MANAGEMENT OF AGRICULTURAL PESTICIDE RINSATE USING A BIOBED UNDER MANITOBA CONDITIONS

by

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ABSTRACT

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A dual-celled biobed was built at the Ian Morrison N. Research farm in Carman, MB to degrade and retain the pesticides from pesticide rinsate derived at the farm. The biobed was designed for Manitoba conditions with considerations taken from existing biobeds in the Province of Saskatchewan. Temperature and moisture of the biomixture contained within the biobed was monitored using probes for the duration of the study. Three pesticides (2,4-DB, dicamba, and metrafenone) were detected in the biomixture prior to pesticide input from rinsing in 2020 but had degraded by the end of the season and were no longer detected. The Influent of the biobed was analyzed for a suite of pesticides and 23 pesticides and one pesticide metabolite were detected. In Effluent One only 11 of the 24 compounds were detected, and six compounds were detected in Effluent Two. Total pesticide concentrations significantly decreased for Influent > Effluent One > Effluent Two. Pesticides detected in the effluents tended to have higher GUS or Koc values. In 2021, a large influx of prepared spraying liquid containing glufosinate (approximately 680L) overburdened the biobed and resulted in reduced degradation and increasing mean concentrations from Influent > Effluent One > Effluent Two. Glufosinate and seven other pesticides detected in the influent were not measured in biomix due to method limitations. There were three pesticides (bifenthrin, trifloxystrobin, and diuron) detected in the

biomixture samples in 2021, and only diuron, which had the highest GUS and Koc values, was still detected at the end of the season.

Keywords: Biobed; Dual-cell; Single-cell; Pesticide; Pesticide rinsate; Rinsate management system; Biomixture

FOREWARD

This thesis was written according to the guidelines of the Department of Soil Science at the University of Manitoba. It is comprised of four chapters: an introduction (Chapter 1), two stand-alone research chapters (Chapters 2 & 3), and an overall synthesis chapter (Chapter 4).

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I am very grateful to everyone who provide their assistance and expertise in establishing and constructing the biobed in Carman, Manitoba. Thank you to Dr. Tom Wolf, Alvin Iverson, Terri Anseeuw, and Evan Derdall for participating in the tour of biobeds in Saskatchewan and for providing valuable feedback on the biobed design. Thank you to all the farm research staff and students at the Ian N. Morrison Research Farm who helped in any way with the biobed construction and operation. I would like to express my enormous gratitude to Terri Anseeuw for providing me with countless hours of help, guidance and hands-on training for plumbing, carpentry, electrical wiring, welding and more. Terri was instrumental in me being able to build the biobed system. I would also like to thank Dr. Annemieke Farenhorst, Phoenix Nakagawa, Andrej Kao, and Dr. Alistair Brown for helping during biobed sampling and construction.

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LIST OF ABBREVIATIONS

AAFC – Agriculture and Agri-Food Canada

AI – Active Ingredient

Biomix – Biomixture

CH_4 – Methane

CO_2 – Carbon Dioxide

Conc - Concentration

DT_{50} – Soil Degradation (Half-Life)

F – Fungicide

F_{ghg} – Greenhouse Gas Flux

FTIR – Fourier-Transform Infrared Spectroscopy

GUS – Groundwater Ubiquity Score

H – Herbicide

I – Insecticide

IUPAC – International Union of Pure and Applied Chemistry

K_{OC} – Soil Adsorption Coefficient

LOQ – Limit of Quantification

LSM – Least Square Means

NH_3 – Ammonia

NO_2 – Nitrogen Dioxide

N_2O – Nitrous Oxide

NTU – Nephelometric Turbidity Unit

PMRA – Pest Management Regulatory Agency

VWC – Volumetric Water Content

1. INTRODUCTION

1.1 Pesticide Use and Environmental Detections

Pesticides are used to control pests that are interfering with crop health or yield. Pesticides are also used in many other applications, including to ensure public safety. Health Canada's Pest Management Regulatory Agency (PMRA) is responsible for pesticide regulation and reports on the sales of pesticide active ingredients in Canada. In recent years, at least 100 million kg of active ingredients are sold annually for agricultural (71.2%), non-agricultural (24.3%) and domestic (4.5%) applications (PMRA 2021). The herbicide glyphosate is among the top pesticide active ingredients sold in Canada (PMRA 2021). Glyphosate is widely used in many countries across the world, but the European Union has imposed restrictions for its use in both agricultural and non-agricultural applications (Székács and Darvas 2018). Although relatively immobile in soil (Lewis et al. 2016), glyphosate has been detected in various environmental matrices, including streams and drinking water sources (Battaglin et al. 2014; Guo et al. 2019)

The prairie provinces Alberta, Saskatchewan, and Manitoba account for 82.3% of total farm area in Canada (Government of Canada 2022) and an estimated 85% of the total pesticide usage in Canada (Statistics Canada 2016). Almost all of Canada's canola (~99%), spring wheat (~98%) and barley (~96%) are grown on the Prairies (Government of Canada 2022). A wide range of pesticides are being applied to these and other crops in Prairie agriculture. Almost all of the field crop producers in the province of Manitoba (96%) apply pesticides (Manitoba Agriculture 2023). Consequently, large percentage of the crop land area in Manitoba receives herbicides (97%), fungicides (83%) and insecticides (68%) (Manitoba Agriculture 2023).

Studies conducted in the Canadian prairies have detected pesticides in drinking water sources (Donald et al. 2007), groundwater (Munira et al. 2018), wetlands (Rawn and Muir 1999), and rivers (Gamhewage et al. 2019). Current-use pesticides have been shown to account for 96% of the pesticides detected in the water-column and 76% in sediments of Manitoba rivers (Gamhewage et al. 2019). Auxin herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) are widely used in Prairie agriculture and more frequently monitored for than other pesticides. Auxin herbicides 2,4-D (95%), MCPA (95%), and clopyralid (88%) are almost always detected in water-column obtained from Manitoba rivers (Fatema et al. 2022). Auxin herbicides have also been detected in Prairie groundwater, including at concentrations greater than the Canadian Drinking Water Guidelines (Miller et al. 1994; Hill et al. 1996; Munira et al. 2018). Fewer studies have monitored for pesticides in bottom sediments but for samples obtained from Manitoba rivers, fungicides tebuconazole (72%), boscalid (67%) and propiconazole (61%) were the most frequently detected pesticides (Fatema et al. 2022). The herbicides MCPA (68%) and glyphosate (55%) were also frequently detected in these bottom sediments.

The water contamination by pesticides is a result of both nonpoint- and point-source pollution. Nonpoint sources responsible for pesticide transport to surface water in the Canadian Prairies include snowmelt and rainfall runoff, as well as atmospheric dry and wet deposition (Donald et al. 1999; Messing et al. 2011; Main et al. 2016). Recommended practices for reducing pesticide non-point source pollution include riparian buffers around water ways to minimizing runoff entry into surface waters and improving on pesticide spraying equipment to minimize atmospheric drift (Gagnon et al. 2016). Management practices such as contour farming and reduced tillage is

recommended to reduce soil erosion, thereby also minimizing pesticide environmental pollution (Gagnon et al. 2016).

Pesticides are an important component of sustainable agriculture in Canada, but their handling areas can be a major cause of pesticide point-source pollution to surface and ground water (Braul and Sheedy 2018). The release of wastewater effluent is another example of a point source responsible for polluting Prairie rivers (Sheedy et al. 2019). Pesticide handling areas are locations used for the filling and cleaning of pesticide spraying equipment and can contribute to up to 90% of the total pesticide loadings in water (Frede et al. 1998; Neumann et al. 2002). It is common to dispose of rinsate on soil surfaces, thereby creating high pesticide concentrations in soil. Schoen and Winterlin (1987) conducted a laboratory to examine which factors contributing to pesticide degradation in such soil disposal zones, including soil characteristics, microbial activity, and pesticide concentration. They concluded that pesticide concentrations were the driving force, with degradation rates being much slower in soil exposed to high (1,000 mg/kg) than lower (100 mg/kg) pesticide concentrations.

Among the most promising methods of reducing point-source pollution associated with the cleaning of pesticide spraying equipment is the adoption of biobeds on farms (Torstensson 2000). Biobed systems are operational on many farms in Europe and Latin America (Karanasios et al. 2012b), but it was not until recently that the Government of Canada has started to develop guidelines for the establishment of biobeds in Canada (Braul and Sheedy 2018).

1.2 Biobeds for Pesticide Rinsate Management

Biobeds are a Swedish invention that aim to mitigate pesticide contamination risks by disposing of pesticide rinsate (influent) in a system to generate cleaner effluent (Castillo et al. 2008). Biobed designs vary substantially, but generally the system is a pit or container filled with a biomixture (biomix) usually consisting of a mixture of dry plant material (e.g., straw, wood chips), humified organic matter (e.g., peat, compost) and soil (Castillo et al. 2008). The performance of biobeds is dependent on the ability of the biomixture to retain and degrade pesticides. Biomixture composition, homogeneity, age, temperature, and moisture are factors that can affect the retention and degradation capabilities of biobed systems (Castillo et al. 2008). Laboratory studies have shown that dissipation of herbicides in biomixtures intensify with increasing temperature (Castillo and Torstensson 2007; Karanasios et al. 2012a; Cessna et al. 2017). Higher temperatures cause enhanced microbial activity as well as increased solubility of certain pesticides, thereby making chemicals more bioavailable to microorganisms (Castillo et al. 2008). Biomixture moisture content is also very important as it affects oxygen availability and therefore microbial communities and their activities such as pesticide degradation (Castillo and Torstensson 2007). The influence of moisture content on pesticide degradation in soils has been widely studied and, in most cases, pesticide degradation rates are largest when moisture conditions are at or slightly below field capacity (Castillo and Torstensson 2007; Shymko and Farenhorst 2008; Camargo et al. 2013). In contrast, prolonged saturated conditions can lead to decreased herbicide degradation rates and increased pesticide transport, including in biomixture (Castillo and Torstensson 2007; Shymko and Farenhorst 2008).

It is typically recommended that the biomixture consists of straw, peat and local soil at a respective ratio of 50:25:25% (Castillo and Torstensson 2007). These biomixture materials are important because straw serves as the main substrate for microbial activity, the peat supplies sorption capacity for pesticides, and the soil supplies pesticide degrading microorganisms as well as additional sorption capacity (Castillo and Torstensson 2007). By having peat comprise 25% of the biomixture, peat lowers the pH to a level more suitable for lignin-degrading fungi that produce phenoloxidases, thereby increasing enzyme-catalyzed pesticide degradation (Castillo and Torstensson 2007). Alternate known biomixture materials, that have practical applications in the Canadian Prairies and are known to work well, are compost and wood chips (excluding cedar) (Braul and Sheedy 2018). Studies elsewhere, utilized locally available biomixture materials for biobed research, such as vermicompost and pine litter in Brazil (Dias et al. 2021), coconut fibres in Costa Rica (Chin-Pampillo et al. 2015), and bagasse (a residue of sugar cane) in Guadeloupe, France (de Roffignac et al. 2008).

Pesticide applications are seasonal in the Prairies with peak times generating the largest influxes of rinsate to biobeds. Collecting, storing, and routinely applying pesticide rinsate to the biobed surface through drip irrigation allows for more controlled application of the rinsate and prevents saturated conditions from occurring. Saturated conditions are not desired as it creates anaerobic conditions that might lead to decreased activities of microorganisms involved in pesticide degradation (Shymko and Farenhorst 2008). According to AAFC (Agriculture and Agri-Food Canada), a daily pesticide rinsate application rate of 10 L/m^2 from rinsate storage to the biobed surface is recommended but this rate can be increased to 20 L/m^2 with little reduction in the pesticide removal efficiency (Braul and Sheedy 2018).

1.3 Single- and Dual-Celled Biobeds

Biobeds are typically built as either single- or dual-cell systems. Single-cell biobeds have one biobed container in which the influent percolates, whereas a dual-cell system has the effluent of the initial biobed cell percolating through an additional second cell. Efficacy of both single- and dual-cell biobeds in the Canadian prairies have been promising in preliminary studies. This includes a study conducted on a series of single-cell biobeds in the Province of Alberta, and dual-cell biobeds in the Province of Saskatchewan that concluded that, under Prairie conditions, the biobeds were effective at retaining and/or degrading many pesticides (Fatema et al. 2022). As well, AAFC reported that such single-cell biobeds removed 90% of pesticides, while dual-celled biobeds removed 98% of pesticides (Braul and Sheedy 2018). Another study demonstrated the biobed efficacy of these biobeds in the Prairies and reported removal rates ranging from 97% to 99% for the single- and dual cell biobeds in Alberta and Saskatchewan, respectively (Bergsveinson et al. 2018).

