more slowly than it was produced, which results in a net increase in AMPA over time (S. Wang et al., 2016).

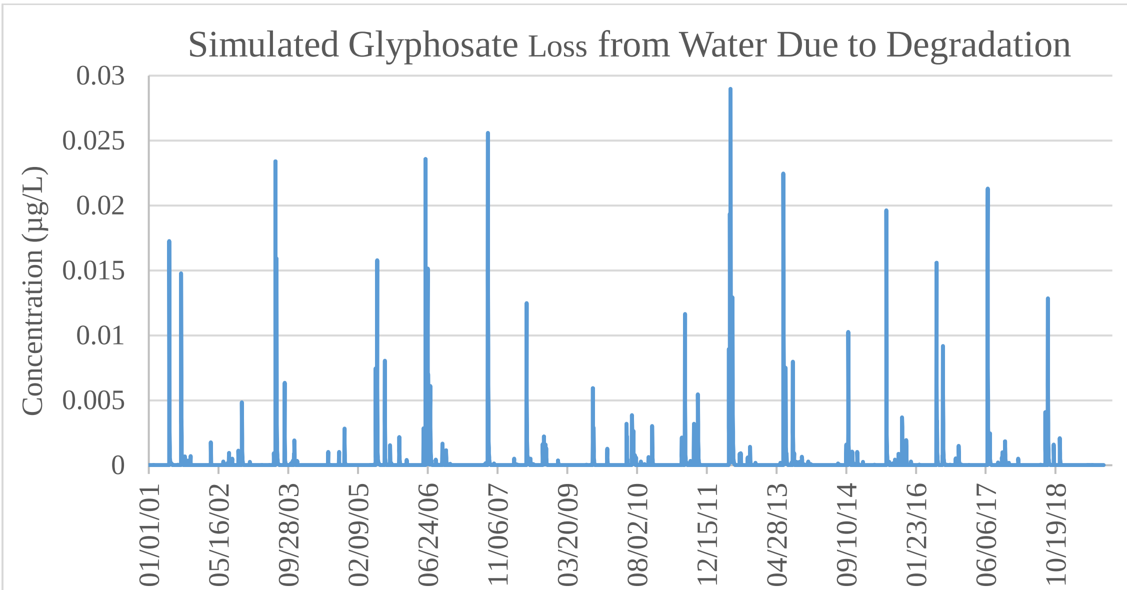

Figure 4-8 Glyphosate loss from water due to degradation. The majority of this glyphosate loss will yield AMPA, which degrades more slowly than it is produced from glyphosate degradation.

4.3.3.2 Simulated Spatial Distribution of Glyphosate Presence

Figure 4-9 presents the average predicted glyphosate concentrations in the inflow and outflow of each subbasin in the watershed for the duration of the simulation. Both glyphosate soluble in water and sorbed to sediment are shown. For soluble glyphosate, only two subbasins (3, 28) in the watershed were predicted to have an average concentration above the LC-MS/MS limit of detection, 0.02 µg/L, in both inflow and outflow. For simulated glyphosate sorbed to sediment, subbasins 2, 3, and 28 were predicted to have average concentrations higher than the EU standard. Subbasin 28 is just downstream of sampling locations in Spanish Lookout (subbasins 31 and 35). Subbasins 2 and 3 are located in the northeastern part of the watershed, close to the outlet. Additionally, subbasins 4, 5, 8, 10, 12, 26 and 27 had simulated concentrations below the EU standard, but above the limit of detection.

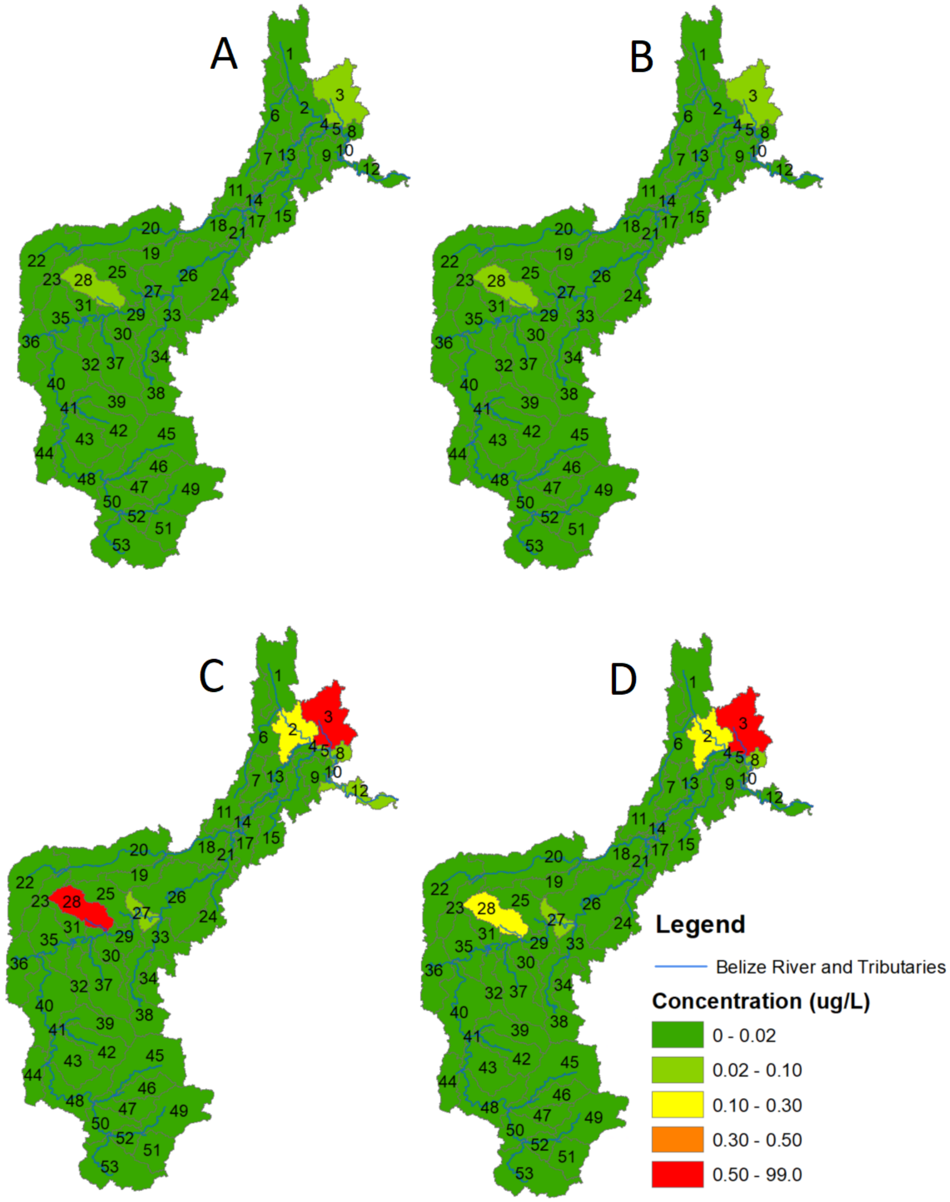

Figure 4-9. A) Average concentrations of soluble glyphosate in the inflow to each subbasin in the watershed. Only subbasins 3 and 28 had detectable average concentrations, all other subbasins had average concentrations below the detection limit. B) Average concentrations of soluble glyphosate in the outflow of each subbasin in the watershed. Only subbasins 3 and 28 had detectable average concentrations, all other subbasins had average concentrations below the detection limit. C) Average concentrations of glyphosate sorbed to sediment in the inflow of each subbasin in the watershed. Higher average concentrations were seen in subbasins 3 and 28 than compared to soluble concentrations. 2, 3, and 28 had concentrations above the EU standard. D) Average concentrations of glyphosate sorbed to sediment in the outflow of each subbasin in the watershed. Higher average concentrations were seen in subbasins 3 and 28 than compared to soluble concentrations. 2, 3, and 28 had concentrations above the EU standard.

Figure 4-10 presents the average simulated concentrations entering and leaving each reach in the watershed during the dry season for both glyphosate soluble in water and sorbed to sediment. During the dry season, it was predicted that there were no subbasins with soluble glyphosate concentrations within a detectable range, in either subbasin inflows or outflows. For simulated glyphosate sorbed to sediment, it was predicted that only two subbasins had concentrations within a detectable range in inflow, decreasing to just one subbasin for outflow. Overall, glyphosate concentrations in the Belize River are predicted to be within an undetectable or safe range during the dry season, according to the model.

Figure 4-11 presents the average simulated concentrations entering and leaving each reach during the wet season for both soluble and sorbed glyphosate. During the wet season, increases to detectable levels and average concentrations exceeding the EU standard were predicted in certain subbasins throughout the watershed. Subbasins 28 and 3 experienced predicted increases in average concentrations of soluble glyphosate to within a detectable range. For glyphosate sorbed to sediment, 4, 5, 8, 10, 12, 26, and 27 had average simulated concentrations that would have been within a detectable range and 2, 3, and 28 had average concentrations exceeding the EU standard. All other subbasins had predicted concentrations below a detectable level for both soluble and sorbed glyphosate.

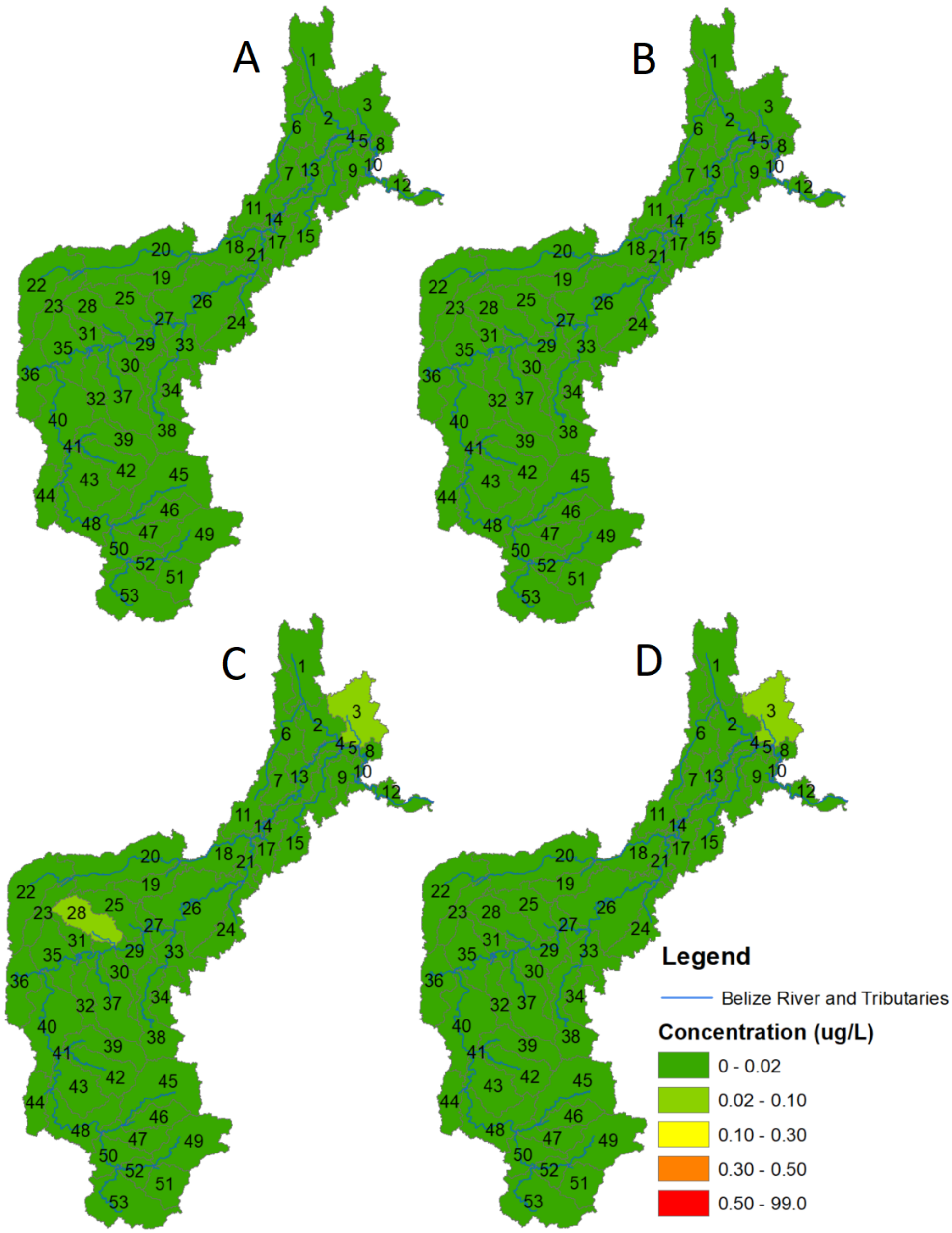

Figure 4-10 A) Dry season average concentrations of soluble glyphosate in the inflow to each subbasin in the watershed. All subbasins had average concentrations below the detection limit. B) Dry season average concentrations of soluble glyphosate in the outflow of each subbasin in the watershed. All subbasins had average concentrations below the detection limit. C) Dry season average concentrations of glyphosate sorbed to sediment in the inflow of each subbasin in the watershed. Detectable average concentrations were only seen in subbasins 3 and 28. D) Dry season average concentrations of glyphosate sorbed to sediment in the outflow of each subbasin in the watershed. Detectable average concentrations were only seen in subbasin 3.