The Groundwater Ubiquity Score (GUS) is a tool used to estimate the relative ranking of pesticide leachability in soils (Gustafson 1989). GUS is calculated taking into consideration the normalized organic carbon sorption coefficient (K_{oc}) and the soil half-life (DT50) of the pesticide active ingredient that is studied. Pesticides that have larger GUS values have a greater potential to move to groundwater than pesticides with small or negative GUS values. The four GUS categories are: extremely low (GUS < 0), low (0-1.8), moderate (1.8-2.8), and high (> 2.8) (National Pesticide Information Center 2023). Fatema et al. (2022) applied the GUS concept to the single- and dual cell biobeds in Alberta and Saskatchewan and found that more than one-half of the effluent samples collected contained pesticide active ingredients that have GUS values >

2.8. Therefore, it appears that GUS can also be used to predict the relative mobility of pesticide active ingredients in a biobed system.

There are no reported studies on the construction, use and efficacy of biobeds in the Province of Manitoba. The goal of this M.Sc. research was to build a functional biobed for use on a research farm in Manitoba, and to test its efficacy by investigating pesticide concentrations in effluents from both the first versus second biobed cell. The dual-cell l biobed was installed at the the Ian Morrison N. Research Farm in Carman, Manitoba (49°30'9" N, 98°00'6" W) and its configuration was, in part, based on the evaluation of three biobeds systems in the Province of Saskatchewan that had a variety of styles and designs (Appendix One).

1.4 Research Objective and Hypotheses

The objective of this M.Sc. research was to investigate the design, construction, and efficacy of a biobed system under Manitoba conditions.

Hypothesis: Both biobed effluents will have a reduction in pesticide detections but a dual-cell biobed design will be a better surface and ground water protection strategy compared to a single-cell biobed design

H_0: Effluent One and Effluent Two have similar pesticide active ingredients detections.

H_A: Effluent Two will have lesser pesticide concentrations than Effluent One.

1.5 Thesis Outline

This thesis was written according to the University of Manitoba Department of Soil Science guidelines. It is comprised of four chapters, which include an introduction (Chapter 1), two stand-alone research chapters (Chapters 2 & 3), and an overall synthesis chapter (Chapter 4).

Chapter 2: Research Biobed System Design and Construction

This chapter outlines the unique design and construction of the research biobed that was constructed at the Ian N. Morrison Research Farm in Carman, MB.

Chapter 3: Efficiency of a Farm Biosystem to Process Twenty-Three Pesticide Active Ingredients Used in the Canadian Prairies

This is the first study to look at the efficacy of a biobed system under Manitoba conditions. It is also the first study to investigate greenhouse gas fluxes from a biobed system in Manitoba.

My contributions to Chapters 2 and 3 included: planning, designing, and constructing the research biobed site in Carman, MB; facilitating discussions between Manitoba biobed stakeholders during the process of establishing the site; initiating the biobed system in the spring for use on the farm; maintaining and repairing the operation of the biobed system; decommissioning (winterizing) the biobed system in the fall in preparation for winter; determining standard operating procedures for the biobed site, sharing operating procedures with farm personnel, and posting the procedures on site; planning and collecting biomix, soil, rinsate and gas sampling from the field; maintaining samples and ensuring appropriate storage and shipping of samples; data management and statistical analysis; preparing tables and figures as

well as facilitating the interpretation of data; writing, editing, and finalizing manuscripts and thesis; addressing comments and feedback; and presenting the work at conferences (e.g. Manitoba Soil Science Society, International Union of Pure and Applied Chemistry, Manitoba Agronomist's Conference, and Canadian Ecotoxicity Workshop).

1.6 Contributions of Authors

This research was funded by research grants awarded to Dr. Annemieke Farenhorst by the Natural Sciences and Engineering Research Council of Canada (NSERC) through its Discovery Grant program, as well as by the Canadian Agricultural Partnership through its Ag Action Manitoba program. Sarah Johnson designed the biobed system in collaboration with her supervisor Dr. Farenhorst, as well as Dr. Claudia Sheedy (deceased in 2020), Dr. Tom Wolf and farm manager Alvin Iverson. Ms. Johnson build the biobed at Carman with the assistance of Dr. Farenhorst, Mr. Iverson, and farm technician Terri Anseeuw. Ms. Johnson carried out all data collection and sampling at the site. Pesticide residue analyses were completed by the Pesticide Chemistry group at Agricultural and Agri-Food Canada under the supervision of Dr. Jonathan Challis. Ms. Johnson completed all further data processing, including statistics and interpretations. Ms. Johnson wrote the thesis with review and editorial support provided by Dr. Farenhorst. A copy of the finalized thesis was also reviewed by advisory committee members Dr. Wolf and Dr. Ayush Kumar, who provided additional suggestions for refinement. Manuscripts arising from the M.Sc. thesis and related work will recognize the above authors as appropriate.

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2. RESEARCH BIOBED SYSTEM DESIGN AND CONSTRUCTION

2.1 Abstract

A dual-biobed system was constructed to degrade and retain the pesticides from pesticide rinsate derived from a Research Farm that utilizes diverse pesticide products and active ingredients. The dual-cell biobed at the Ian Morrison N. Research Farm was designed based on biobeds already operational in the Province Saskatchewan. This study demonstrates design and construction aspects selected for a biobed in the Canadian Prairies. The support of the research farm community to establish the on-farm biobed system was an important component to why the research biobed system design and construction was successful. Temperature and moisture had similar trends for the two biobed cells between the two years. The moisture data of the biomixture was less reliable when the temperatures were below zero degrees Celsius. Three pesticides, which included two herbicides (2,4-DB, and dicamba), and one fungicide (metrafenone), were present in the Biomixture prior to the biobed having pesticide rinsate applied and have GUS values that range from 0.91 to 1.94. These pesticides were degraded by the end of the biobed operational season as they were no longer detected at the end of the season.

2.2 Introduction

This study focuses on designing a biobed system for a research farm operated by the University of Manitoba Faculty of Agriculture and Food Sciences under the Department of Plant Science. Prior to establishing a biobed system at the University of Manitoba Ian Morrison N. Research Farm (49°30'9" N, 98°00'6" W), the practice of disposing pesticide rinsate was to deposit it on the soil surface in a small area centrally located on the farm. It has been suggested that the high

level of pesticide concentrations remaining in soil, results in slower pesticide degradation rates (Schoen and Winterlin 1987), possibly because the zones are no longer conducive to support a diverse soil microbial population. It has been estimated that such point-source locations can account for 90% of the pesticide loadings to water (Frede et al. 1998; Neumann et al. 2002).

This study commenced in September 2019. A key advisor to this study was Dr. Claudia Sheedy[1], who is recognized by the 2020 Orville Yanke Award for her contributions to establishing biobed systems across Canada. As one of her key collaborators highlighted in an oral presentation at the 49th Annual Canadian Ecotoxicity Workshop in 2022: "*Dr. Sheedy has been a highly recognized scientist and her sudden passing on July 12 in 2020 at 45 years of age brought shock and sadness to many in our scientific community"* Dr. Sheedy was instrumental in arranging for student Sarah Johnson, farm manager Alvin Iverson, and farm technician Terri Anseeuw to visit the three biobeds systems in the Province of Saskatchewan so that the research team in Manitoba was able to better understand the variety in biobed design (Appendix One).

The dual-cell biobed research site is located on the Ian Morrison N. Research Farm and its exact location (49°29'56.2" N, 98°01'54.9" W) was chosen in 2020 by Mr. Alvin Iverson (Farm Manager, University of Manitoba; *now retired in 2021*) based on ease of use for farm machinery, the site conditions (e.g., not likely to flood in Spring) and accessibility (e.g., to water lines on the farm). The location also did not border the waterway on the property or border any neighbouring properties (Figure 2.1). The site was already accessible by an existing farm road that would not require reinforcements to withstand the traffic of the large spraying equipment and tractors that would be visiting the biobed site for rinsing after pesticide field applications.

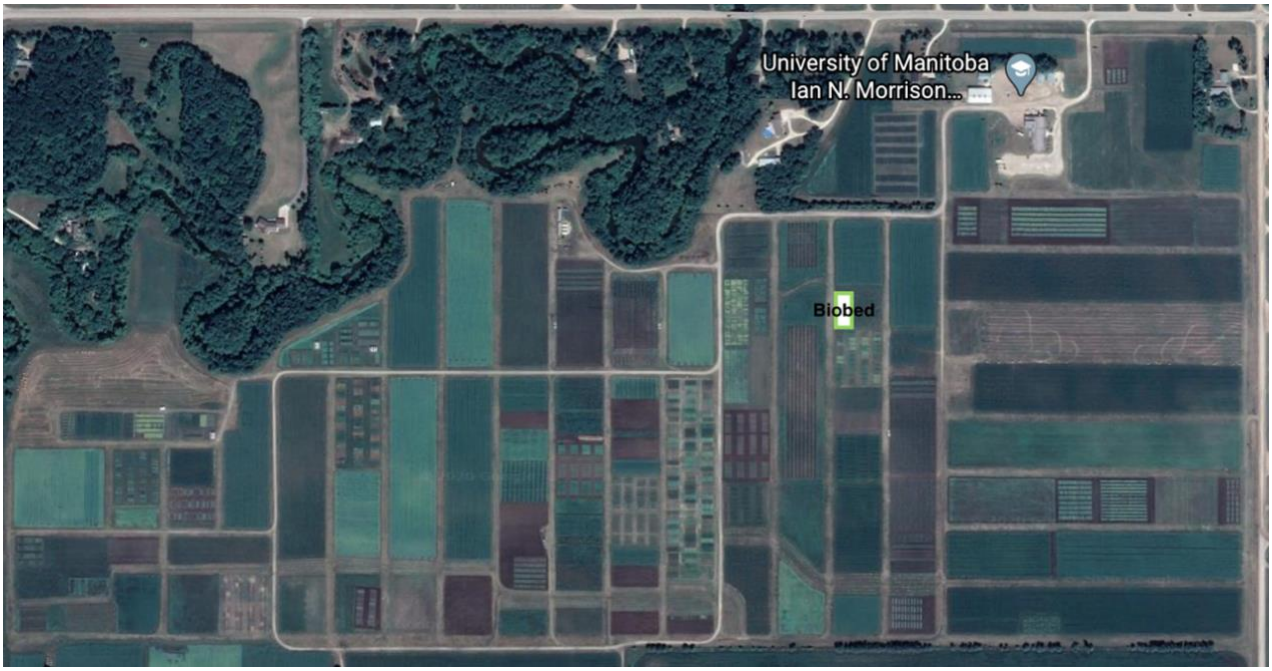

Figure 2.1 Biobed location on Ian N. Morrison Research Farm. (Map Data ©2024 Google)

The objective of this M.Sc. research was to investigate the design of a biobed system for Manitoba conditions and construct a dual-cell biobed at the University of Manitoba Ian Morrison N. Research Farm.

2.3 Material and Methods

2.3.1 Biobed System Design

Three existing biobeds systems in the Province of Saskatchewan were visited, and these had a variety of styles and designs (Appendix One). The three biobed systems were near Rosthern (52°39'45" N, 106°20'2" W), Saskatoon (52°08'28" N, 106°39'5" W), and Outlook (51°29'17" N, 107°03'30" W). Based on observations made at the Rosthern site, it was decided that the Manitoba biobed system should have a plastic roof cover over top of the biobed surface to prevent any precipitation from entering the biomix. It was also decided that the Manitoba biobed

system should have an automated pumping system so that the system can operate automatically and without daily interventions. Decisions for the design that were inspired by the Outlook site were that the Manitoba biobed system should have an effective rinsate application system with drip emitters along with a series of water meters throughout the system to monitor and quantify the liquid travelling through the system. The need for the biobed to have an automated system was reiterated after observing the extent of manual interventions required to sustain the operation of the Saskatoon biobed, which would not be economical or efficient with the larger scale of expected activity on the research farm in Carman. Seeing these biobeds in operation and having discussions with the personnel that built and operated them was extremely advantageous in designing the biobed that was constructed in Carman, Manitoba.