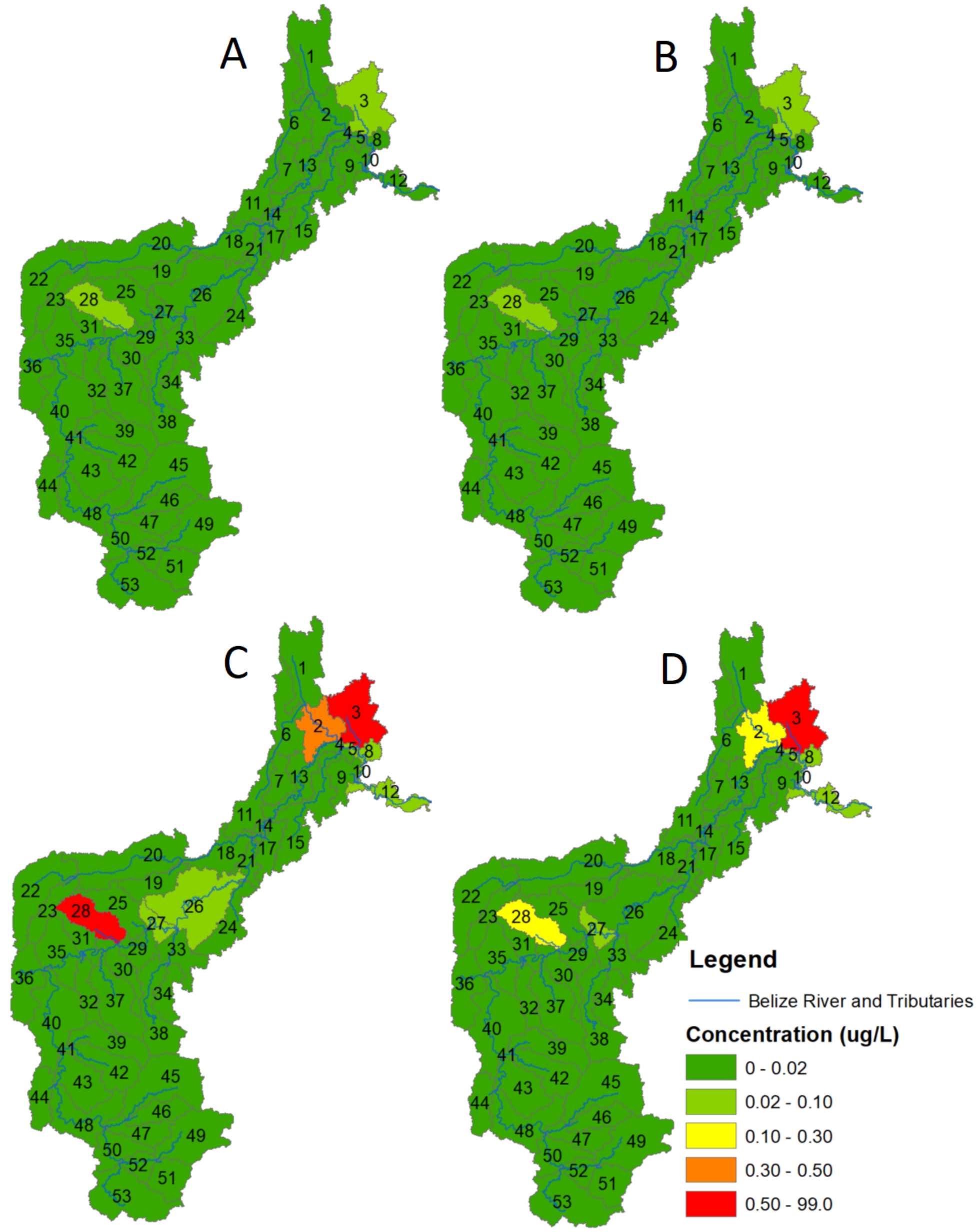

Figure 4-11 A) Wet season average concentrations of soluble glyphosate in the inflow to each subbasin in the watershed. Only subbasins 3 and 28 had detectable average concentrations, all other subbasins had average concentrations below the detection limit. B) Wet season average concentrations of soluble glyphosate in the outflow of each subbasin in the watershed. Only subbasins 3 and 28 had detectable average concentrations, all other subbasins had average concentrations below the detection limit. C) Wet season average concentrations of glyphosate sorbed to sediment in the inflow of each subbasin in the watershed. Subbasins 2, 3, and 28 had concentrations above the EU standard. D) Wet season average concentrations of glyphosate sorbed to sediment in the outflow of each subbasin in the watershed. Concentrations in subbasins 2, 3, and 28 were above the EU standard, though concentrations in subbasins 2 and 28 had decreased from their inflow concentrations.

4.3.3.3 Comparing Model Predictions to Observed Results

Simulated glyphosate concentrations were evaluated at the two subbasins in which the sampling sites are located. No glyphosate was predicted to be present in either soluble or sorbed phases, at all Bullet Tree sampling locations in subbasin 36. Spanish Lookout sampling locations are divided among two subbasins, with the upstream sampling point within subbasin 35, and the abstraction site and drinking water system within subbasin 31. Glyphosate was not predicted to be present in subbasin 35 in either soluble or sorbed phases for the duration of the simulation. However, in subbasin 31, glyphosate was occasionally predicted to be present throughout the simulation. Simulated soluble glyphosate concentrations in the inflow would have exceeded the EU standard 0.06% of the time, while outflow concentrations never were predicted to exceed the standard. Simulated sorbed glyphosate concentrations in the inflow and outflow were predicted to exceed the EU standard 1.05% and 0.70% of the time, respectively. However, during the month of July 2019, soluble and sorbed concentrations were all below 0.005 ppb. There predictions are consistent with the samples collected from the same locations which did not yield detectable concentrations. However, subbasin 28, which was predicted to have a wet season average concentration of soluble glyphosate within a detectable range, and a wet season average concentration of sorbed glyphosate above the EU standard, is just downstream of where samples were collected.

To understand the modeled distribution of glyphosate presence in the watershed during the time that sample collection occurred, average soluble and sorbed glyphosate concentrations were calculated from predicted glyphosate loads for the month of July 2019 and illustrated in maps shown in Figure 4-12.

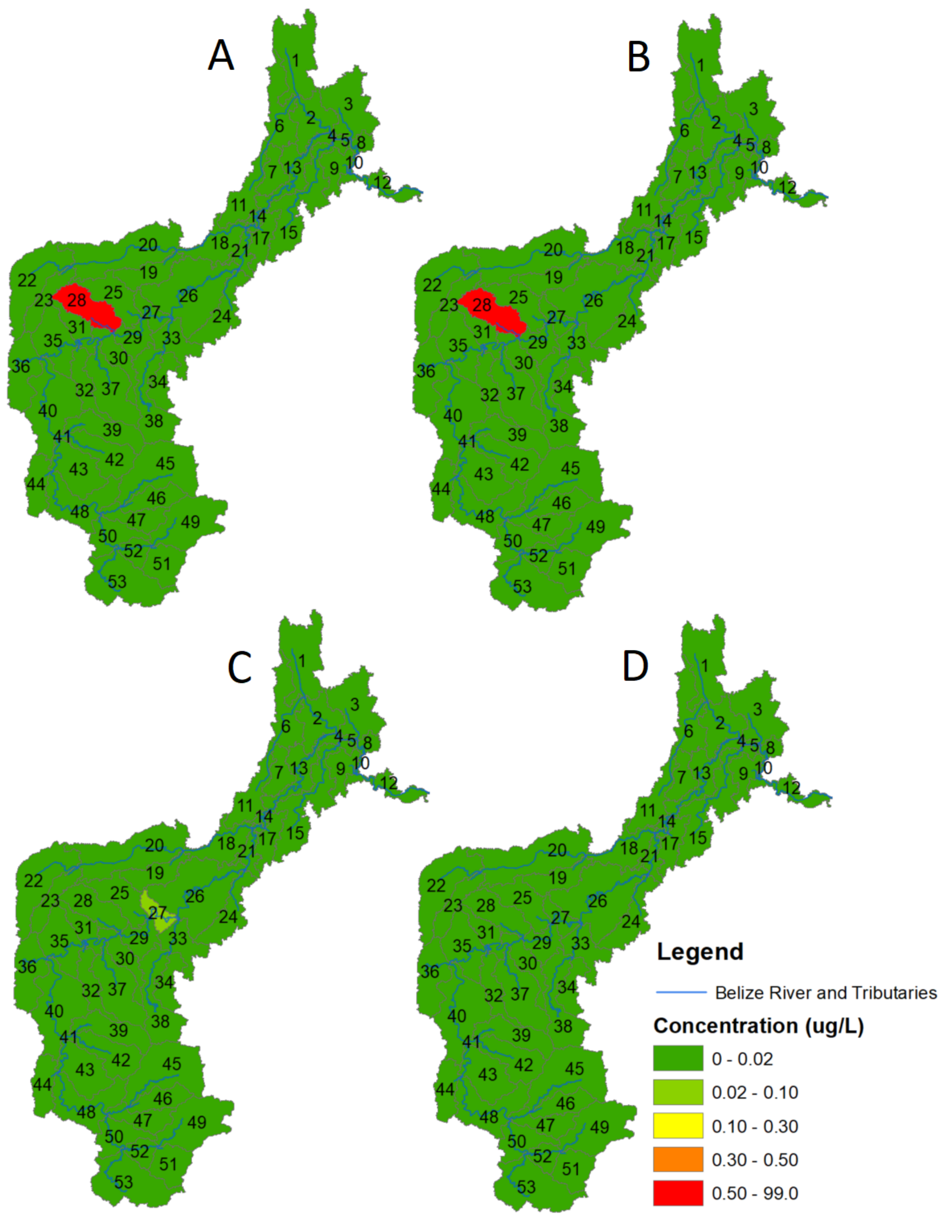

Figure 4-12 A) Average soluble glyphosate concentrations in the inflow to each subbasin during July 2019. Concentrations were all below the detection limit, with the exception of subbasin 28 which had a concentration of 0.65 ppb, above the EU standard. B) Average soluble glyphosate concentrations in the outflow of each subbasin during July 2019. Concentrations were all below the detection limit, with the exception of subbasin 28 which had a concentration of 0.65 ppb, above the EU standard. C) Average concentrations of glyphosate sorbed to sediment in the inflow to each subbasin during July 2019. Concentrations were all below the detection limit, with the exception of subbasin 27, which was less than the EU standard. D) Average concentrations of glyphosate sorbed to sediment in the outflow of each subbasin during July 2019. Concentrations were all below the detection limit.

For average simulated concentrations of soluble glyphosate for the month of July 2019, all subbasins were below the limit of detection apart from subbasin 28, which was predicted to have a concentration of 0.65 µg/L for both inflow and outflow, above the EU standard. Subbasin 28 is just downstream of subbasins 35 (containing the Spanish Lookout upstream sampling point) and subbasin 31 (containing the Spanish Lookout abstraction site and drinking water sampling locations), as shown in Figure 4-13. One of the ELISA kit samples that was quantified to have a concentration over the method detection limit of 0.05 ppb was taken from the Spanish Lookout upstream site, though no conclusions were able to be made due to the other two triplicates being below the detection limit.