Design and planning for the dual-cell biobed in Carman, Manitoba began in June 2019, and the physical construction began Sept 2019 but was paused during the Winter months. Construction resumed in the Spring of 2020 until the completion with the first pesticide application to the biobed occurring on August 25th, 2020. The surface area and volume of each biobed cell are equal and were chosen on the basis that an estimated total of 10,160 L of rinsate needs to be disposed on the farm each year. This is within the range suggested by a manual developed for the operation of biobeds in Canada (Braul and Sheedy 2018). This AAFC manual recommends pesticide rinsate application rates between 10 L/m^2 to 20 L/m^2/day of biomixture surface area, to not significantly alter pesticide sorption or degradation rates. Assuming a 100-day operational period per year, and a maximum application rate of 20 L/m^2/day, a container with an exposed surface area of 8 m^2 was deemed appropriate for the needs of the research farm it services, with a maximum potential of processing of 16,000 L per year. This container was corrugated to add

strength and was comprised of polyethylene. The estimated 16,000 L per year is well above that expected based on Ian N. Morrison Research Farm records (10,160 L).

2.3.2 Biobed Probes and Water Addition

Three 60 cm long Sentek Drill & Drop Soil Moisture Probes were installed in each biobed cell (Figure 2.2). The Sentek probe is a 60 cm cylindrical probe with sensors to take hourly measurements of the volumetric water content (vwc) and temperature at six depths each 10 cm apart. With the biomixture depth being approximately 50 cm, and the probe length being 60 cm, a portion of the probe did not penetrate the biomixture. As such, all the measurements from the first sensor of the Sentek probe were disregarded. In conjunction with the soil probes, an iMetos IMT280-US Weather Station unit was installed adjacent to the biobed site to monitor field conditions using the same supplier as the moisture probes. Dataloggers for the moisture probes reported the measurements directly to a personal computer through an online portal. This chapter summarizes the results of the moisture and temperature measurements by depth and over a two-year time period.

Following the installation of the probes and weather station, over 1,950 liters of water was run through the biobed system between July 24, 2020, and August 24, 2020, to prepare the system and generate effluent at the second biobed cell that did not yet contain any added pesticides from rinsing activities. The effluent was analyzed for pesticide residues as described in Chapter 3 and provided for an indicator of pesticide residues that were potential present in the biomixture and desorbed. This ‘clean’ effluent was sampled on August 17, 2020, and August 24, 2020. The water added induced favourable moisture conditions for microbial populations in the biomixture

prior to commencement of biobed operation and application of pesticide rinsate. The water was obtained on the farm from a water line sourced from municipal water. During this period of applying municipal water to the biobed system, the rinsate storage tank had free and total chlorine concentrations that were between 0.10 mg/L and 0.13 mg/L. This is within the expected range of free chlorine concentrations of drinking water distribution systems in Canada which is 0.04 to 2.0 mg/L (Health Canada 2009). The water line sourced from municipal water is also used to prepare spraying tank solution and to rinse spraying tanks, on the research farm.

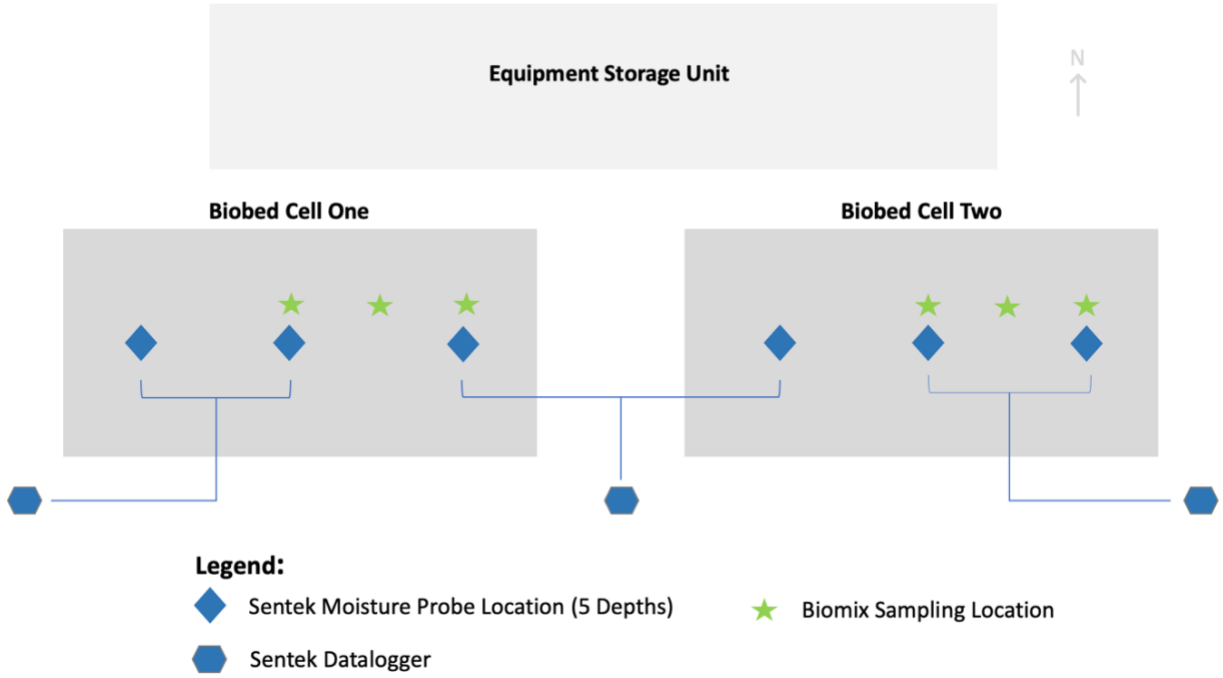

Figure 2.2 Sentek probe locations and biomixture sampling locations in biobed cells.

2.3.3 Biobed Properties and Weed Species Measurements

To establish a baseline, the biomixture in the biobed cells were sampled on August 17, 2020. This was during the period when only municipal water had been run through the system. Pesticide rinsate did not enter the system until August 25, 2020. Biomixture samples were

collected from two depths, 0-15 cm, and 15-30 cm, to quantify a range of nutrients and soil properties. Analyses were completed by ALS Laboratories in Winnipeg, Manitoba. In addition, biomixture samples were transported in coolers with icepacks to the laboratory, and frozen at -20°C until pesticide residue analyzes. Prior to extractions and further analyses, biomixture samples were freeze-dried over a period of 48 hours and ground using a mortar and pestle. Details of the pesticide residue analysis and quantification are described in Chapter 3.

Due to COVID-19 restrictions, research was severely restricted on the farm, delaying the completion of the biobed system and plot studies and hence the generation and collection of pesticide applications and rinsate. Consequently, because no pesticide rinsate was applied to the biobed system in spring and early summer 2020, weeds were able to establish themselves on each biobed cell surface (Figure 2.3). A quadrat study was conducted on each biobed cell to determine the weed species present before the biobed became operational. There were four quadrats applied to each biobed cell and weed surveys were conducted on August 7, 2020.

Figure 2.3 Biobed surface with weed coverage (photos taken after quadrat study).

2.4 Results and Discussion

2.4.1 Biobed Site Preparation

An overview of the biobed site is presented in Figure 2.4 and includes a collection area comprised of a collection trough and a collection tank, a rinsate collection tank, an equipment storage unit, and two biobed cells with their effluent tanks. As part of the site preparation, the topsoil of the site was first levelled using the bucket of a front-end loader. A non-woven geotextile fabric was then placed on the ground and covered with ¾" down gravel. Fifteen concrete blocks for each biobed cell were placed to provide for their foundation, with the gravel foundation being at an 8% grade so that the rinsate would naturally drain towards the exit of each biobed cell into the respective effluent tanks (Figure 2.5, Figure 2.4). The concrete blocks also raised the biobed cells making it easier to collect effluent samples from a sump tank below the biobed level without any of the effluent tanks needing to be below the ground level.

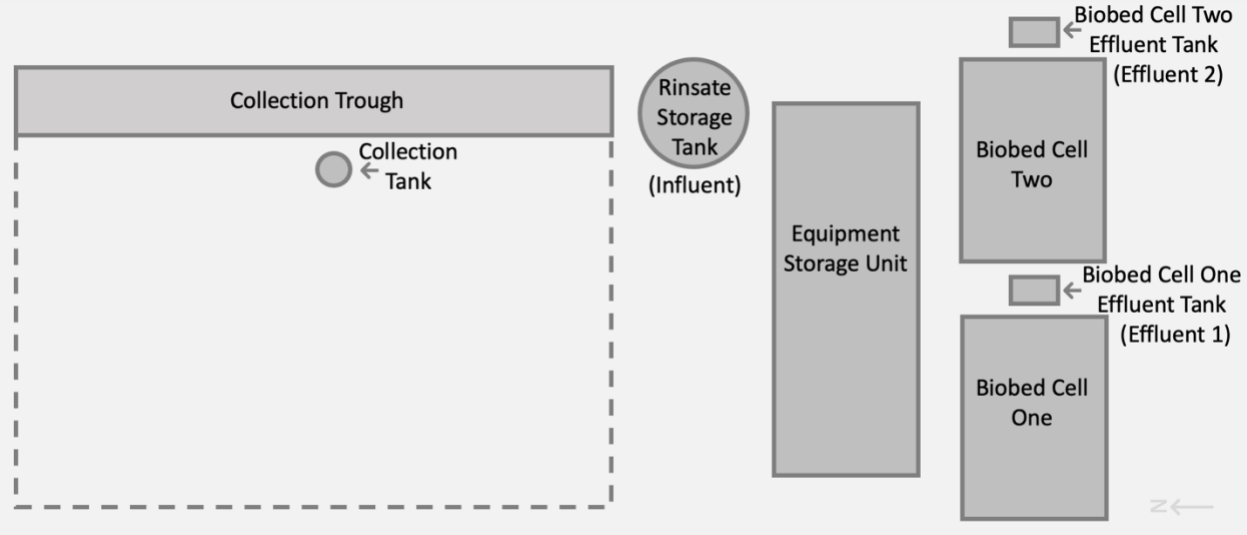

Figure 2.4 Biobed site components and layout.

Figure 2.5 Site preparation including concrete blocks and biobed container placement.

2.4.2 Biomixture Preparation and Properties

The biomixture or "biomix" was made from a combination of straw, soil, and peat at a ratio of 2:1:1, respectively. Soil and straw substrates were sourced from the Ian N. Morrison Research Farm. Sunshine Sphagnum Peat Moss, which is Canadian sourced, was used as the third substrate and was purchased through Homestead Co-op in Carman, Manitoba. Wheat straw was chopped into finer fragments ranging approximately from 3 to 7 cm by processing it through a combine multiple times prior to mixing into in biomixture. Smaller straw pieces help create a more homogenous biomixture and avoids risk for the development of preferential flow paths (Castillo et al. 2008).

The AAFC biobed manual recommends a loam soil with a relatively high organic matter content rather than a clay soils that could reduce water movement or sandy soils that lack the organic matter needed to provide for pesticide sorption (Braul and Sheedy 2018). The Ian N. Morrison Research Farm has a wide range of soil series. The Denham series was selected for use in biomix, primarily because of its suitable soil properties, but also because it was situated in an area in which the topsoil could conveniently be removed without interfering with the research

plot studies taking place on the research farm. The Denham series represent loamy texture soils that are non-eroded, non-saline and have a medium water holding capacity, a high soil organic matter content, and a high natural fertility (Mills and Haluschak 1993).

The biomixture was prepared by combining and mixing the straw, soil, and peat at a respective ratio of 50:25:25% at the soil extraction site and leaving it to compost in a pile for 30 days which follows the recommended maturation duration of up to 8 weeks by Agriculture and Agri-Food Canada (Braul and Sheedy 2018). The pile was rotated and mixed weekly during the maturation period, and moisture content was also observed while rotating the biomix. According to recommendations by Braul and Sheedy (2018), the biomixture must be moist but not wet during the maturation process (Figure 2.6). It was observed that the biomixture was moist but not wet throughout this maturation period, so no additional water was supplemented during the maturation period.

Figure 2.6 Biomixture being rotated during maturation and mature biomixture.