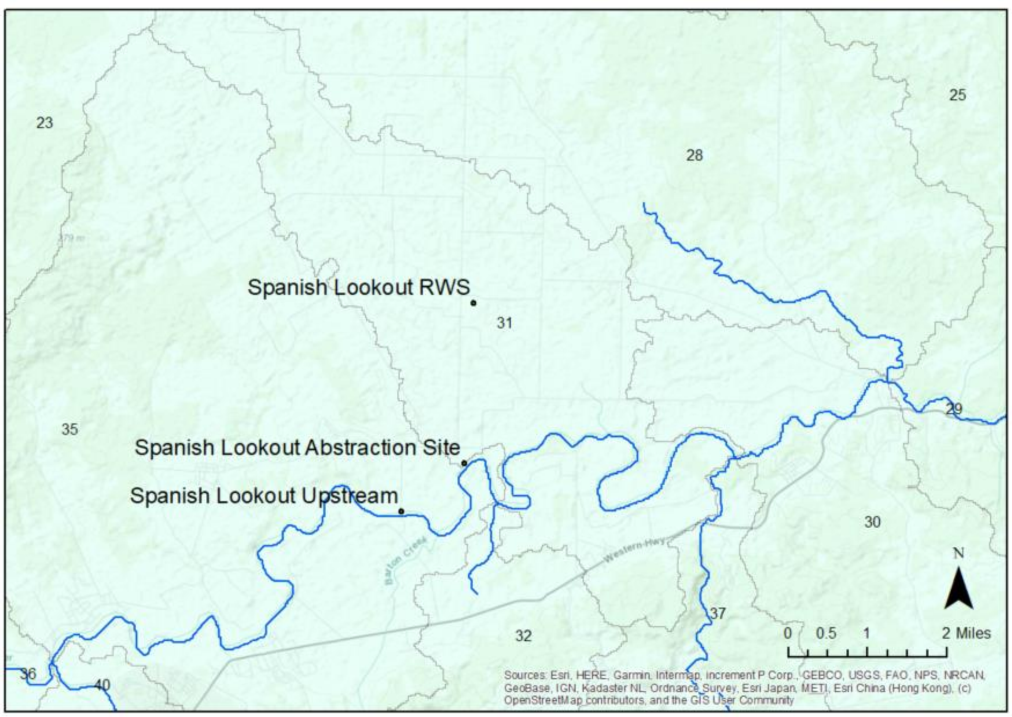

Figure 4-13 Zoomed in map of Spanish Lookout area. RWS is rudimentary water system, where drinking water is distributed. Subbasin 28, which contributes the most glyphosate to the river, is located just downstream from the Spanish Lookout RWS.

For glyphosate sorbed to sediment, all simulated concentrations were below the detection limit, apart from inflow to subbasin 27 only, which was predicted to have a concentration of 0.06 µg/L, below the EU standard. These results are inconsistent with the average wet season concentrations for the entire simulation and may be due to the unusually dry climate and late wet season that Belize was experiencing during that time. One study that quantified the occurrence of glyphosate in water bodies of Mexico, with a similar climate to Belize, found significantly higher concentrations in the dry season as opposed to the wet season, and concluded that these higher concentrations were due to less dilution by rainfall (Ruiz-Toledo et al., 2014). The dry climate at the time would also explain the decreased simulated concentrations of glyphosate sorbed to sediment, as less rainfall would result in decreased erosion and sediment loads to the river.

4.3.3.4 Subbasins with Elevated Simulated Glyphosate Concentrations

Model results indicate that subbasins 2, 3, and 28 may have the highest likelihood for glyphosate concentrations above the EU standard. Additionally, subbasins 4, 5, 8, 10, 12, 26, and 27 were predicted to have detectable concentrations of glyphosate and may be areas that should also be considered for future monitoring. Simulated soluble and sorbed glyphosate concentrations in subbasin 2 over time are shown in Figures 4-14 and 4-15, respectively. In subbasin 2, sorbed concentrations were predicted to be significantly greater than soluble concentrations (p-value<0.0), and concentrations in the inflow were predicted to be greater than concentrations in the outflow (p-value<0.0). Simulated soluble concentrations in the inflow and outflow exceeded the EU standard 1.05% and 0.45% of the time, respectively. Simulated sorbed concentrations in the inflow and outflow exceeded the EU standard 9.73% and 7.92% of the time, respectively. The land use in this subbasin is predominantly agriculture, and the crop type is corn.

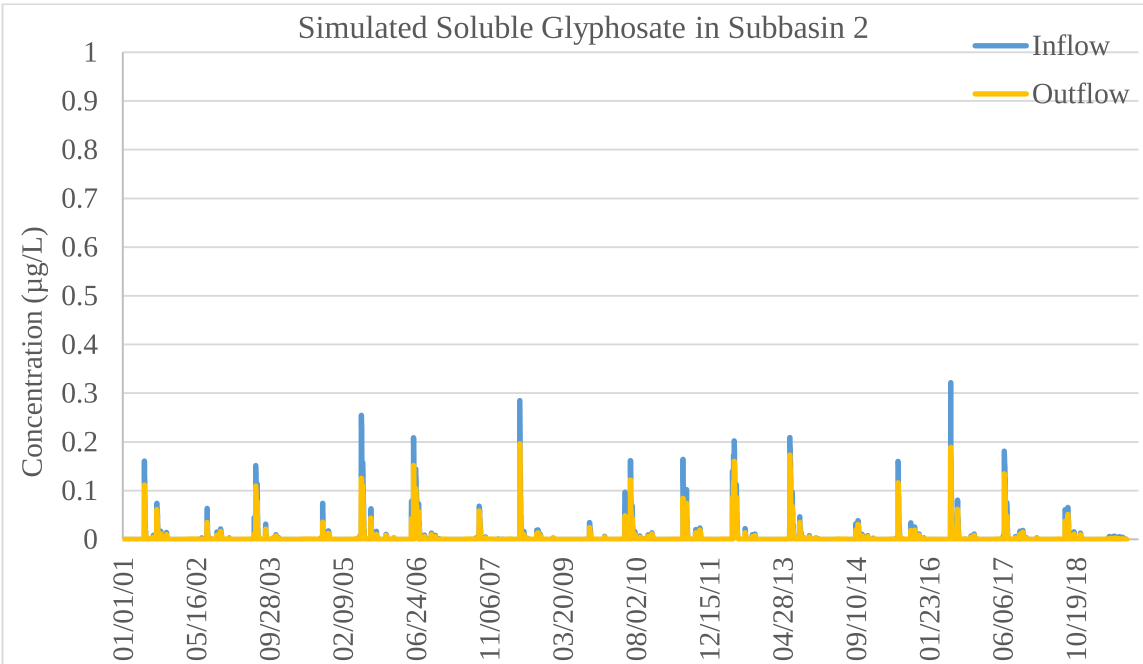

Figure 4-14 Simulated soluble glyphosate in the inflow and outflow of subbasin 2. Soluble concentrations in the inflow and outflow exceeded the EU standard 1.05% and 0.45% of the time, respectively.

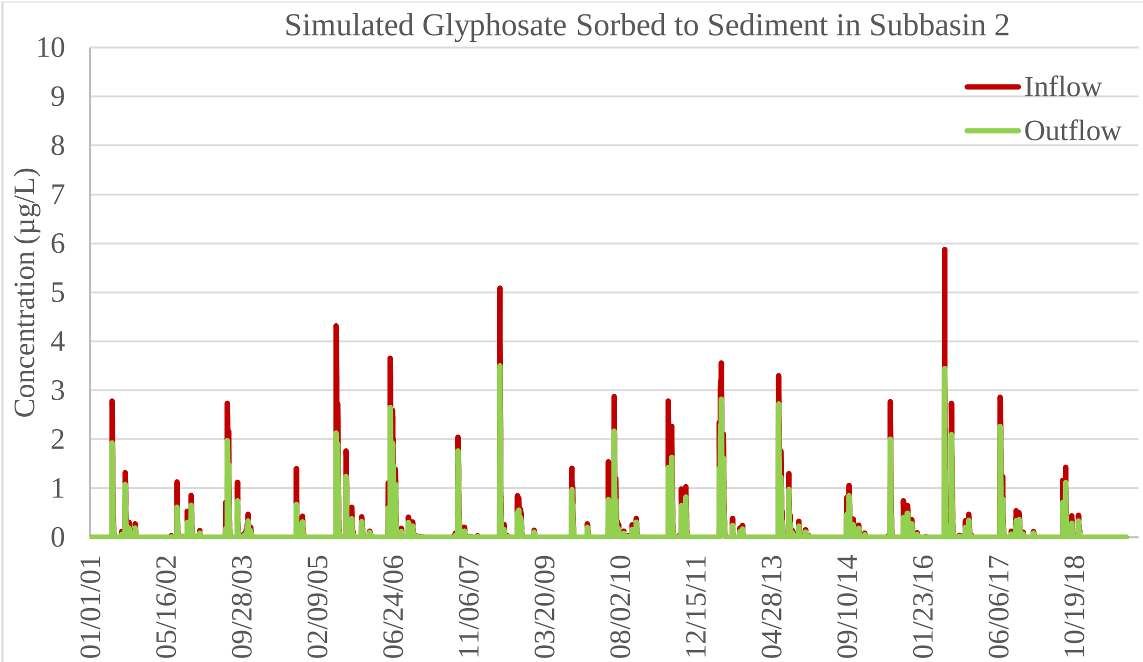

Figure 4-15 Simulated glyphosate sorbed to sediment in the inflow and outflow of subbasin 2. Sorbed concentrations in the inflow and outflow exceeded the EU standard 9.73% and 7.92% of the time, respectively.

Simulated soluble and sorbed glyphosate concentrations in subbasin 3 over time are shown in Figures 4-16 and 4-17, respectively. In subbasin 3, simulated sorbed concentrations were also significantly greater than soluble concentrations (*p*-value<0.0), and concentrations in the inflow were predicted to be greater than concentrations in the outflow (*p*-value<0.0). Simulated soluble concentrations in the inflow and outflow exceeded the EU standard 4.34% and 1.58% of the time, respectively. Simulated sorbed concentrations in the inflow and outflow exceeded the EU standard 17% and 12.79% of the time, respectively. The land use in this subbasin was also predominantly agriculture, and the crop type is corn.

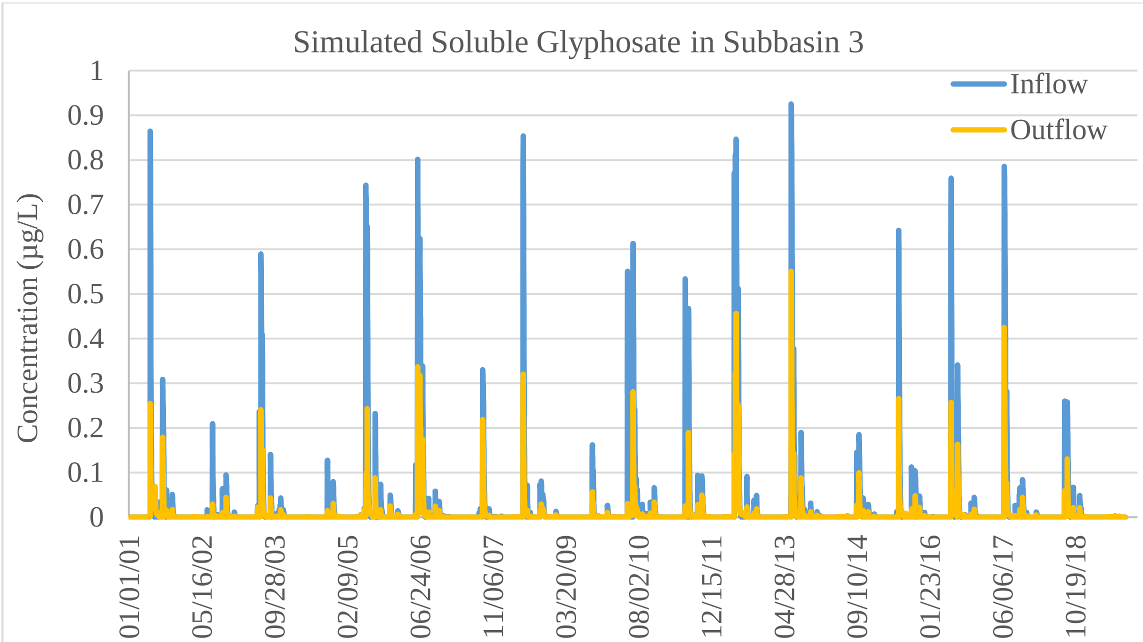

Figure 4-16 Simulated soluble glyphosate in the inflow and outflow of subbasin 3. Soluble concentrations in the inflow and outflow exceeded the EU standard 4.34% and 1.58% of the time, respectively.

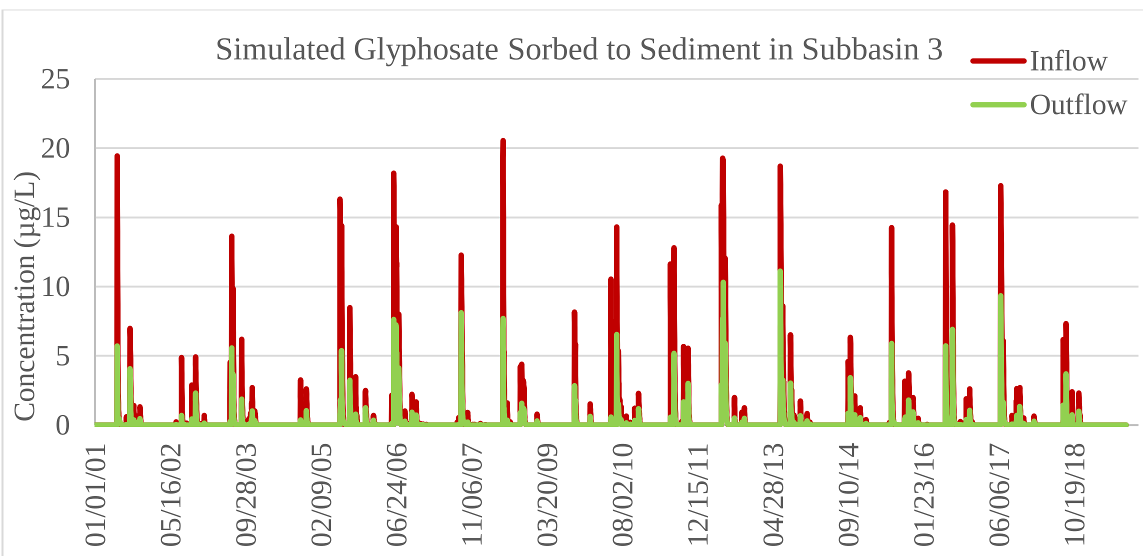

Figure 4-17 Simulated glyphosate sorbed to sediment in the inflow and outflow of subbasin 3. Sorbed concentrations in the inflow and outflow exceeded the EU standard 17% and 12.79% of the time, respectively.

Simulated soluble and sorbed glyphosate concentrations in subbasin 28 over time are shown in Figures 4-18 and 4-19, respectively. In subbasin 28, simulated sorbed concentrations were significantly greater than soluble concentrations in the inflow. However, simulated sorbed and soluble concentrations are not statistically different in the outflow. While simulated inflow concentrations of sorbed glyphosate were significantly greater than outflow concentrations as seen with other subbasins (p-value$<$0.0), simulated soluble concentrations of glyphosate were actually greater on average in the outflow than inflow (p-value$<$0.0). Because the outflow of soluble glyphosate in this subbasin was predicted to be greater than the inflow, subbasin 28 may be a significant contributor of soluble glyphosate to the Belize River system. According to the model, subbasin 28 is the most significant source of glyphosate to the Belize River, as compared to subbasin 2 (p-value <0.0) and subbasin 3 (p-value <0.0).

Simulated soluble concentrations largely remained below 5 ppb, apart from one modeled event in September 2001 when simulated outflow soluble concentrations showed a large spike up to over 28 ppb. Around this same time, simulated sorbed concentrations in the outflow also experienced a large spike, exceeding inflow concentrations at the time. Simulated soluble concentrations in the inflow and outflow exceeded the EU standard 12.53% and 11.65% of the time, respectively. Simulated sorbed concentrations in the inflow and outflow exceeded the EU standard 4.47% and 4.10% of the time, respectively. The land use in this region was also predominantly agriculture, and consists of corn, sugarcane, soybean, and pinto bean production.

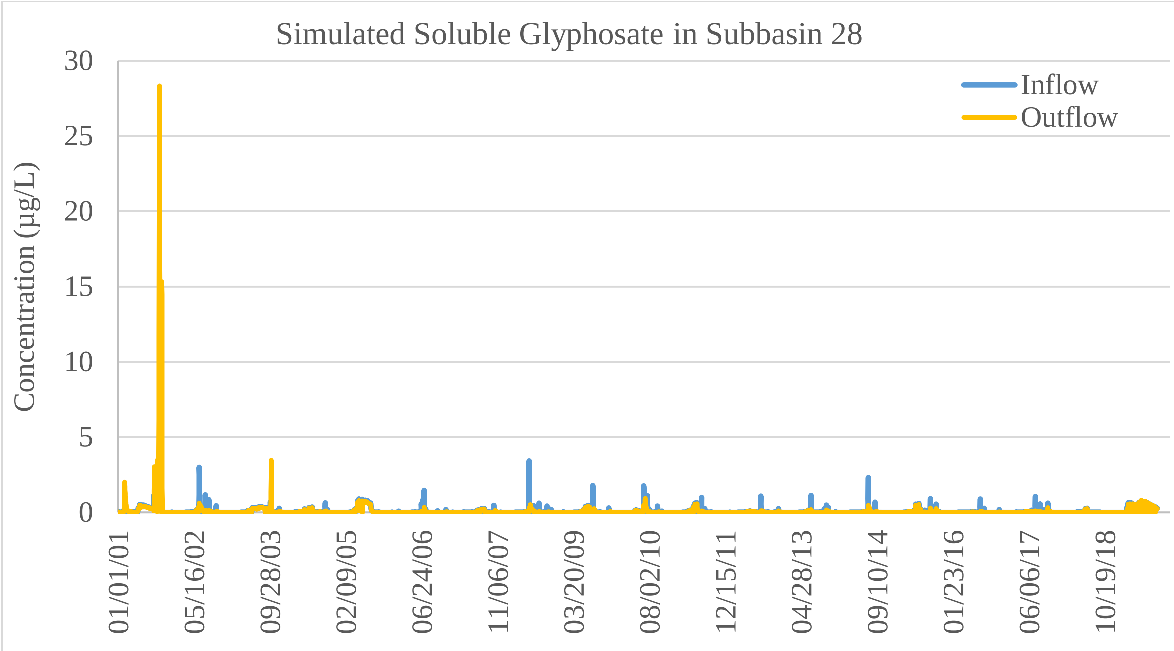

Figure 4-18 Simulated soluble glyphosate in the inflow and outflow of subbasin 28. Outflow concentrations are greater than inflow concentrations, meaning that that this subbasin may be contributing significant amounts of soluble glyphosate to the river. Soluble concentrations in the inflow and outflow exceeded the EU standard 12.53% and 11.65% of the time, respectively.

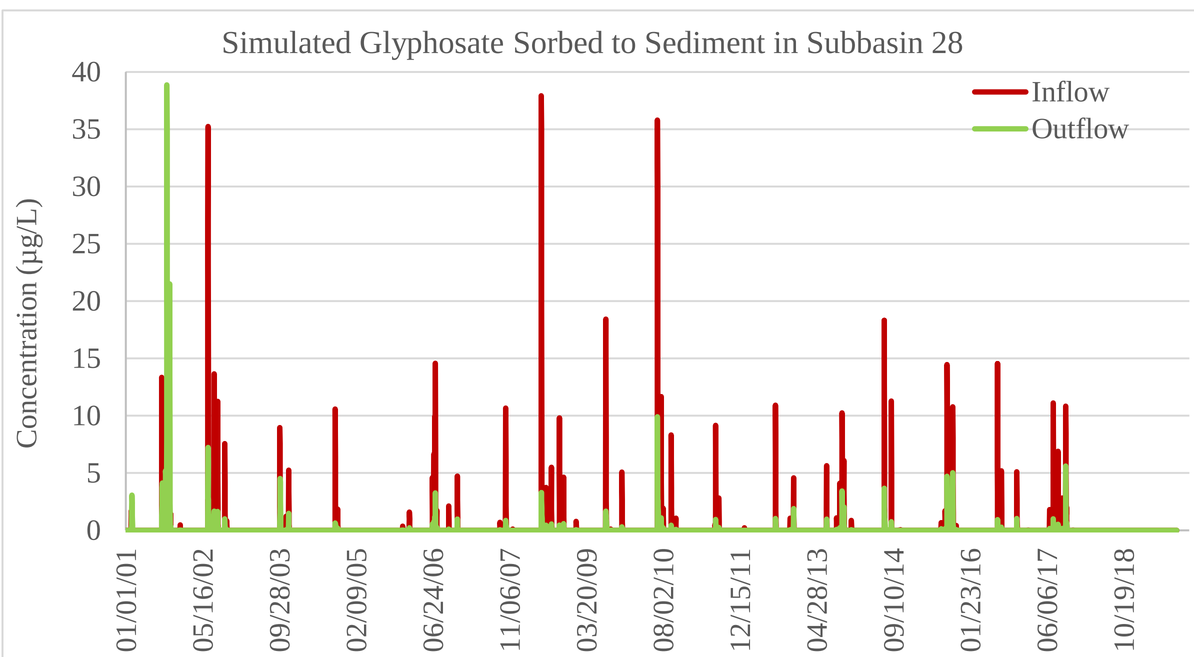

Figure 4-19 Simulated glyphosate sorbed to sediment in the inflow and outflow of subbasin 28. Sorbed concentrations in the inflow and outflow exceeded the EU standard 4.47% and 4.10% of the time, respectively.

4.4 Model Limitations

It is important to note that the glyphosate application operations indicated for each crop in the simulation were determined according to industry recommendations and is likely to be very conservative for estimating actual glyphosate use in the area. A lack of stringent regulation typically results in applicators using much more herbicide than the recommended amounts, as revealed during discussions with the Pesticide Control Board in Belize. Additionally, for the model, it was assumed that no glyphosate tolerant genetically engineered crops were grown in the watershed and thus application only occurs before the planting of crops. This may also be a conservative estimate, as it is known that genetically modified crops have been imported into the country but are currently not legal to cultivate (Alam, 2019; Jacobs, 2016).

A major limitation of the model is the current lack of available data to calibrate and validate the model. Model results were compared to available data for observed sediment and nutrient concentrations and was shown to not perform well for these parameters. These results can be seen in Appendices 1-3. This is an indicator that the model may not be accurately simulating runoff and erosion conditions in the watershed, which is likely to impact the accuracy of the glyphosate results as well. More data is needed to calibrate the model for nutrients and sediments. The lack of existing glyphosate monitoring data makes it impossible to definitively conclude whether the model is accurately representing glyphosate transport in the watershed. An estimation of the average glyphosate concentration in the watershed outlet based on the total glyphosate imports during 2009, the model estimated volume of water leaving the watershed in 2009, and the assumption that a third of the glyphosate imports would be applied in the watershed based on the knowledge that the watershed supplies water to about a third of the population, revealed an average concentration of glyphosate leaving the watershed of 337 ppb (Basel Convention Regional Centre for Training and Technology Transfer, 2015; Carrias et al., 2018). While this value is only an estimate, it is orders of magnitude greater than the model predicted glyphosate concentrations at the watershed outlet. This suggests that the model is likely to be underpredicting glyphosate loads to the river. While much more data is required to demonstrate model accuracy, this research serves as a starting point in the application of this technique for modeling pesticide transport and a framework for future use and development.

It is likely that the land use data used for the model is also underpredicting glyphosate transport. The available spatial dataset does not include urban areas in the watershed, which has a significant impact of glyphosate transport. Previous studies have demonstrated the importance of developed areas on quicker overland flow transporting glyphosate with greater efficiency to water sources, and have claimed that near-site land use may be a better predictor of glyphosate presence in water than generalizing land use across a watershed (Kolpin et al., 2006; Medalie et al., 2020). This suggests that higher resolution land use data, incorporating developed land, is needed for accurate simulations. In addition, it has been shown that wastewater treatment effluent serves as a source of glyphosate to waterways (Desmet et al., 2016; Kolpin et al., 2006). At least two wastewater treatment plants are located along the Belize River, and may also be contributing glyphosate to the river, resulting in model underprediction. However, SWAT does have the capability to model point sources, which may useful for future work.

An important missing link in the results generated by the model is quantification of the glyphosate's main degradation product, AMPA. While SWAT calculates the amount of glyphosate lost to degradation during each time step, it does not directly quantify or characterize degradation products. Because AMPA is more persistent than glyphosate, and may also pose human health and environmental risks, it is critical to also understand AMPA transport in the environment.