2.4.3 Biobed Assembly

After biomixture maturation, the biobed containers that were placed on concrete blocks were filled with biomixture (Figure 2.7). The biobed containers were acquired from a local liquid handling products supplier, Polywest Ltd. The containers were a low-profile tank comprised of polyethylene that were modified by removing the tops of the tanks to utilize them as the biobed containers.

Figure 2.7 Photos of biobed containers at field site filled with gravel and biomix.

The biobed containers were filled with 10 mm pea gravel, to a depth of 15 to 20 cm, which depth varied due to corrugation of the tank bottom (Figure 2.7). The gravel layer at the base of the container allows drainage of the biobed cell which prevents saturated conditions. A non-woven geotextile fabric was placed on top of the pea gravel layer and then the remainder of the volume of the containers were filled with biomixture to a depth of 50 cm thick. The geotextile fabric allows for the continued separation of the biomixture and the gravel and prevents the tank bung that is required for drainage from getting plugged. Heating tubing was placed inside biomixture layer at 20 cm above the geotextile fabric layer to heat biobed, as needed, for example to prevent freezing in the Fall or promote thawing in the Spring, if required (Figure 2.8). This would be

accomplished by pumping heated glycol through the heat tubing to warm the biobed. To prevent precipitation from entering system, a roof cover constructed from lumber and plastic roof sheeting was built over both biobed cells (Figure 2.7). The flow of liquid through the biobed system design is shown in Figure 2.8.

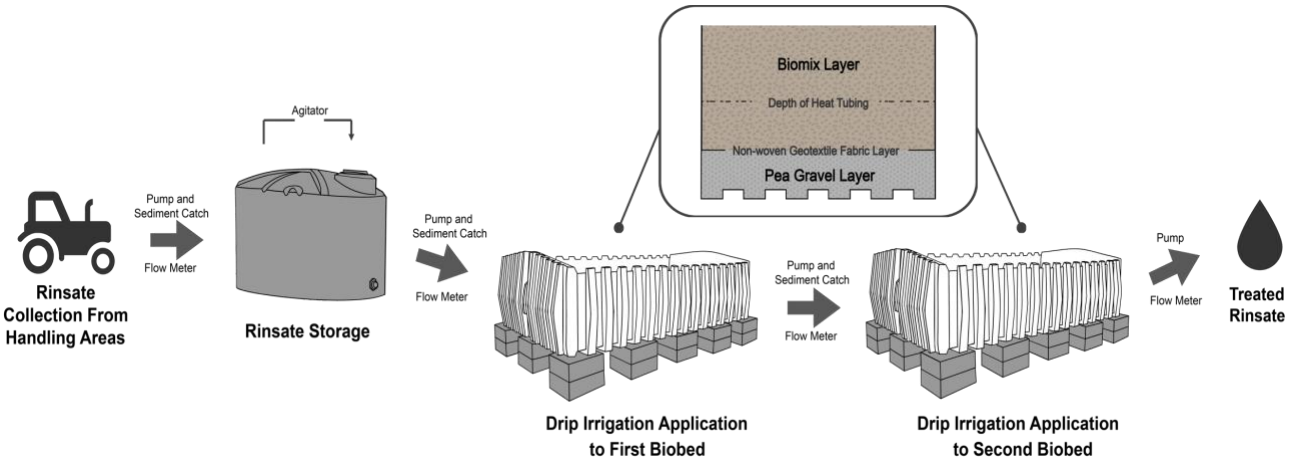

Figure 2.8 Schematic diagram of biobed system in Carman.

2.4.4 Rinsate Collection System

Collection of pesticide rinsate from the rinsing of agricultural spraying equipment was executed by building a collection trough with a moveable small secondary collection tank (Figure 2.9). The trough was wide enough to fit the width of all sprayer booms used on the farm, and personnel could simply back the sprayer boom overtop the trough so that any rinsing from flushing the nozzles would collect into the rough during routine rinsing (Figure 2.9). This trough was constructed by cutting and welding five-cylinder tanks to create an over 12-meter-long trough. Metal footings were welded together and placed on concrete blocks to support the trough. A backsplash comprised of leftover roofing materials was constructed behind the trough to restrict the effect of wind on the sprayer nozzles and prevent liquid from being blown away from

the trough while the nozzles are flushing. The additional, moveable collection tank could be positioned below the sprayer tank filter to collect any liquid that resulted from rinsing the sprayer filter. This tank drained into the collection trough so that all rinsate was collected. Pesticide rinsate was pumped into a rinsate storage tank so that a controlled application the pesticide rinsate could be accomplished.

Figure 2.9 Sprayer rinsing and depositing pesticide rinsate into collection trough after field application.

2.4.5 Biobed System Operation and Maintenance

A drip irrigation manifold was chosen to apply the rinsate to ensure there was a controlled application of rinsate to each biobed cell (Figure 2.10). Two identical drip irrigation manifolds were constructed for each biobed cell with drip emitters placed 30 cm apart. An irrigation timer (Figure 2.11) was installed to control the pump that applied the stored rinsate through the irrigation pipes from the rinsate storage tank to the biomixture surface of the first biobed cell. At a set time every day the pump could be programmed to run for a set duration so that consistent

daily applications could be achieved. A separate pump that cycled the rinsate within the rinsate storage tank for the purpose of agitation was also wired into the irrigation timer so that it could run for five minutes prior to the application of the first biobed to increase the homogeneity of the influent solution being applied.

Figure 2.10 Irrigation pipes with drip emitters for rinsate application onto biobed cell surface.

From July 24th to September 28th, 2020, an average application rate of 5.75 L/m^2/day was applied to the first biobed cell. In 2020 some of what was applied was water as the pesticide rinsate application did not begin until August 25, 2020. In 2021, between June 11th to June 25th, June 25th to July 27th, and July 27th to October 14th the average application rate was 16, 25, and 9.6 L/m^2/day respectively. The change in application rate was due to fluctuations in the available pesticide rinsate that was collected. Application rates were increased when pesticide rinsate storage was near capacity to ensure that rinsate collection continue to be collected so that all rinsate produced at the farm could be collected. Application rate was also decreased when the volume of collected pesticide rinsate was low and/or water was being added to the system during periods where rinsate was not being added due to low amounts spraying activities on the farm.

The added water allowed the biobed to remain moist and in operation until fall spraying activities were completed on the farm. Rinsate supply was supplemented with municipal water in 2020 and from September 22nd to October 14th in 2021.

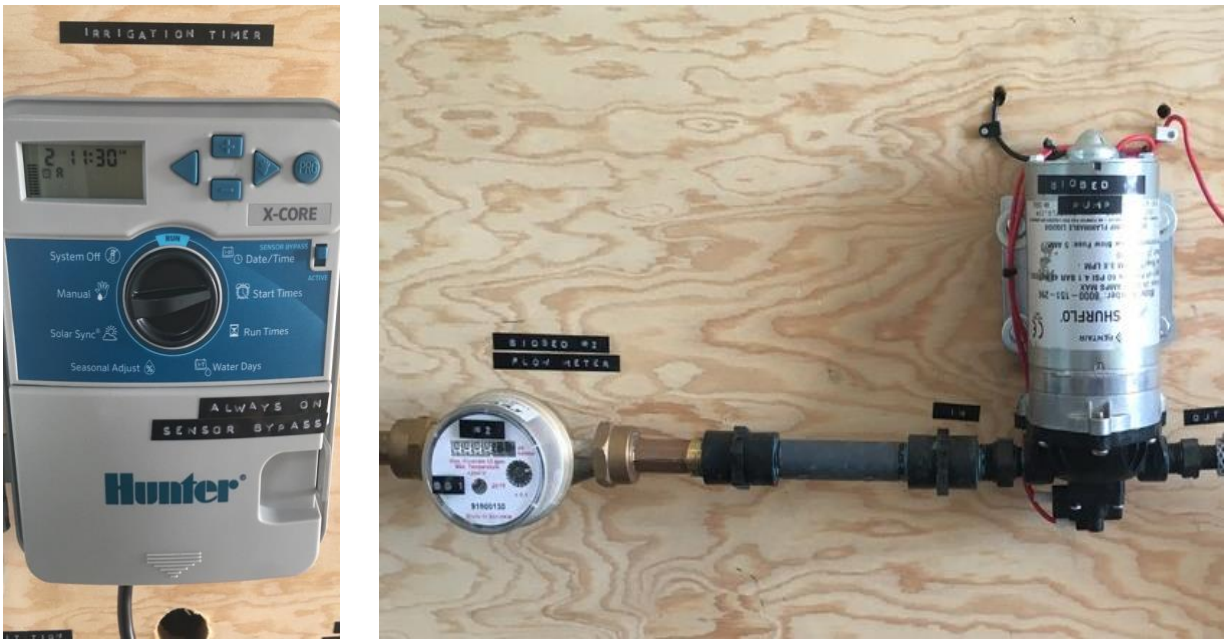

Figure 2.11 Irrigation timer, flow meter and pump before first biobed cell.

Once the influent has been applied to the first biobed cell, liquid would percolate though the biobed cell and then drains into the effluent one tank which was then applied to the second biobed cell through another pump. Once there is enough effluent volume present in the effluent one tank, a float switch in the tank was triggered which starts a pump that was located inside the equipment storage container, which was connected to the irrigation manifold on the second biobed cell, causing the effluent of biobed cell one to be applied by drip irrigation to the surface of the second biobed cell. The effluent of the first biobed cell then percolated through the biomixture of the second cell and drained into the effluent two tank. The liquid from the effluent two tank, is pumped onto an adjacent vegetated covered area that was not in crop production.

Following the biobed construction, as planned, there was a large pile of surplus biomixture from the original mixture used to fill the biobed cells remained at the site for future use. Biobeds naturally experience some volume loss due to breakdown of components, settling, and compaction of the biomixture during their operation which is usually greatest in the first year of biobed use (Braul and Sheedy 2018). This volume loss was mitigated by adding some of the surplus biomixture to the surface of the biobed in spring.

The entire biobed system is powered through a solar system. Four solar panels are secured with custom welded angle iron mounts to keep them stationary and stable to the top of the equipment storage unit with the solar panels facing South at a tilt angle of 38°. These solar panel supports were built on site from welding angle iron pieces and were subsequently secured to the equipment storage unit roof using bolts. A solar converter and batteries are wired to the panels to convert and then store power for the biobed system and are protected within the storage unit. A tilt angle of 38° was selected for the solar panel installation to optimize energy production for summer when site operations would be at its peak. According to the Government of Manitoba, a tilt angle of 35 to 40° from the horizontal is ideal for solar systems being used during summer months. (Government of Manitoba n.d.).

2.4.6 Biobed Properties and Weed Survey Results

The baseline chemical and physical properties of the biomixture from the samples collected on August 17, 2020, are in Table 2.1. These baseline samples were taken from the biomixture after the mixture had been placed in the biobed cell containers. The biomixture was high in both

micronutrients and macronutrients with boron consistently sampling at lower rates than other nutrients from a field crop perspective (Table 2.1).

Table 2.1 Average biomixture baseline properties of both biobed cells from August 17, 2020 (prior to pesticide application). Averages are determined from the depth of 0-15 cm excluding Nitrogen, and pH, which include the depths of 0-15 and 15-30 cm.

Nutrient/Property	Unit	Average for Biobeds
Nitrogen	*ppm*	21.83
Phosphorus	*ppm*	26.00
Potassium	*ppm*	382.5
Sulfur	*ppm*	17.50
Boron	*ppm*	0.65
Zinc	*ppm*	3.62
Iron	*ppm*	164.9
Manganese	*ppm*	12.60
Copper	*ppm*	0.87
Magnesium	*ppm*	365.0
Calcium	*ppm*	1712
Sodium	*ppm*	38.0
Organic Matter	*%*	4.85
Carbonate (CCE)	*%*	0.70
pH	*pH*	5.88
Cation Exchange Capacity	*meq*	17.95

Of the weed species present on the biobed cell surfaces, grass species were over ten times more abundant in the quadrat study than broadleaf species (Figure 2.12). The weeds identified on the biobed surface are typical for the area according to a 2016 weed survey of the province (Leeson et al. 2017). Of the seven weed species observed on the biobed surface, four species (green

foxtail, barnyard grass, yellow foxtail, and redroot pigweed) were in the top 10 list for the relative abundance index with two of the remaining species (smartweed and lamb's quarters) being in the top 20 (Leeson et al. 2017). The species detected that had a lowest relative frequency index, purslane, has a species frequency map that indicated that the biobed location was in an area of the province that experienced the highest frequency for this species (Leeson et al. 2017). More grass species appeared in the top relative abundance index which supports the observation that there were more grass species detected on the biobed surface (Leeson et al. 2017). The weeds observed on the biobed surface appear to be representative of the weeds in the area based on previous observations.