4.5 Recommendations for Future Work

Several identifiable next steps can be taken to continue to improve the accuracy of the model. Acquiring higher resolution land use/cover data that includes urban areas would make a significant difference on model outcomes. If possible, obtaining long term data for TSS so that the model can be calibrated for sediment loads would make the model more robust in its ability to predict glyphosate loads from erosion. Obtaining more local knowledge to get a better idea of how glyphosate is actually used and applied in Belize would decrease uncertainty as well. This may also aid in getting a more specific distribution of crop type in agricultural areas in the watershed, also helping to reduce uncertainty.

An essential next step in validating this method is to obtain a large amount of water and/or sediment samples in the watershed over time, so that the model can be fully validated for glyphosate transport. Proper preservation of these samples is also essential, and LC-MS/MS with solid phase extraction is strongly recommended for quantification due to its accuracy and low detection limit. Future monitoring studies should target the subbasins 2, 3, and 28, as the highest simulated concentrations were predicted to occur there. Useful directions that this work can go next include incorporating the modeling of AMPA fate and transport, simulating different BMPs to evaluate efficiencies, and inputting wastewater treatment plants as point sources.

Monitoring and Modeling Glyphosate Transport in the Belize River Watershed

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***Abstract*—Glyphosate, an effective herbicide used worldwide as a weed control, can be transported from application areas to unintended locations. There is growing concern regarding the health impacts of both glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) as increasing evidence suggests exposure may cause adverse health effects in humans. However, consistent monitoring data is still limited, especially in developing countries like Belize that are heavily reliant upon agriculture and the use of glyphosate. In this study, we use high performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA) kits, and liquid chromatography with tandem mass spectrometry (LC-MS/MS) to quantify concentrations of glyphosate and Soil Water Assessment Tool (SWAT) to model transport of glyphosate in the Belize River Watershed. Water samples were collected from two rural communities with rudimentary drinking water systems, Bullet Tree and Spanish Lookout, located in subbasins 31, 35, and 36. Sampling points were located upstream of the abstraction site, at the abstraction site, and at the site of drinking water distribution. HPLC, ELISA kits, and LC-MS/MS showed that glyphosate was not present in the water samples. The model confirms that glyphosate is not expected to be present in the sampling locations. However, the model did reveal that glyphosate transport to the Belize River may be occurring and that subbasins 2, 3, and 28 are most likely to have elevated concentrations due to having the highest percentages of days exceeding the EU standard for glyphosate of 0.1 µg/L. Subbasin 28, located just downstream of the Spanish Lookout drinking water system, was predicted to be the most significant contributor of soluble glyphosate to the river, as compared to soluble glyphosate concentrations in subbasins 2 (*p*-values <0.0) and 3 (*p*-values <0.0). Simulated soluble glyphosate concentrations in subbasin 28 inflow and outflow exceeded the EU standard by 12.53% and 11.65% of the time, respectively. Additionally, simulated concentrations of glyphosate sorbed to sediment were significantly greater than soluble glyphosate in surface runoff (*p*-values <0.0). Higher sorbed concentrations may still be concerning due to the potential of glyphosate to be re-released from sediment into the water column. This work demonstrates a framework for applying SWAT for pesticide transport modeling in developing countries and has the potential to be a powerful and accessible tool for watershed management and measuring sustainable development progress when monitoring data is unavailable.**

Keywords*—*sustainable development, water quality, pesticide transport, glyphosate, watershed modeling

INTRODUCTION

Two of the United Nations Sustainable Development Goals (SDGs) are to provide clean, accessible water and sanitation to all and achieve global food security by 2030 (United Nations). The use of pesticides has greatly increased agricultural productivity and has proven to be a useful tool for increasing food security. However, pesticide use may have unintended impacts on the environment and public health. Glyphosate is one of the most widely used herbicides in agriculture, with the introduction of glyphosate tolerant crops causing a 15-fold increase in use globally (Benbrook, 2016). While glyphosate was previously believed to be immobile in the environment and not hazardous to human health, it is now known that glyphosate can migrate to unintentional locations from runoff and erosion, and the herbicide is now listed as “probably carcinogenic to humans” by the World Health

Organization (Daouk et al., 2013; International Agency for Research on Cancer, 2017). Glyphosate has been shown to induce oxidative stress, DNA damage, and endocrine disruption, and has been correlated to a range of adverse health effects such as liver damage, kidney damage, cancer, and reproductive problems (Camacho & Mejía, 2017; De Roos Anneclaire et al., 2005; Gasnier et al., 2009; Woźniak et al., 2018). There is also evidence that the primary and more persistent degradation product of glyphosate, aminomethylphosphonic acid (AMPA), may cause similar adverse health effects (Woźniak et al., 2018). Therefore, it is critical to consider the role that glyphosate and other pesticides play in achieving clean water and food security SDGs. Widespread use has resulted in the prevalence of glyphosate in water bodies in developing and developed nations alike. However, the human health effects of glyphosate may be more severe in developing countries with limited access to improved water treatment systems. Additionally, pesticide and environmental regulations are not always strictly enforced, and watershed management is often limited in these regions (Carrias et al., 2018; Ecobichon, 2001). Accurate determination of glyphosate in environmental samples is also complex and costly, and consistent monitoring is not feasible in most low to middle income countries. These compounding factors make it more likely for glyphosate to be transported

Fig. 1. Map of Belize, Belize River Watershed, and sampling locations

to water resources undetected and evade removal before distribution of drinking water. In order to understand the state of water quality in a developing region, evaluate the efficacy of environmental policies and regulations, and measure progress towards achievement of the SDGs, large high-quality data sets are extremely valuable. To obtain such, innovative means of data collection and analysis are required. Modeling has the potential to be an extremely useful tool in developing countries to supplement a lack of data and better understand water quality problems. The Soil and Water Assessment Tool (SWAT) is a widely used hydrodynamic model that has been employed for thousands of published watershed modeling studies. However, the application of SWAT for pesticide modeling only makes up about 50 of these studies, with less than a third of these taking place outside of the US due to the ease of application in the US (R. Wang et al., 2019). To the authors' knowledge, there are no published applications of SWAT in Belize, and only one published study modeling glyphosate in the US (Love et al., 2011). The objective of this work is to develop a framework for modeling glyphosate transport in developing countries to understand its transport across watersheds and inform the management of watersheds and pesticide use. This framework has been demonstrated by applying SWAT for modeling glyphosate transport in the Belize River Watershed.

METHODOLOGY

A. Case Study Location Background

Belize is a developing Central American nation that relies heavily on agriculture for its economy. Glyphosate is the most commonly used agricultural chemical in Belize, being the largest fraction of its pesticide imports and applied in the production of crops such as sugarcane, citrus, bananas, soybeans, corn, and dry beans (Basel Convention Regional Centre for Training and Technology Transfer, 2015; Kaiser, 2011). However, due to human health concerns, glyphosate was recently added to Belize's list of Restricted Use Pesticides, and discussions with regulatory agencies in Belize have revealed an interest in investigating the presence of glyphosate in drinking water resources (Pesticide Control Board, 2019). The Belize River Watershed (Fig. 1) is a major source of drinking water to over a third of the population of Belize (Carrias et al., 2018). Water treatment plants in urban centers draw water from the Belize River for treatment and distribution to city residents. However, rural regions largely rely on rudimentary drinking water systems, water systems that have little to no treatment (Grau &

Rihm, 2013). Approximately 87% of Belize's rural population relies on these rudimentary systems (Grau & Rihm, 2013). These rural systems are often located where glyphosate is most often applied. Additionally, a watershed management plan compiled by the University of Belize reported severely degraded riparian zones along the river allowing for increased erosion and runoff, including in areas where large volumes of pesticides are applied (Carrias et al., 2018). Spanish Lookout and Bullet Tree Falls are two villages that use rudimentary water systems and draw surface water from the Belize River. These communities were selected for sampling due to their proximity to agricultural activity and reliance on surface water for rudimentary drinking water systems. Spanish Lookout is an agricultural community with a population of 2,253 residents and 482 households (The Statistical Institute of Belize, 2013). The primary drinking water system in the community is managed by a poultry production facility, Quality Poultry Products. The system pumps water to its production facility and diverts drinking water to be distributed throughout Spanish Lookout and two neighboring villages. Drinking water is filtered and passed through two settling ponds before distribution. There is no disinfection treatment. Discussions with locals revealed that most Spanish Lookout residents use private filter systems or rely solely on bottled water. However, it is likely that lower income households in Spanish Lookout consume water without further treatment. It was not disclosed how many residents of neighboring villages consume this water, or if there is any further treatment of the water supply in either village. Bullet Tree Falls is a rural village located in the upper reaches of the Belize River Watershed, with a population of 2,124 residents, and 426 households (The Statistical Institute of Belize, 2013). The drinking water system employs automatic chlorination before distribution throughout the village.

B. Sample Collection

Samples were collected from Spanish Lookout and Bullet Tree Falls in July 2019. Surface water and sediment samples were collected at two points in each community: upstream of the drinking water intake, and at the drinking water intake. Surface water samples were collected and preserved in accordance with the U.S. EPA operating procedure for surface water sampling and Section 8 of U.S. EPA Method 547 for determination of glyphosate in drinking water (U.S. Environmental Protection Agency, 1990, 2013). The sediment sampling method used was based on the U.S. EPA operating procedure for sediment sampling (U.S. Environmental Protection Agency, 2014). Surface water samples were collected either by wading in or using a Niskin Bottle sampler, and stored in two 125 mL amber opaque plastic bottles and one 1 L clear plastic bottle. Plastic was used instead of glass as recommended in EPA Method 547, because glyphosate has the potential to bind to glass. Collected water samples were immediately placed inside a cooler with ice packs, and frozen. Sediment samples were collected either by wading in and scooping bed sediment or using a Ponar grab sampler. Sediment samples were quartered to ensure homogenization, stored in quart sized Ziploc bags, placed in a cooler with ice packs, and frozen as soon as possible. Drinking water samples were collected and preserved in accordance with EPA Method 547 (U.S. Environmental Protection Agency, 1990). At each community drinking water system, water samples were collected in two 125 mL amber opaque plastic bottles and one 1 L clear plastic bottle. Bottles were immediately placed inside a cooler with ice packs. 100 mg/L sodium thiosulfate was added to drinking water samples from Bullet Tree to neutralize chlorine and prevent glyphosate degradation. All samples were kept frozen until the time of shipment. The 125 mL water samples and the sediment samples were packaged in a cooler with icepacks and shipped to Brookside Laboratories in New Bremen, Ohio. The 1 L bottles were packaged in coolers with icepacks and shipped to University of Kentucky.

C. Water Quality Analysis

A YSI multiparameter meter was used in the field to determine temperature, conductivity, dissolved oxygen, salinity, total dissolved solids, chloride, and ammonia levels at each sampling point. Nutrient concentrations and pH were measured at University of Kentucky. Nutrient concentrations were determined using the orthophosphate [method PO-19 (224800) and PO-19A (224801)] and nitrate [method NI-11 (146803)] test kits included in the Hach Surface Water kit. The Mettler Toledo Benchtop FP20 pH/mV Meter was used to measure pH.

D. Glyphosate Quantification

The 125 mL water and sediment samples shipped to Brookside Laboratories were analyzed using High performance liquid chromatography (HPLC) in accordance with EPA method 547 (U.S. Environmental Protection Agency, 1990) with a detection limit of 25 ppb.

The 1 L water samples sent to University of Kentucky were analyzed using enzyme-linked immunosorbent assay (ELISA) kits. Abraxis Glyphosate Microtiter Plate kits were used for this analysis. To determine glyphosate concentrations, the mean absorbance for each of the provided standards was divided by the absorbance for the zero standard. These values were plotted against each respective log glyphosate concentration to determine a regression line, from which the concentration of each sample could be determined.

Water samples were also analyzed by another laboratory at the University of Kentucky using Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). Samples were filtered using vacuum filtration with 0.7 µm pore size glass fiber filter papers, allowing about 200 mL of sample to pass through before sample was collected. The lab developed a method based on the USGS method 5-A10 for determination of glyphosate and its degradation products aminomethylphosphonic acid and glufosinate by isotope dilution, online solid-phase extraction, and LC-MS/MS (Meyer, 2009). However, this developed method deviated from USGS method 5-A10 by eliminating the solid-phase extraction step. This resulted in the occurrence of matrix interference which increased the method detection limit from 0.02 ppb to 0.19 ppb.

E. Modeling Approach

1) Model Set up

SWAT Version 2012 and the ArcSWAT interface were used to set up the watershed model. A 30 m digital elevation model was used to delineate the watershed (World Bank -European Space Agency Partnership, 2018). Streams and outlet points were defined, with two additional outlet points added manually for the sites at which observed flow rate data exists. The watershed was delineated into 53 subbasins. Hydrologic response units (HRUs) in SWAT represent areas of the watershed that are homogenous in soil type, land use, and slope, and can therefore be assumed to respond similarly to various hydrological conditions (Winchell et al., 2013). Land use data was converted to land use types listed in the SWAT 2012 database and reclassified to the respective SWAT land use code (Central American Commission on et al., 1998). Soil data used the Food and Agriculture Organization (FAO) soil classification system, so the user soil table in the SWAT 2012 database using the United States Department of Agriculture (USDA) soil taxonomy system was changed to FAO classification (FAO/UNESCO, 2020). A soil database using FAO classification could be found in MWSWAT 2009, an older version of SWAT for a different user interface. This table was imported into the SWAT 2012 database. The slope geoprocessing tool in ArcMap was used to determine the ranges to be used for the slope classification step of HRU analysis. The number of slope classes selected was 3, and ranges were determined to be 0-14%, 14-32%, and 32% and up. These layers were then overlaid, and an HRU feature class was created. To define HRUs, a threshold of 20% land use, 10% soil, and 20% slope was indicated. These thresholds were used because they have been shown to be adequate for most applications (Winchell et al., 2013). Land use classification was further refined to split agricultural land use into four crops; corn, sugarcane, soybean, and beans (represented in SWAT as kidney beans). These crops were selected based on local knowledge and by recommendation of the Pesticide Control Board of Belize. It was assumed that there was an equal distribution of these four crop types. 181 HRUs were created.

The weather generation user table of the SWAT 2012 database was edited to incorporate weather station data provided by the National Meteorological Service of Belize. The WGNmaker4 excel macro tool was used to calculate temperature and precipitation statistics given the observed data. Information regarding hourly maximum rainfall, solar radiation, wind speed, and dew point are also required for this table, although these data weren't available for Belize. However, the SWAT 2012 database contains these statistics for weather stations in the United States. A weather station in Key West, Florida was selected to supplement the missing data being that it is the US weather station closest in proximity and climate. Weather input files were written for daily observed precipitation data from Ladyville, Belmopan, and Spanish Lookout weather stations, and for daily maximum and minimum temperature at the Belmopan and Ladyville stations.

2) Model Calibration and Validation

The SWAT Calibration and Uncertainty Program (SWAT-CUP) and the Sequential Uncertainty Fitting Version 2 (SUFI-2) procedure were used to calibrate the model. The model was calibrated for flow since a long-term glyphosate monitoring dataset is nonexistent. These programs were selected based on their repeated use in literature and demonstrated efficiency with large scale models (Abbaspour et al., 2015). Latin Hypercube sampling is used to obtain a distribution of outputs to create an uncertainty band called the 95% prediction uncertainty (95PPU), with the goal of containing the largest fraction of observed data within this uncertainty band (P-factor), while minimizing the average thickness of the uncertainty band (R-factor) (Abbaspour, 2015; Khalid et al., 2016). To calibrate, input parameters and respective ranges of feasible values were selected based on recommendations for similar applications in literature (Moriasi et al., 2007). The number of simulations per calibration iteration was specified to be 500, as recommended (Abbaspour, 2015). Observed daily discharge data used for calibration were provided by the Belize National Hydrological Service for two locations in Belize: Double Run Water Treatment Plant (subbasin 8) from 2001-2009 and Big Falls Ranch (subbasin 14) from 2001-2005. The Nash-Sutcliffe (NS) function was specified as the objective function for calibration, and a threshold of 0.5 was indicated. Nash-Sutcliffe efficiency is an indicator of the goodness of fit of hydrologic models and is commonly used in literature for similar applications (Moriasi et al., 2007). NS values in the range of 0.5 to 0.65 are indicative of satisfactory model performance (Moriasi et al., 2007). An acceptable value of 0.56 was achieved for the Nash-Sutcliffe (NS) efficiency of subbasin 8 after 6 iterations. However, subbasin 14 was poorly simulated and not able to meet the threshold, with a NS efficiency of 0.15 in the sixth iteration. Because flow at subbasin 8 was well simulated, it was determined to move forward with validation using the parameter ranges from the sixth iteration.

Model validation was conducted by inputting the parameter ranges that resulted in successful calibration and daily observed discharge data for subbasin 8 for a period from 2010 to 2013, and running a single iteration of 500 simulations to evaluate how well the model performs for data not used in calibration. The model was validated using flow rate data for subbasin 8 only, since more data from subbasin 14 were not available. Validation resulted in a NS efficiency of 0.64, meaning that model performance for flow can be considered satisfactory. These parameter ranges were then used to simulate glyphosate transport in the watershed.

3) Glyphosate Transport Simulation

The management input file in SWAT was edited to simulate the use of glyphosate in the watershed. Application rates were estimated based on literature and industry recommendations: 0.87 kg/ha for corn, 0.87 kg/ha for soybean, 2.36 kg/ha for beans, and 4.93 kg/ha for sugarcane (Love et al., 2011; Sugar Research Australia, 2017; University of Kentucky Research and Education Center at Princeton). It was assumed that “Round-up Ready” crops genetically modified to be resistant to glyphosate are not grown in the region, because the cultivation of these crops are not yet permitted in Belize (Jacobs, 2016), simulated glyphosate application was scheduled to occur before the planting of crops. Default physiochemical properties of glyphosate from the SWAT pesticide database were applied. The routing pesticide option in the general watershed data input file was edited to allow for the transport of glyphosate through the channel network. A simulation was then run on a daily time step for the period of January 1, 1999 to September 30, 2019. A warmup period of 2 years was specified to allow the watershed parameters to come to a reasonable state, as recommended (Winchell et al., 2013).

F. Analysis of Results

The segment of river or stream within each subbasin is known as the reach. SWAT reports pesticide loads of both soluble glyphosate and glyphosate sorbed to sediment transported with water into and out of each reach in units of mg active ingredient per time step. Glyphosate loads were converted to concentrations by converting average daily flow rate into and out of each reach per time step to volume of water into and out of each reach per time step, and then dividing glyphosate load per time step by volume of water per time step to yield glyphosate concentrations in water in mg/L. These concentrations were then converted to µg/L. Average glyphosate concentrations in each subbasin were calculated using data from the entire simulation. Because the climate in Belize consists of two seasons, rainy and dry, average concentrations in each subbasin were also calculated for each season. The dry season typically lasts from November to May, with November and May being transition periods. The wet season typically lasts from May to November, with the onset of the wet season ranging from early May in Northern Belize to early June in Southern Belize. For the purposes of determining average concentrations across the watershed for both seasons, the dry season was established as December to April, and the wet season was established as May to November. Data were analyzed using a single factor ANOVA test with a significance level $\alpha = 0.05$ to determine significant differences based on site, season, or type of glyphosate load.

RESULTS & DISCUSSION

A. Water Quality

Table 1 presents the results of water quality analyses of each sample. Belize does not yet have national standards for drinking water quality or river and stream health, and instead has effluent limitations for industry wastewater discharge and follows the World Health Organization guidelines for drinking water. Therefore, observed data were compared to these standards as well as to EPA guidelines for rivers and streams to consider non-point source pollution. Total dissolved solids and chloride are within the recommended ranges for the Belize Effluent Limitations, WHO Guidelines for Drinking Water, and the National Secondary Drinking Water Regulations set by the EPA (Belize Department of Environment, 2003; US EPA, 2009; World Health Organization, 2017). The observed dissolved oxygen levels of all samples are above the EPA recommended minimum levels for warm water aquatic life, meaning that eutrophic activity is unlikely (US EPA, 1986). Ammonia and chloride concentrations also are within the ranges recommended for freshwater aquatic life by the EPA (US EPA, 2004, 2013). pH in each sample meet the EPA recommended criteria for aquatic life, the Belize Effluent Limitations, and the EPA Secondary Drinking Water Standards (Belize Department of Environment, 2003; US EPA, 2004, 2009). Phosphate and nitrate concentrations are all below the Belize Effluent Limitations for phosphate (5 mg/L) and nitrate (3-10 mg/L) (Belize Department of Environment, 2003). EPA standards for total phosphorus and total nitrogen in rivers and streams vary across the United States. The criteria for total phosphorus in rivers and streams ranges from 10 to 128 µg/L, and the observed concentrations of orthophosphate reported as phosphorus, exceed the criteria in some of these regions (US EPA, 2002). However, when compared to ecoregion XII, the region in the US most similar to the climate of Belize, the observed concentrations fall below the standard of 40 µg/L (US EPA, 2002). However, it is important to note

TABLE I. WATER QUALITY ANALYSIS RESULTS

Sampling Point	BTU[a]	BTA[b]	BTDW[c]	SLU[d]	SLA[e]	SLDW[f]
Dissolved Oxygen (mg/L)	7.93	7.77	7.64	8.4	13.43	7.15
TDS (mg/L)	259.88	259.91	255.92	233.05	234.37	223.53
Orthophosphate (µg/L)	80	40	40	40	80	40
Orthophosphate as Phosphorus (µg/L)	26.09	13.04	13.04	13.04	26.09	13.04
Nitrate (mg/L)	4	4	2	2	2	4
Nitrate as Nitrogen (mg/L)	0.9	0.9	0.45	0.45	0.45	0.9

[a] Bullet Tree Upstream
[b] Bullet Tree Abstraction Site
[c] Bullet Tree Drinking Water
[d] Spanish Lookout Upstream
[e] Spanish Lookout Abstraction Site
[f] Spanish Lookout Drinking Water

TABLE II. SUMMARY OF GLYPHOSATE DETERMINATION RESULTS

Sampling Point	HPLC[a]	ELISA[b]	LC-MS/MS[c]
Bullet Tree Upstream	ND[d]	0.05±0.01	0.01
Bullet Tree Abstraction Site	ND	0.04±0.01	0
Bullet Tree Drinking Water	ND	0.04±0.02	0.11
Spanish Lookout Upstream	ND	0.04±0.01	0.01
Spanish Lookout Abstraction Site	ND	0.04±0.01	0
Spanish Lookout Drinking Water	ND	0.03±0.01	0

[a] High-Performance Liquid Chromatography detection limit: 25 ppb
[b] Enzyme Linked Immunosorbent Assay detection limit: 0.05 ppb
[c] Liquid Chromatography with Tandem Mass Spectrometry detection limit: 0.19 ppb
[d] Non-detect

that orthophosphate as phosphorus does not include organic forms of phosphorus. Measured nitrate was reported as nitrogen concentrations, ranging from 0.45 to 0.90 mg/L. EPA standards for total nitrogen vary from 0.12 mg/L to 2.2 mg/L (US EPA, 2002). In some regions in the US, the observed nitrogen concentrations would exceed EPA standards. When comparing to the standard for total nitrogen in ecoregion XII, concentrations in the samples from Bullet Tree upstream, Bullet Tree abstraction site, and Spanish Lookout drinking water are equal to the standard of 0.9 mg/L. This means these areas are most likely exceeding the total nitrogen standard when factoring in nitrite and ammonia concentrations as well. Nitrate concentrations are all below the US EPA standard for nitrate in drinking water (10 mg/L) and the WHO guideline for nitrate in drinking water (50 mg/L), which protect against Methemoglobinemia (US EPA, 2009; World Health Organization, 2017). In summary, these results indicate that water quality in these locations is acceptable by Belize, US, and WHO standards for drinking water and aquatic life. However, nutrients may be higher than recommended and could be indicative of the occurrence of agricultural runoff and erosion.

B. Glyphosate Determination

1) HPLC Results

Glyphosate and AMPA were not detected in any of the sediment or water samples analyzed at Brookside Laboratories. However, the detection limit using HPLC (25 ppb) is significantly higher than the concentrations reported in a previous monitoring study in Belize, with average glyphosate concentrations ranging from 0.2 to 1.7 ppb (Kaiser, 2011). This may have been due to unforeseen difficulties with U.S. Customs preventing the samples from being delivered on time. As the half-life of glyphosate ranges from 2 to 91 days in water , and it is recommended to either store samples at 4 °C for analysis within two weeks or to keep frozen if storing for longer than two weeks, it is likely that any glyphosate present would have degraded during shipping time (W.A. Battaglin et al., 2014; U.S. Environmental Protection Agency, 1990). Though AMPA presence was likely due to its greater persistence, AMPA concentrations are likely to have been below 25 ppb.