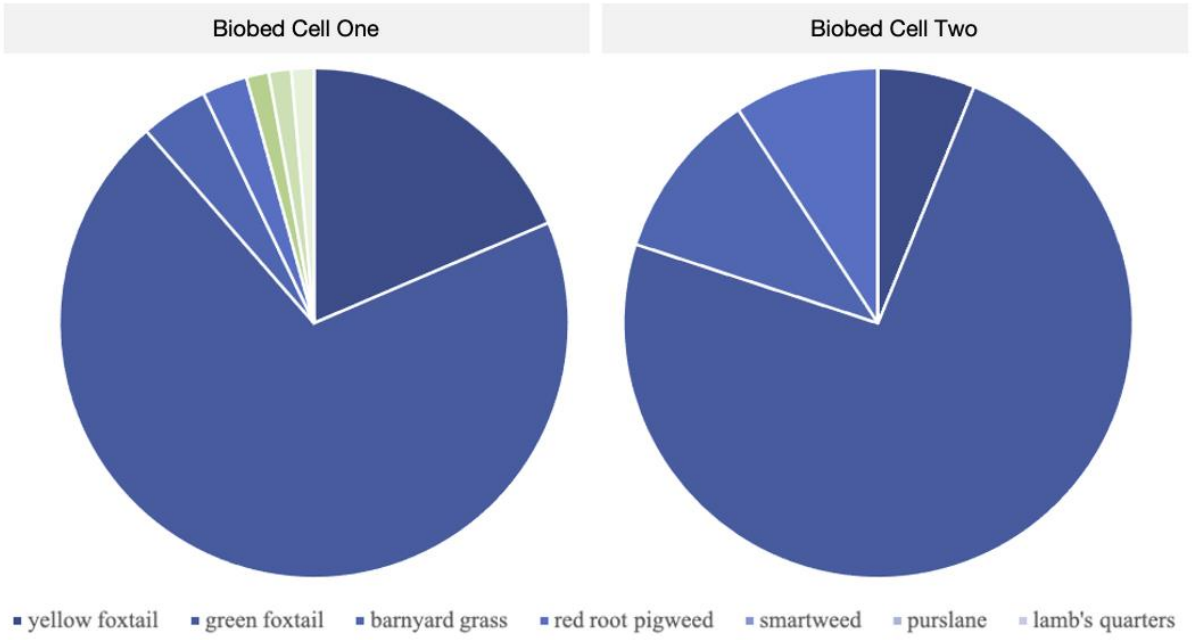

Figure 2.12 Average weed count for biobed cell one and biobed cell two in Fall 2020 before pesticide application. Blue represents grass species and green represents broadleaf species.

2.4.7 Initial Pesticide Detections in the Biomixture

Biomixture samples of the biobed prior to pesticide application, which were taken on August 17, 2020, were analyzed for pesticide content. Two herbicides (2,4-DB, and dicamba), and one

fungicide (metrafenone) were detected at a frequency of 58%, 25% and 50% out of the 12 samples respectively (Table 2.2, Figure 2.13). Given that no rinsate had been applied prior to the August 2020 sampling round, the pesticide concentrations detected in the biomixture must have been pesticide residues already present in the straw, peat or soil used in preparing the biomix. This is plausible being that both 2,4 DB and dicamba are present in an inventory of chemicals present on the farm. Metrafenone is a pesticide that is permitted for use in Canada, so it may have been used on the farm previously. These pesticides have K_{oc} values ranging from 224 to 7061 L kg^{-1}, Soil DT_{50} ranging from 3.9 to 200.9 days and GUS values ranging from 0.91 to 1.94. None of the pesticides detected in the initial sampling were detected in samples after biobed was operational, on October 5, 2020, suggesting the degradation of the pesticides initially present in the biomixture.

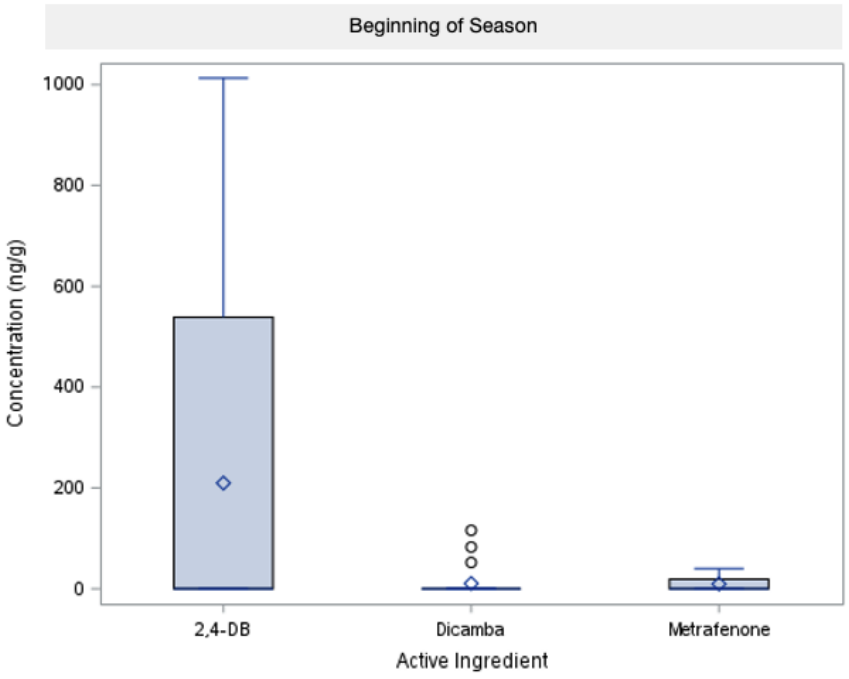

Figure 2.13 Concentration of Active Ingredients detected above LOQ in biomixture at the beginning of the season in 2020. There were no detections above the LOQ at the end of the season in 2020. Mean indicated with diamond and median indicated with line.

Table 2.2 Pesticide detections above LOQ for biomixture samples collected in 2020 (GUS=Groundwater Ubiquity Score, LOQ=Limit of Quantification, H=Herbicide, F= Fungicide). K_{oc}, Soil DT_{50}, and GUS values were obtained from Lewis et al. (2016).

Active Ingredient	K_{oc} *L kg^{-1}*	Soil DT_{50} *d*	GUS	LOQ	Number of Detections	Detection Frequency *%*	Avg. Conc. *ng g^{-1}*	Min. Conc. *ng g^{-1}*	Max. Conc. *ng g^{-1}*
Beginning of Season (n=12)									
2,4-DB (H)	224	2.87 to 15.6	1.68	17.6	7	58	209.6	484.3	1013
Dicamba (H)	N/A	3.9 to 9.62	1.94	9.50	3	25	10.45	52.07	115.8
Metrafenone (F)	7061	62 to 200.9	0.91	9.30	6	50	9.53	37.4	39.9
End of Season (no detections)									

2.4.8 Biomatrix Temperatures by Depth and over Time

The monthly temperatures in biobed cell one and biobed cell two followed the same trend as the air temperature that was measured by the nearby weather station (Figure 2.14, Figure 2.15). In 2020, air temperatures recorded by the weather station were lowest in December (median air temperature of -5.6℃) and highest in July (median air temperature of 20.5℃). Similarly, December 2020 had the coldest median biobed temperatures for all depths, -5.4℃ and -5.5℃ in biobed cell one and biobed cell two respectively (Figure 2.14). July 2020 was the warmest month when the median biobed temperature for all depths were 23.7℃ and 24.1℃ in biobed cell one and biobed cell two respectively (Figure 2.14). In general, average temperatures in the biomixture numerically decreased slightly with depth in the spring and summer months. For example, the median temperatures were 21.6℃, 21.9℃, 21.7℃, 21.4℃, and 20.8℃ for 10cm, 20cm, 30cm, 40cm, and 50cm in biobed cell one respectively in June 2020 (Figure 2.14). In contrast, the median temperatures in the biomixture numerically increased with depth in the fall and winter months. For example, the median temperatures were -5.9℃, -5.9℃, -5.3℃, -5.1℃, and -5.1℃ for 10cm, 20cm, 30cm, 40cm, and 50cm in biobed cell one in December 2020.

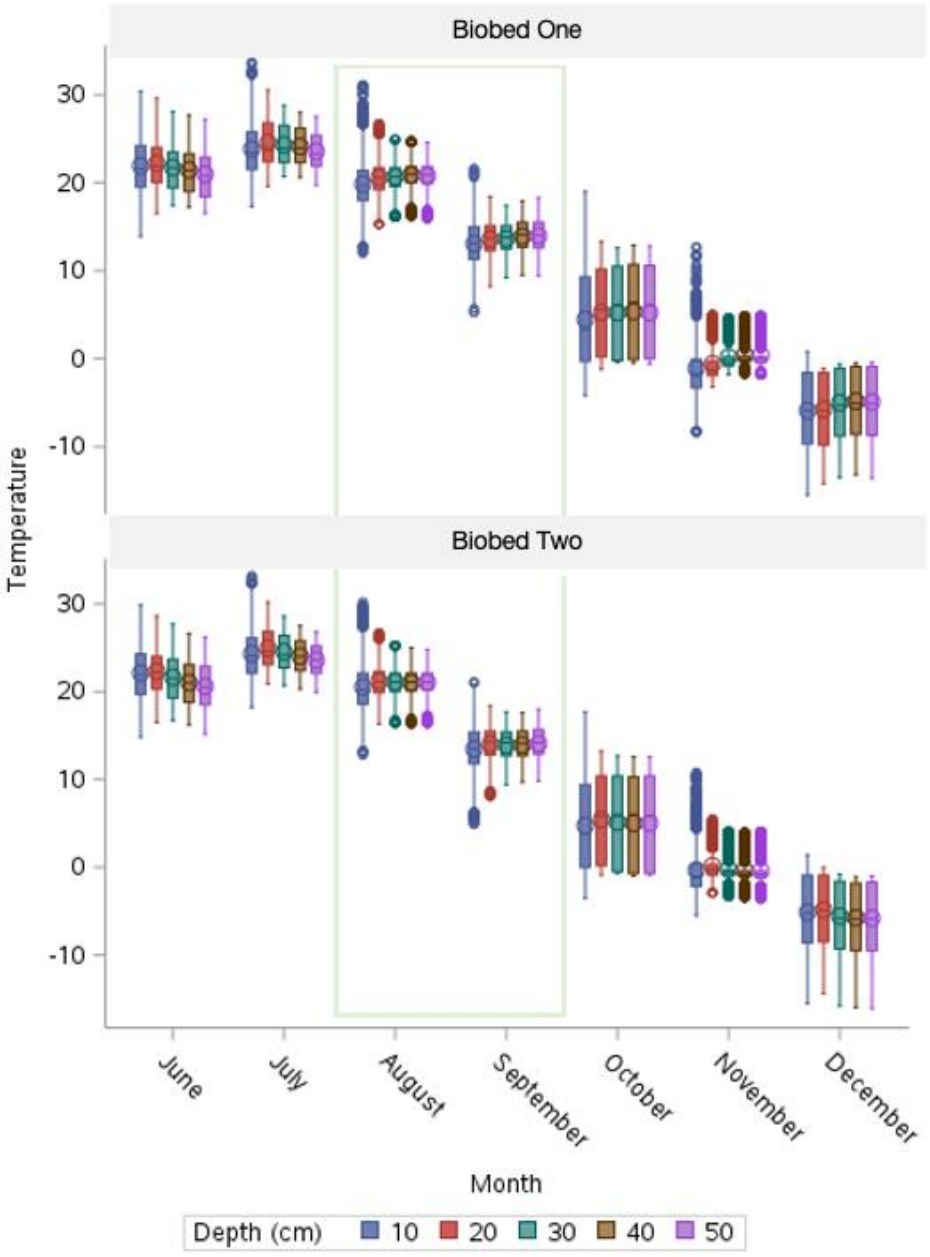

Figure 2.14 Monthly temperature of biomixture (℃) at 5 depths for two biobed cells during 2020. Biobed operational period is highlighted with a green box. Median is indicated with circle and Mean is indicated with line.

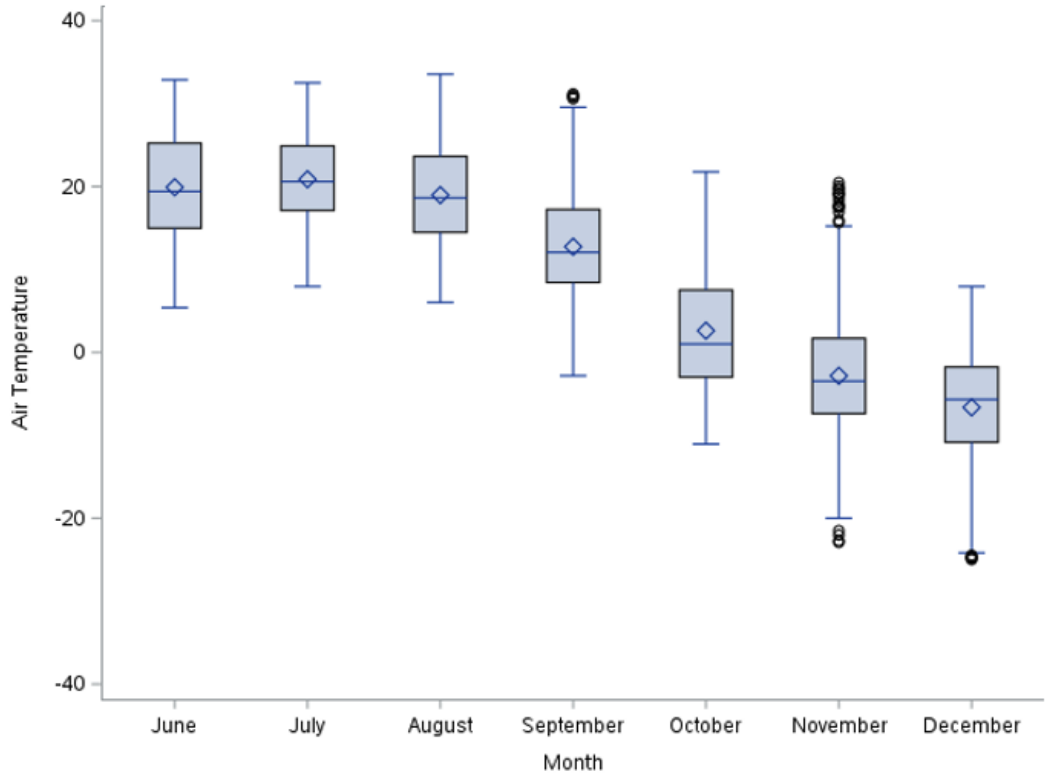

Figure 2.15 Monthly air temperature (℃) at biobed weather station in 2020. Mean indicated with diamond and median indicated with line.

In 2021, the monthly temperatures of biobed cell one and biobed cell two again followed the same trend as the air temperature that was measured by the nearby weather station (Figure 2.17, Figure 2.16). Air temperatures were coldest in February 2021with a median air temperature of -17.7℃ and warmest in July 2021 with a median air temperature of 21.5℃ (Figure 2.16). February 2021 was also the coldest month when median biobed temperatures for all depths were -14.7℃ and -13.9℃ in biobed cell one and biobed cell two respectively (Figure 2.16). The warmest month for both biobed cells was July 2021, when the median biobed temperature for all depths were 23.4℃ and 23.1℃ in biobed cell one and biobed cell two respectively (Figure 2.16). In general, average temperatures in the biomixture again numerically decreased with depth in the spring and summer months. For example, the median temperatures were 21.5℃, 21.6℃, 21.3℃,

21.1℃, and 20.8℃ for 10cm, 20cm, 30cm, 40cm, and 50cm in biobed cell one respectively in June 2021 (Figure 2.14). The trend of median temperatures in the biomixture again numerically increased with depth in the fall and winter months. For example, in October 2021, the median temperatures were 8.3℃, 8.7℃, 8.6℃, 8.9℃, and 8.8℃ for 10cm, 20cm, 30cm, 40cm, and 50cm in biobed cell one. In addition, the monthly trends for each biobed cell were similar in 2020 and 2021 when comparing June through to December, which were the months for which data was available for both year (Figure 2.14, Figure 2.16).

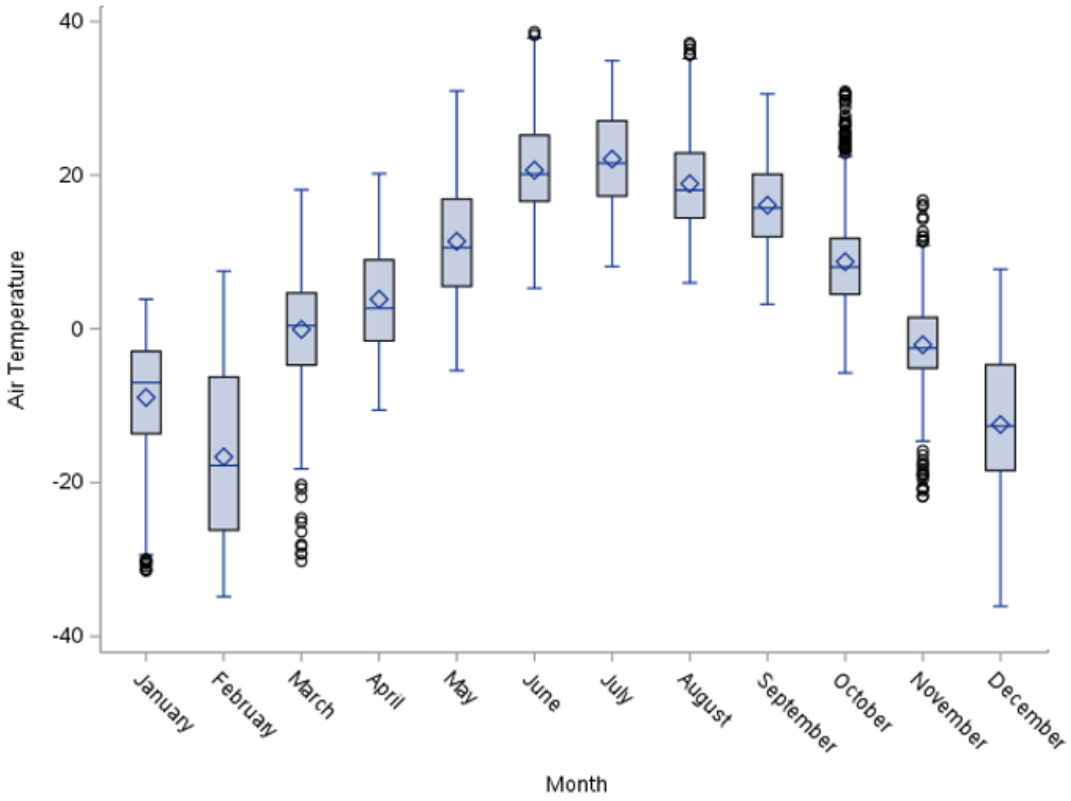

Figure 2.16 Monthly air temperature (℃) at biobed weather station in 2021. Mean indicated with diamond and median indicated with line.

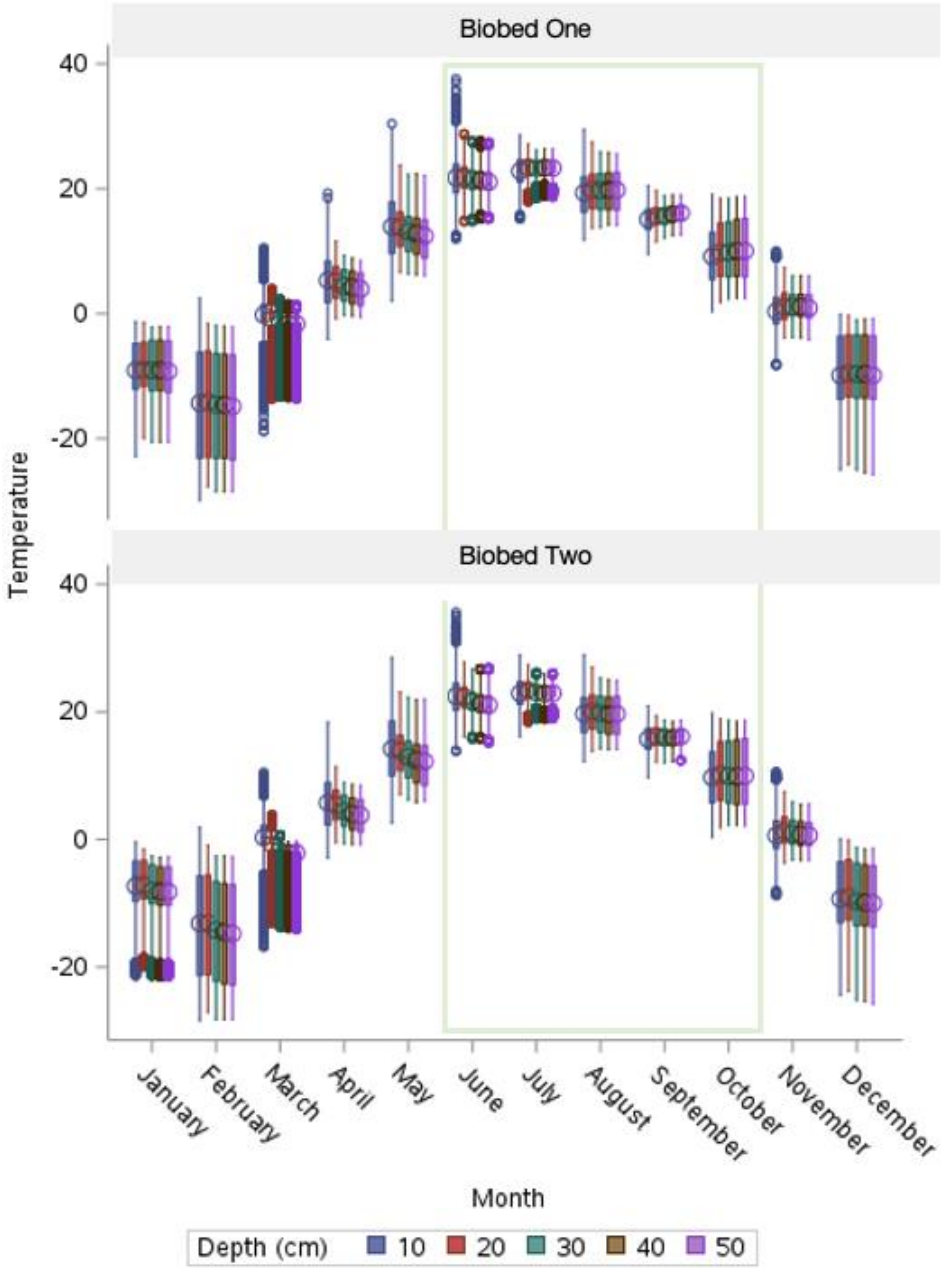

Figure 2.17 Monthly temperature of biomixture (℃) at five depths for two biobed cells during 2021. Biobed operational period is highlighted with a green box. Mean indicated with circle and median indicated with line.

2.4.9 Biomatrix Moisture by Depth over Time

Biobed moisture trends for the five probe depths in 2020 and 2021 are summarized in Figure 2.18 and Figure 2.19 respectively. The moisture content remained consistently larger at deeper

depths compared to the shallower depths in both biobed cells and in both years (Figure 2.18 and Figure 2.19). Additionally, in both years, biobed cell one tended to have numerically higher moisture measurements than biobed cell two. For example, the median moisture for all depth measurements in biobed cell one was 19.7% and 17.1% in 2020 and 2021, respectively, whereas for biobed cell two medians were 17.7% and 15.2%, respectively (Figure 2.18 and Figure 2.19).