2) ELISA Kit Results

ELISA kit results yielded glyphosate concentrations below the range of quantitation in water (0.075 ppb) as well as the limit of detection (0.05 ppb) for each sample. While the calculated concentrations of some individual triplicates were at or slightly above the limit of detection, none of the average concentrations were above the limit of detection, so it was concluded that the concentrations in these samples were all non-detectable. These higher triplicates were from Bullet Tree Upstream at 0.05 ppb, Bullet Tree Abstraction Site 0.05 ppb, Bullet Tree Drinking Water 0.06 ppb, and Spanish Lookout Upstream 0.05 ppb. This analysis was conducted three months after samples were collected. They remained frozen after delivery, apart from being thawed, tested, and refrozen on three occasions for other analyses. According to EPA Method 547, glyphosate has been shown to remain stable in frozen samples for up to 18 months (U.S. Environmental Protection Agency, 1990). However, thawing and refreezing may have impacted the preservation. Additionally, there are some limitations to ELISA kits as they have the potential for cross-reactivity with other compounds possibly present in environmental samples.

3) LC-MS/MS Results

Glyphosate was not detected in any of the samples analyzed by LC-MS/MS. This analysis was conducted five months after sample collection and four incidences of thawing and refreezing, so degradation of any originally present glyphosate is highly likely. AMPA was not measured but may have been detectable at these concentrations.

4) Summary of Glyphosate Determination Results

After using three methods of analysis, it is concluded that glyphosate was not present in any of the water samples in concentrations within a detectable range. A summary of all results is shown in Table 2. This is unexpected due to the proximity of the two sample locations to agricultural activity, extensive glyphosate application, and results from previous studies reporting widespread glyphosate presence in surface water under similar conditions. As mentioned earlier, Kaiser (2011) reported all samples to be positive for glyphosate ranging from 0.2 to 1.7 ppb. Another monitoring study conducted in Mexico reported glyphosate concentrations in water ranging from <0.13 to 36.71 ppb (Ruiz-Toledo et al., 2014). A second study conducted in Mexico quantifying glyphosate in groundwater and drinking water found concentrations ranging from 0.44 to 1.41 ppb in groundwater and 0.35 to 0.65 ppb in drinking water (Rendon-von Osten & Dzul-

Caamal, 2017). These results indicate that it is likely if glyphosate was present in the samples, concentrations would have been below the 25-ppb detection limit. While the ELISA and LC-MS/MS analyses would have been able to detect similar concentrations, these analyses occurred several months after sample collection, and preservation may have been impacted by thawing and refreezing during that time. Glyphosate has a wide-ranging half-life, and one experiment investigating glyphosate biodegradation in a water sediment system reported that glyphosate was completely removed from water and only present in sediment after 40 days (S. Wang et al., 2016). If preservation was compromised, it is very likely that glyphosate would have been degraded or sorbed to particulate matter in the samples by the time analysis occurred. Because the ELISA and LC-MS/MS analyses did not investigate glyphosate in sediment or AMPA concentrations, and samples were filtered through 0.7 µm filters before LC-MS/MS analysis, it is possible that these methods would not have been able to capture any glyphosate processes occurring at that time.

C. Comparing Simulated and Observed Results

Simulated glyphosate concentrations were evaluated in the subbasins in which the sampling sites are located. Glyphosate was not present in either soluble or sorbed phases, at all Bullet Tree sampling locations in subbasin 36. Spanish Lookout sampling locations are divided among two subbasins, with the upstream sampling point in subbasin 35, and the abstraction site and drinking water system in subbasin 31. Glyphosate was not present in subbasin 35 in either soluble or sorbed phases. However, glyphosate was occasionally present at detectable levels throughout the simulation in subbasin 31. Soluble glyphosate concentrations flowing into the subbasin exceeded the EU standard of 0.1 µg/L 0.06% of the time, while outflow concentrations never exceeded the standard. Sorbed glyphosate concentrations in the subbasin inflow and outflow exceeded the EU standard 1.05% and 0.70% of the time, respectively. However, during the month of July 2019 during which samples were collected, all soluble and sorbed concentrations were less than 0.005 ppb. This is all consistent with the nondetectable concentrations

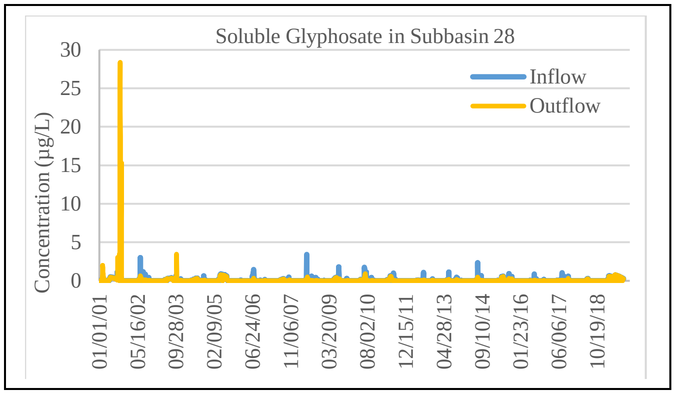

Fig. 3. Soluble glyphosate concentrations in the inflow and outflow of subbasin 28. This subbasin has the highest percentage of days with concentrations above the EU standard.

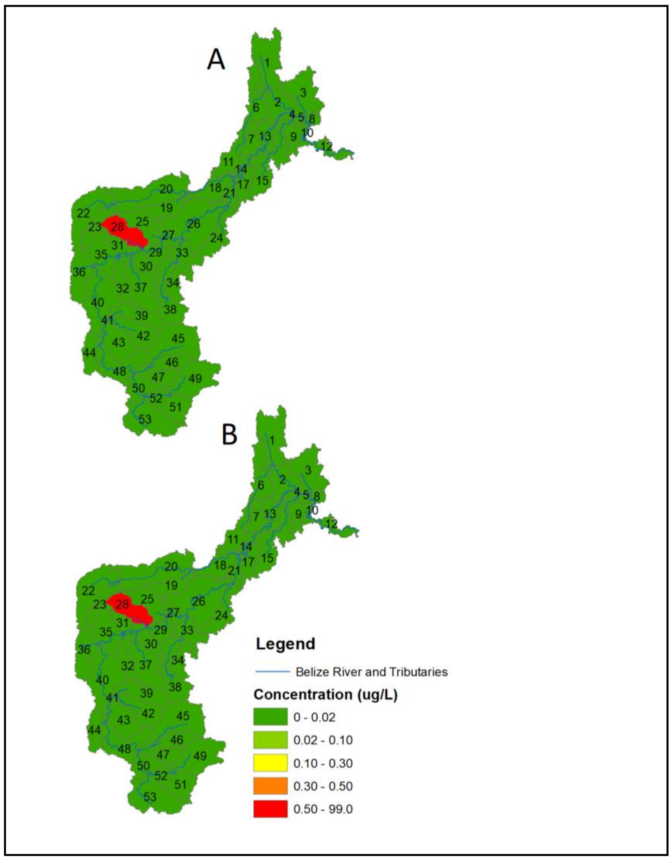

Fig. 2. Average simulated soluble glyphosate concentrations in each subbasin during month samples were collected. A) Average soluble glyphosate in subbasin 28 inflow was 0.65 ppb B) Average soluble glyphosate in subbasin 28 outflow was also 0.65 ppb

observed in the samples collected Bullet Tree Falls and Spanish Lookout.

To understand what may have been occurring in the rest of the watershed at this time, average concentrations in each subbasin during the month of July 2019 were calculated and displayed spatially as shown in Fig. 2. This revealed that subbasin 28, just downstream of where samples were collected in Spanish Lookout, was the only subbasin to have a detectable amount of soluble glyphosate at 0.65 µg/L, which exceeds the EU standard. These elevated concentrations may be due to the unusually dry climate and late wet season that Belize was experiencing during that time. One study that measured glyphosate in water bodies of Mexico over the course of a year found significantly higher concentrations in the dry season as opposed to the wet season, and concluded that these higher concentrations were due to less dilution by rainfall (Ruiz-Toledo et al., 2014).

Soluble glyphosate concentrations in subbasin 28 over time are shown in Fig. 3. Concentrations are greater on average in the subbasin outflow than inflow (p-value<0.0). Soluble concentrations largely remain below 5 ppb, apart from one event in September 2001 when outflow soluble concentrations experienced a large spike up to over 28 ppb. Soluble concentrations in the inflow and outflow exceeded the EU standard 12.53% and 11.65% of the time, respectively. The land use in this region is predominantly agriculture, consisting of corn, sugarcane, soybean, and pinto bean production.

D. Model Predictions for the Rest of the Belize River Watershed

Average concentrations for the wet season, dry season, and entire simulation in each subbasin were also calculated for both soluble and sorbed glyphosate. According to the model, concentrations of glyphosate soluble in water across the watershed are generally non-detectable or below the EU standard, and soluble glyphosate is significantly less than sorbed glyphosate (p-value<0.0). Based on these results, the risk of glyphosate contamination in drinking water is low, especially if water filtration is employed to remove glyphosate sorbed to particulates. However, higher concentrations of glyphosate sorbed to sediment entering the Belize River is still of concern as glyphosate in sediment has the potential to be desorbed and re-released into the water column (Pandey et al., 2019). Model results indicate that subbasins 2, 3, and 28 have the highest likelihood of glyphosate concentrations that exceed the EU standard, suggesting that monitoring in these regions should be considered in future studies. Subbasin 28 is the most significant source of glyphosate to the Belize River, when compared to subbasin 2 (p-value <0.0) and subbasin 3 (p-value <0.0).

E. Model Limitations

A major limitation of the model is the current lack of glyphosate monitoring data, and a lack of a large enough dataset for nutrient and sediment data to calibrate and validate the model for these parameters. Model results were compared to available data for observed sediment and nutrient concentrations and was shown to not perform well for these parameters. This is an indicator that the model may not be accurately simulating runoff and erosion conditions in the watershed, which is likely to impact the accuracy of the glyphosate results as well. Additionally, glyphosate application operations estimated for each crop in the simulation are likely to be very conservative for estimating actual glyphosate use in the area. Applicators typically apply much more herbicide than what is recommended, as revealed during discussions with the Pesticide Control Board. Additionally, the assumption that no glyphosate tolerant genetically engineered crops were grown in the watershed and thus application only occurs before the planting of crops may also be conservative, as it is known that genetically modified crops have been imported into the country but are currently not legal to cultivate (Jacobs, 2016). It is likely that the land use data used for the model is also underpredicting glyphosate transport. The available spatial dataset used for this model does not include urban areas in the watershed, which has a significant impact on glyphosate transport. Previous studies have demonstrated the relationship between developed areas and quicker overland flow transporting glyphosate to water sources, and have claimed that near-site land use may be a better predictor of glyphosate presence in water than generalizing land use across a watershed (Medalie et al., 2020). This suggests that incorporating developed land is needed for a more accurate simulation.

F. Future Work

A crucial next step is to obtain a large dataset of glyphosate concentrations over time so that the model can be fully validated for glyphosate transport. LC-MS/MS is strongly recommended for future studies due to its accuracy and low detection limit. Future monitoring studies should target the subbasins 2, 3, and 28, as they were predicted by the model to have the highest likelihood of elevated concentrations. Acquiring more accurate land use data that includes urban areas would make a significant difference on model outcomes. Obtaining more local knowledge to get a better idea of how glyphosate is actually applied in Belize would decrease uncertainty as well. Useful directions that this work may go next include incorporating the modeling of AMPA fate and transport, simulating different best management practices to evaluate efficiencies, and inputting wastewater treatment plants as point sources as they have also been shown to contribute to glyphosate loads (Kolpin et al., 2006).

CONCLUSION

A combined detection, monitoring and modeling approach was applied in the Belize River Watershed to determine if glyphosate was present in the drinking water resources of agricultural regions and whether glyphosate transport in the watershed could be modeled using SWAT. HPLC, ELISA kits, and LC-MS/MS all corroborated that glyphosate was not present in any of the samples collected from Bullet Tree Falls or Spanish Lookout. Modeling results for the same areas supported this finding, simulating no detectable glyphosate concentrations at the time that samples were collected. However, what was evident from the model was that just downstream of sample collection sites were elevated concentrations of glyphosate and a subbasin that is predicted to be the most significant contributor of soluble glyphosate to the watershed. The model also predicted low, safe levels of glyphosate for the vast majority of the watershed, apart from two other higher risk areas; subbasins 2 and 3. Supplementing a very limited amount of field and lab data with an informed, robust model allowed for the identification of potential risks and areas to target for future studies. This work demonstrates the application of watershed modeling for more efficient and informed analysis of water quality in watersheds of developing regions, which can be extremely useful for designing studies to measure progress towards SDGs and helping developing countries monitor and manage glyphosate transport.

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CHAPTER 6. CONCLUSION

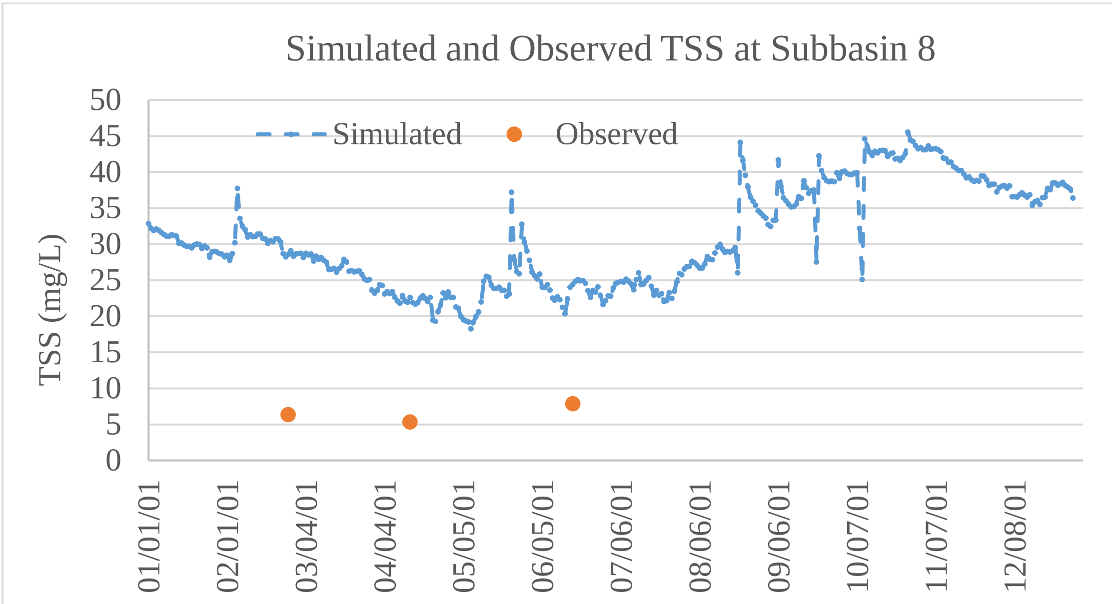
Glyphosate is one of the most widely used herbicides in history and is considered by the World Health Organization to be a "probable carcinogen" to humans. While the extent of the risk associated with glyphosate exposure is still disputed, the problem of glyphosate transport in erosion and runoff from application areas to unintended locations is clear. Glyphosate is widely used in Belize, and there is growing concern among Belizean regulatory agencies regarding the safety of continued glyphosate use. Glyphosate concentrations are not currently monitored in waterways of Belize, and conducting consistent, costly analysis is not feasible at this time.

The first objective of this study was to determine if glyphosate is present in the Belize River. After using three methods of varying levels of precision, glyphosate was not detected in any of the water samples. Sediment samples were only analyzed using HPLC, and glyphosate was not detected in any sediment samples in concentrations above 25 ppb. However, lack of glyphosate presence cannot be definitively ruled out from these results for several reasons. Difficulties with transporting the samples in a timely manner may have impacted the preservation of any glyphosate that may have been present in the samples. Additionally, the thawing and re-freezing may have played a role in expediting degradation of any glyphosate in the samples. Since AMPA has a longer half-life than glyphosate, it is likely that if any glyphosate had been present, AMPA could have been detected in the samples. However, when samples were analyzed for AMPA, the detection limit was much higher than what was expected to be seen in the region. Additionally, the study was limited to only one day of sampling in two villages, which is not nearly enough to be representative of the entire watershed and the wide variation in concentrations due to changes in flow rate and climate, as demonstrated by the model. Furthermore, sampling occurred during an unseasonably dry time, which is not indicative of the typical climate and runoff during the wet season. However, model results indicated that no detectable glyphosate should have been present in the two locations in which samples were collected, which is reflected in the observed results.

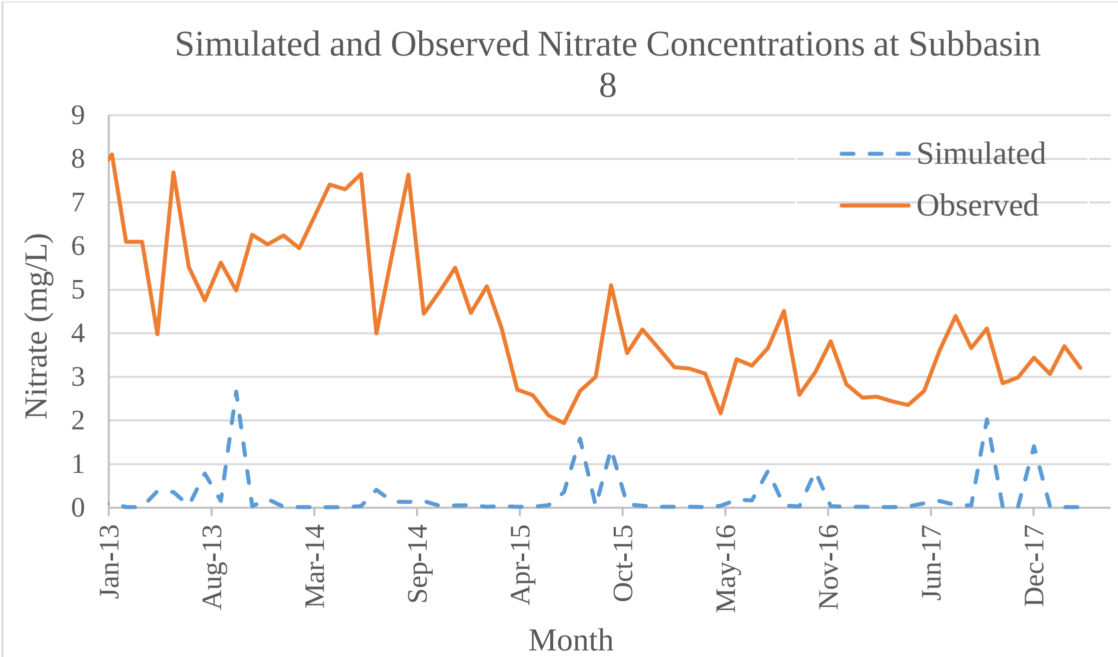
The second objective was to determine whether SWAT is an effective tool for simulating glyphosate fate and transport at the watershed scale. The lack of long-term glyphosate concentration data makes it impossible to definitively conclude whether the model is accurately simulating glyphosate transport across the watershed. However, the capability of the model to simulate other parameters was used to evaluate how well the model represents the Belize River Watershed overall. The model was able to be calibrated for flow in two subbasins, and satisfactory model performance was achieved for flow within subbasin 8, while flow at subbasin 14 was not well simulated. In addition, nutrient and sediment concentrations were not well represented by the model when compared to limited observation data. Inaccurate nutrient sediment concentrations may mean that runoff and erosion from agriculture areas is being poorly modeled, which could largely influence glyphosate transport from runoff. Overall, more data is needed to demonstrate the accuracy of the model in simulating glyphosate transport to the Belize River.

While the accuracy of these results is still uncertain and pending more comprehensive monitoring data, the simulated results allow for an exploration of model capabilities and what the model is currently predicting to be occurring in the watershed. According to the model, predicted concentrations of glyphosate soluble in water across the watershed should generally be non-detectable or below the EU standard, and soluble glyphosate should be significantly less than sorbed glyphosate. Judging from these preliminary modeling results, the risk of glyphosate contamination in drinking water is probably low, especially if water filtration is employed to remove glyphosate sorbed to particulates. However, the higher concentrations of glyphosate sorbed to sediment entering the Belize River may still be of concern as glyphosate in sediment has the potential to be re-released into the water column. Model results indicate that subbasins 2, 3, and 28 may have the highest likelihood of glyphosate concentrations that could exceed the EU standard and suggest that these regions should be considered in future studies. Subbasin 28 was the largest contributor of modeled soluble glyphosate loads to the river, as compared to other subbasins with elevated concentrations. Model results also show that detectable concentrations should be present in subbasins 4, 5, 8, 10, 12, 26, and 27, and suggest that these regions should be monitored as well.

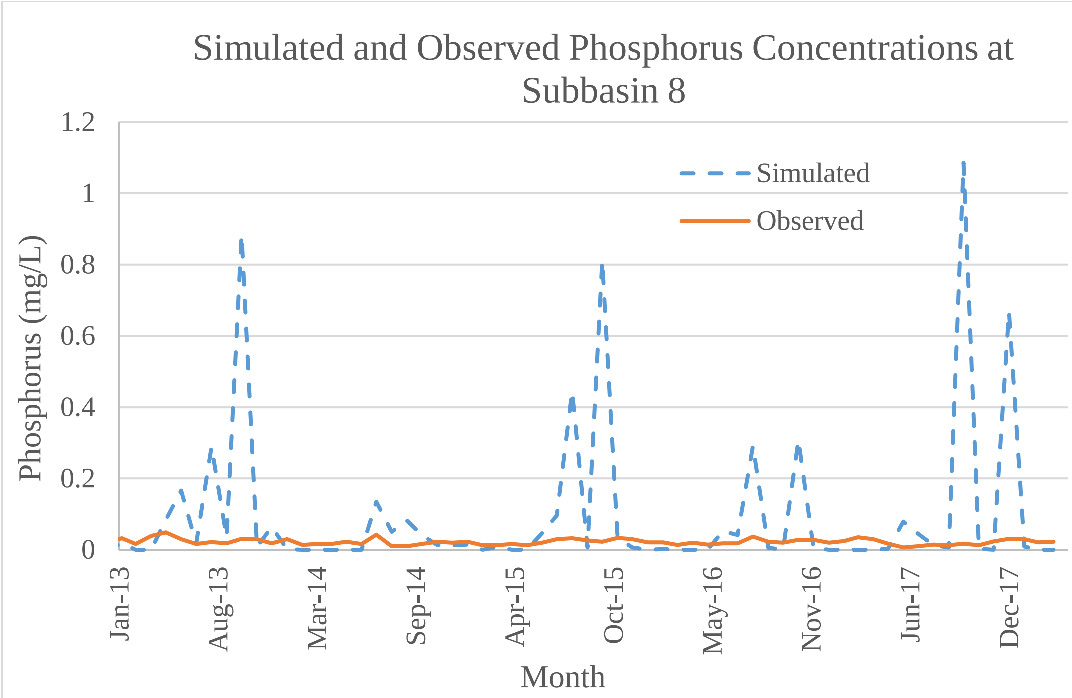
After completing the first known application of SWAT for herbicide transport in Belize, it can be concluded that much more work is needed before the model can be relied upon for accurate results to base management decisions upon. However, the modeling tool shows a lot of potential to be useful for watershed management in places such as Belize. From limited data, a watershed model was developed and used to simulate herbicide use, fate, and transport. From these simulated results, a wide range of analysis was able to be conducted; including estimating concentrations in each subbasin, visualizing herbicide concentration change with time and climate, determining which regions in the watershed are most likely to experience elevated glyphosate concentrations, and evaluating in-stream processes such as diffusion and degradation. These results can be extremely useful in prioritizing next steps for watershed managers and were accomplished with only an internet connection and a computer, making SWAT a powerful and accessible tool. Overall, this work has demonstrated a framework for applying SWAT in Belize to predict glyphosate fate and transport, and that with some improvements and more comprehensive datasets, the model has the potential to be a powerful tool for simulating and managing glyphosate transport in water.



Appendix 1 Comparison of simulated and observed total suspended solids at subbasin 8. Only three measured data points were available, but model overestimates total suspended solid concentrations when compared to these three observations.



Appendix 2 Comparison of simulated and observed nitrate concentrations at subbasin 8 Model underestimates nitrate concentrations. This may be due to inaccuracy in modeling runoff, or additional nitrate input to the river from wastewater treatment plant discharges not accounted for in this model.



Appendix 3 Comparison of simulated and observed phosphorus concentrations at subbasin 8. Model tends to overestimate peak concentrations and underestimate minimum concentrations.

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Publications

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