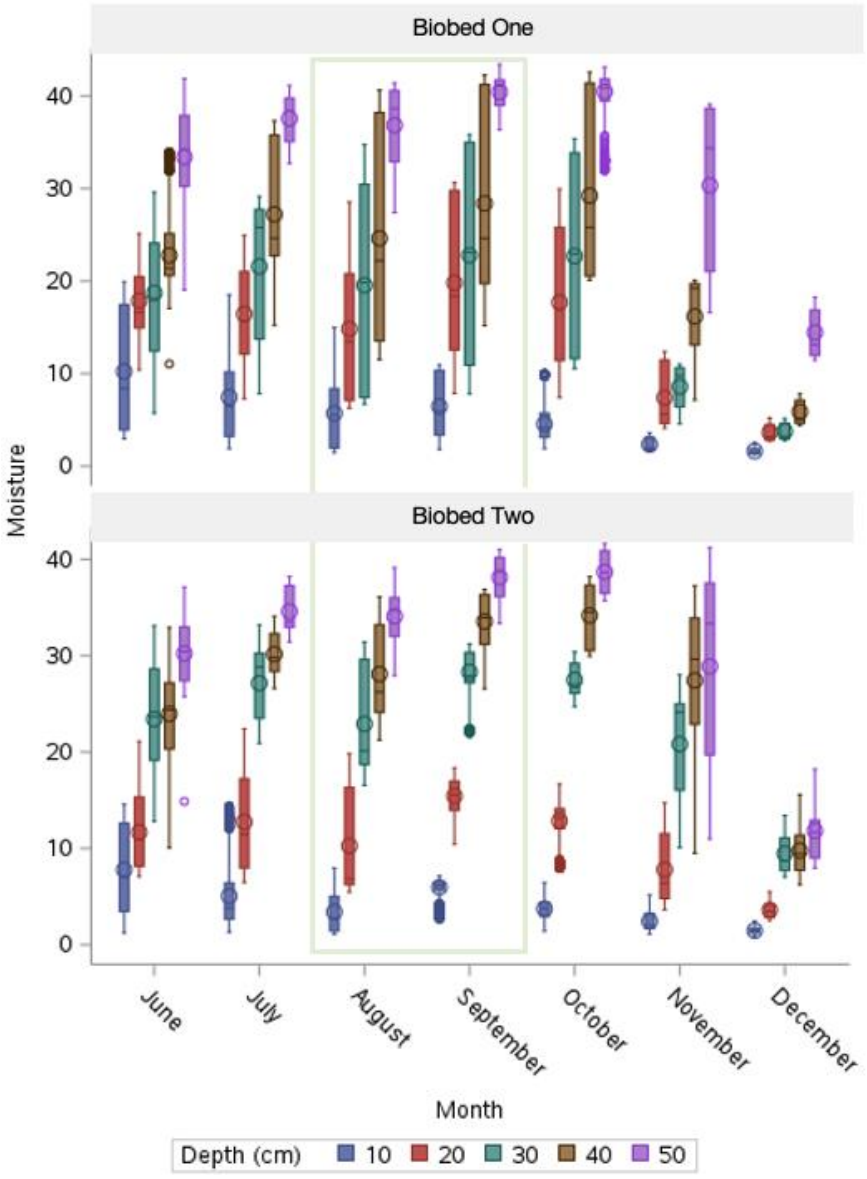

Figure 2.18 Monthly moisture of biomixture (vwc) at 5 depths for two biobed cells in 2020. Biobed operational period is highlighted with a green box. Mean indicated with circle and median indicated with line.

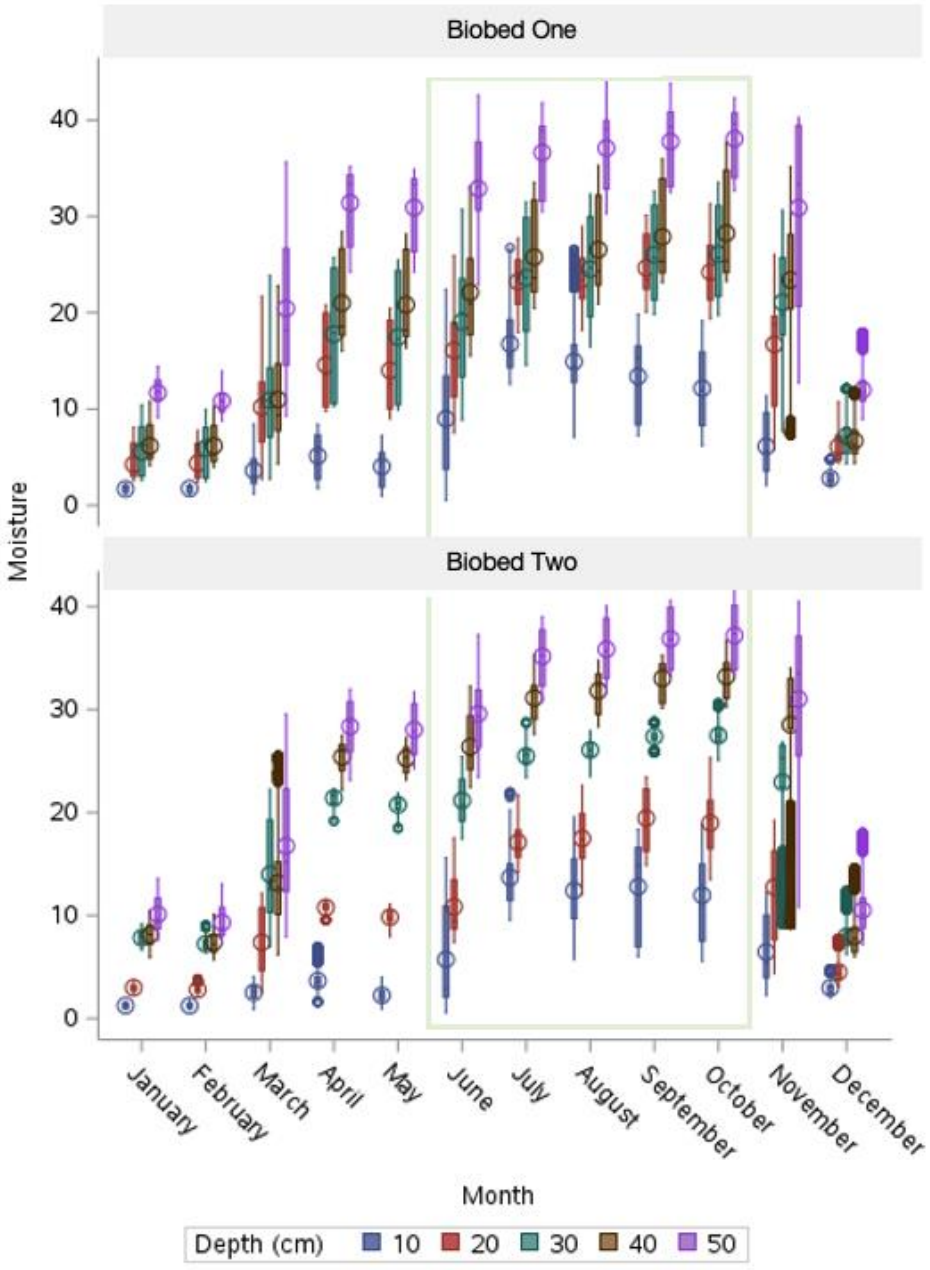

Figure 2.19 Monthly moisture of biomixture (vwc) at 5 depths for two biobed cells during 2021. Biobed operational period is highlighted with a green box. Mean indicated with circle and median indicated with line.

The variability in moisture readings was smallest in the months that biobed temperatures were consistently below zero (i.e. January, February, and December). It should be noted that the median moisture content at 50 cm depth sharply decreased from 33.5% in November 2020 to

12.1% in December 2020 (examples is from biobed cell one). Similarly, this decrease also occurred in 2021 when the median moisture content at 50 cm depth sharply decreased from 33.4% in November 2021 to 11.3% in December 2020 (examples is from biobed cell one). The lower moisture readings during the winter months are likely a result of the probes providing less reliable moisture readings during colder and freezing periods. Despite the moisture measurements of the probes being less reliable during these cold winter months, the temperature measurements taken during periods of freezing from these probes did not seem to be affected.

2.5 Conclusion

The biobed system construction was successfully completed by the graduate student and farm personnel with the help of undergraduate summer students. Construction required the students to learn many skills including welding for building portions of the collection trough and mounting solar panels, carpentry for building backsplash of trough and building roof structure for the biobed cells. Additionally, it required learning how to do the plumbing for the irrigation system and rinsate collection system as well as how to do the wiring for the various pumps of the system and to connect everything to the solar power installed on site. This project provided a unique experience in learning many hands-on skills for the students involved. The pesticides detected in the biomixture at the onset of the study (i.e. present from biomixture components) were degraded by the end of the first season. Temperature data from the two biobed followed the same trends as the weather station that was at the biobed site. Both temperature and moisture data were consistent between the two years; however, moisture data became unreliable at temperatures below zero degrees Celsius.

2.6 References

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3. EFFICIENCY OF A FARM BIOSYSTEM TO PROCESS TWENTY-THREE PESTICIDE ACTIVE INGREDIENTS USED IN THE CANADIAN PRAIRIES.

3.1 Abstract

Influent and effluent samples and biomixture of a biobed constructed in Carman, Manitoba were monitored over the first two years of the operation of a biobed in 2020 and 2021. Three pesticides (bromoxynil, deltamethrin, and metolachlor) were detected in the Influent of the biobed in 2020, but no pesticides were detected in Effluent One or Effluent Two. Twenty-three pesticide active ingredients and one pesticide metabolite were detected in the Influent in 2021. Because only 11 of the 24 pesticide compounds were detected in Effluent One and subsequently only six were detected in Effluent Two, the biobed was considered effective at degrading and retaining the pesticide compounds. The overall concentration of pesticides the Influent, Effluent One and Effluent Two were significantly different which demonstrates the added value of dual-celled biobed designs in the Canadian Prairies. Pesticides detected in the effluents tended to have either a large GUS factor or a longer half-life or both. In 2021, three pesticides (bifenthrin, trifloxystrobin, and diuron) were detected in the biomixture and the beginning of the season but diuron, which had the highest K_{oc} and GUS values of the compounds detected, was the only pesticide active ingredient detected in the end of season biomixture samples. A large influx of approximately 680 L of prepared spraying fluid containing glufosinate was added to the biobed Influent, which overburdened the system which affected degradation and resulted in glufosinate increasing in mean concentration from Influent < Effluent One < Effluent Two. Greenhouse gas fluxes measured in 2021 suggest that biobeds do not pose a large risk for greenhouse gas

emissions because they appeared comparable to surrounding soil. Differences in carbon dioxide fluxes between biobed cells is likely due to the different rates of drip irrigation between the cells.

3.2 Introduction

A biobed system is an in-ground or above-ground structure that is filled with a biomixture usually comprised of a mixture of straw (50%), peat (25%) and soil (25%) (Dias et al. 2021). Biobeds process the rinsate produced when cleaning pesticide spraying equipment. By depositing this rinsate onto the biomixture held in a biobed cell, pesticide active ingredients can be sorbed by the biomixture and/or degraded and mineralized by microorganisms thriving in the biomixture substrate. There are a range of factors influencing biobed efficiency, but most studies report a >90% reduction in pesticide concentrations (Cooper et al. 2016; Bergsveinson et al. 2018). Factors include variables that can influence pesticide sorption and degradation such as pH, moisture, temperature, and nutrient availability for microbial growth, as well as biomixture composition and age (Castillo et al. 2008). Biobed systems are being used on farms and in municipalities across the world, particularly in Europe and Latin America (Dias et al. 2021).

There are a limited number of biobed systems established in Canada, and efforts are made by the Government of Canada to expand on the number of biobed systems in use by farmers and municipalities (Braul and Sheedy 2018). For four biobed systems in the Canadian Prairies, Fatema et al. (2022) reported a highly efficient removal rate for most of pesticide active ingredients (n=59). However, this study reported that biobed efficiency was not sufficient for herbicides clopyralid (~77%; Groundwater Ubiquity Score (GUS)=3.02), metsulfuron-methyl (~75%; GUS=3.28) and metazachlor (~68%; GUS=1.75) (Fatema et al. 2022). The studied dual-

or single-cell biobeds also showed a relatively poor performance for herbicides imazethapyr (GUS=3.90), mecoprop (GUS=2.29), diclofop, (GUS=2.38) and fluroxypyr (GUS=1.03) (Fatema et al. 2022). Fatema et al. (2022) concluded that the poorer performance was more likely to occur for pesticides with a high GUS (> 2.8) rather than pesticides with lower GUS values. As well, this study also detected pesticide active ingredients in effluent that were not applied as rinsate to the biobed system. Fatema et al. (2022) speculated that the biomixture might already contain pesticide residues, and that such pesticide active ingredients can desorb and move into the effluent.

Substrates such as manure, composted manure, and livestock bedding sites are known to be sources of greenhouse gasses such as methane and nitrous oxides (Petersen et al. 2013). Substrates with greater organic matter contents, are known to have greater methane emissions (Chadwick et al. 2011; Petersen et al. 2013; JianHui et al. 2015). Biomixtures (i.e., mixtures of straw, peat, and soil) are comparable with substrates such as manure, composted manure, and livestock bedding in that these are substrates that encourage microbial growth by being sufficient in organic matter content and nutrients. As such, it might be expected that biobeds can function as a source for greenhouse gases. This will contribute to the previous greenhouse gas studies conducted on a dual-celled in Saskatchewan (Ngombe, 2013).

The current study occurred during the COVID-19 pandemic which resulted in a temporary closure of the laboratory used for pesticide residue analyses. Therefore, influent, and effluent samples were collected in both amber glass bottles and polycarbonate (plastic) bottles, and frozen to preserve samples until the reopening of the laboratory. Amber glass bottles represent

the typical way of collecting samples but are prone to breakage upon freezing and the additional plastic bottles were seen as a precaution to prevent sample loss. Several decades ago, a number of studies investigated the specific sorption potential of pesticide active ingredients by plastic and glass surfaces (Sharom and Solomon 1981; Topp and Smith 1992; Giannopolitis and Kati 2008), and more recently a focus has been the potential for pesticides to sorb to microplastic contaminants in the environment (Torres et al. 2021; Fatema and Farenhorst 2022). Insecticide permethrin sorbed to some extent on both glass and plastic materials (Sharom and Solomon 1981). Although legacy non-polar pesticides such as DDT (dichlorodiphenyltrichloroethane) have been shown to sorb readily to microplastic, current-use ionizable herbicides (2,4-D, atrazine, and glyphosate) did not show an appreciable sorption except for glyphosate sorption by polyvinyl chloride (Torres et al. 2021; Fatema and Farenhorst 2022). Glass showed to be a potential absorbent for herbicides trifluralin and glyphosate (Sharom and Solomon 1981; Giannopolitis and Kati 2008). Thus, the use of both glass and plastic bottles in this study also provided an opportunity to test the impact of sampling bottle material on measured pesticide concentrations.

The objective of this study was to assess the efficiency of a two-cell biobed in processing current-use pesticides in Manitoba, and to also investigate its potential for greenhouse gas emissions.

3.3 Materials and Methods

A two-cell biobed system was constructed as described in Chapter 2. The biobed system became operational with the first pesticide rinsate being applied to the system on August 25, 2020. After

rinsate application commenced, influent and effluent samples continued to be collected on a weekly basis until the biobed was shut down on September 28, 2020, and subsequently winterized (Figure 3.1). Samples were taken at three locations (see Chapter 2, Figure 2.4), i.e., from the pesticide rinsate storage tank (Influent), the biobed cell one effluent tank (Effluent One), and the biobed cell two effluent tank (Effluent Two).

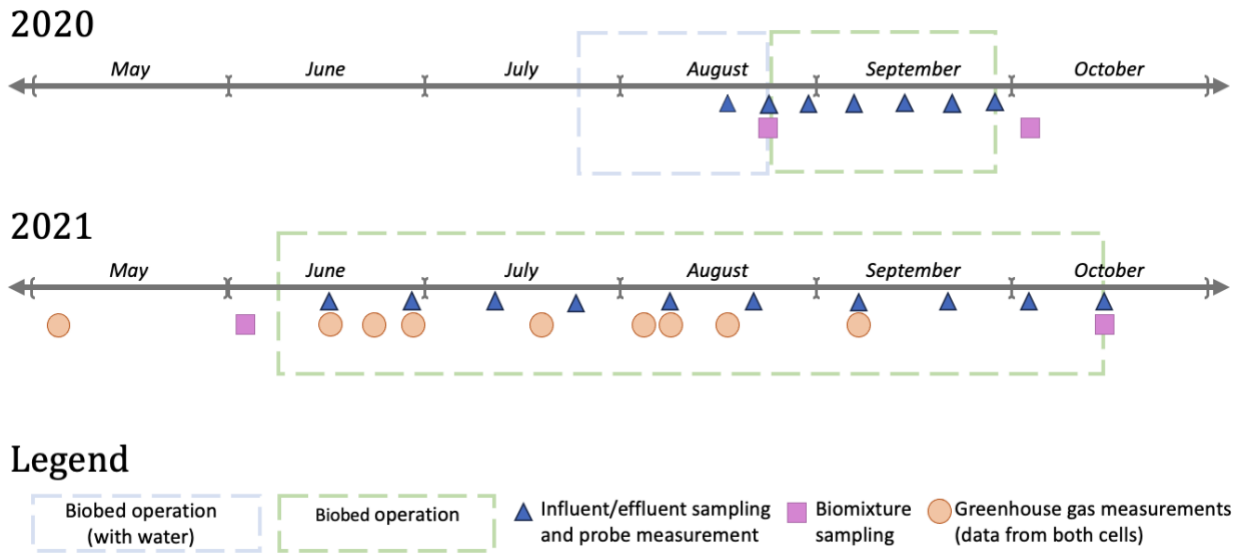

Figure 3.1 Sampling timeline for influent/effluent and biomixture and greenhouse gas measurements.

Rinsate sampling in 2020 was collected in three bottle types, which can be seen in Figure 3.2. For the sample collection, the 1-L amber glass bottles were only filled to 990 mL, leaving a headspace to allow for the expansion of water during freezing. The 1.25L plastic bottle suitable for freezing were filled without leaving a headspace since breakage was not a concern for this bottle type. Influent and effluent samples were also collected in 50 mL Falcon tubes (leaving no headspace) to specifically allow for analysis for herbicides glyphosate and glufosinate, as well as aminomethylphosphonic acid (AMPA, the main metabolite of glyphosate). In 2021, rinsate

samples were collected on a bi-weekly basis from June 11 to October 14 (Figure 3.1). Influent and effluent samples were only collected in amber glass bottles (leaving no headspace) and Falcon tubes (leaving no headspace). This was due to a lack of need for freezing samples due to laboratories re-opening and samples being able to be shipped and received immediately after being acquired.

Figure 3.2 All three bottle types (i.e. plastic, glass and falcon) used for rinsate collection in 2020 that were frozen.

In conjunction with the rinsate sampling, in both 2020 and 2021, portable meters were used to measure the turbidity, free and total chlorine, dissolved oxygen (%), electroconductivity (µS) and pH of influent and effluent samples. A Hach Chlorine Pocket Colorimeter™ II (VWR International, Mississauga, ON, Canada) was used to quantify the free and total residual chlorine concentrations adhering to USEPA DPD Methods 8021 and 8167, respectively (Hach, 2002). An AQ4500 turbidity meter (Thermo Scientific™ Orion™ AQUAfast™, Beverly, MA, USA) was used to determine turbidity. A potable pH/DO meter (Extech DO700, Waltham, MA, USA) was used to quantify measure pH and dissolved oxygen concentrations.

The biomixture was sampled in the Spring of 2020 (see Chapter 2) and 2021 (i.e. prior to the biobed becoming operational for the season), as well as in the Fall of 2020 and 2021 (i.e. after the final rinsate was applied) (Figure 3.1). Biomixture was sampled at three locations per biobed cell, and each location was sampled at two depths: 0 to 15 cm and 15 to 30 cm (See Chapter 2, Figure 2.2). Biomixture samples were transported in coolers with icepacks to the laboratory, and frozen at -20°C until pesticide residue analyzes. Prior to extractions and further analyses, biomixture samples were freeze-dried over a period of 48 hours and ground using a mortar and pestle.

In 2021, greenhouse gas flux measurements were quantified for each biobed cell on a weekly basis from March 24th to Oct 4th in 2021. The days where measurements were produced for each biobed cell is shown in Figure 3.1. A measurement (30-minutes each) was taken at two locations in each biobed cell by placing a greenhouse gas chamber on top of the biobed surface and allowing for a continuous flow of air from the inlet (ambient) to the outlet (biobed) of the chamber. A diode facilitated to switch sequential measurement from the air of inlet and outlet. Fourier-transform infrared spectroscopy (FTIR) (Gasmet) was used to measure concentrations of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), nitrogen dioxide (NO_2), and ammonia (NH_3). Inlet and outlet concentrations data were then further processed to estimate greenhouse gas fluxes (F_{GHG}) from biobed surface, using the following formula:
F_{GHG} (g GHG/m^2/d) = (C_{Outlet} - C_{Inlet}) * AF * (Density / CA), where C_{Outlet} = Average outlet gas concentration for a campaign (ppm); C_{Inlet} = Average inlet gas concentration for a campaign (ppm); AF = Air flow (m^3/s) = velocity (m/s) * duct area (m^2); Density = gas density at sampling

temperature (kg/m^3) = (molecular weight (g/mol) * air pressure (Pa)) / ideal gas constant 8.314 J/K/mol * Temperature (K) / 1000; CA = Chamber area (m^2)

3.4 Pesticide Analyses

Influent and effluent samples were extracted and analyzed for 142 compounds by the ISO17025 federal government laboratory in the Lethbridge Research and Development Centre in Agriculture and Agri-food Canada. The total list of pesticides analyzed are reported in Table A1 (see Appendix Two). A multi-residue method was conducted using validated in-house quantitative methods including multiple reaction monitoring and surrogate internal standards, and this methodology has been previously published. Briefly, samples were filtered using glass wool and then acidified to a pH of 2 using sulfuric acid. Samples were then extracted from water using liquid-liquid extraction (LLE) with dichloromethane (CH_2Cl_2). Subsequently, extracts were dried using sodium sulfate anhydrous (Na_2SO4), methylated using diazomethane and then added to 40 mL of hexane. Diazomethane was evaporated using a rotary evaporator until 10 mL of extract remained. 2 µL injections of the methylated extract was injected in an Agilent 7890B gas chromograph for pesticide quantifications. This chromograph has a 7000C QQQ mass selective detector (MSD) and multiple reaction monitoring. A HP-5MS UI 30 m x .25 mm x .25 µm, p/n 19091S-433UI column was used with temperatures programmed at 70°C for 2 minutes, then increased by 25°C/min until 150°C, then increased by 3°C/min until 200°C, then increased by 8°C/min until 280°C, and then held for 7 minutes for a total analysis time of 38.867 minutes. Method blanks were run with each of the set of samples with a target ion and at least two qualifier ions being monitored. The limit of quantification (LOQ) is the lowest value of the amount of an analyte that can reasonably be distinguished from zero. For pesticide compounds,

the LOQ for the detected pesticides ranged from 2.48 μgL^{-1} to 24.3 μgL^{-1} with a median LOQ of 2.72 μgL^{-1}. Detections that were below the LOQ were considered non-detects.

Influent and effluent samples were also analyzed for glyphosate and glufosinate, as well as aminomethylphosphonic acid (AMPA). These analyses were also executed by the ISO17025 federal government laboratory in the Lethbridge Research and Development Centre, Agriculture and Agri-food Canada. For the extraction of these three chemicals from the samples, an internal standard mixture comprised of glufosinate-d_3, glyphosate-^{13}C, AMPA-^{13}C, concentrated HCl, and acetonitrile was added to the sample. Subsequently the samples were centrifuged at 4,000 rpm for 5 minutes at 5˚C. A borate buffer (comprised of 4.75 g of disodium tetraborate decahydrate + 250 ml of deionized water and adjusted to a pH of 10 using NaOH) and Fmoc-Cl (comprised of 1.5 mg of flurenylmethyloxycarbonyl chloride per 1 ml of acetonitrile), were added to the top layer after centrifuging. After a 30-minute period of sample incubation, formic acid was added, and the extracts were filtered using 1.2 µm PTFE 13 mm acrodisc filters into vials. Analysis of the extracts and quantification of the chemicals was conducted using liquid chromatography tandem mass spectrometry (LC-MS/MS). Similar to the previous analysis, detections of the compounds below the LOQ were considered non-detects. The LOQ of glyphosate, glufosinate and AMPA were 4.4 ng/mL, 5.3 ng/mL, and 4.5 ng/mL respectively. Biomixture samples were extracted and analyzed for 111 compounds by the ISO17025 federal government laboratory in the Lethbridge Research and Development Centre in Agriculture and Agri-food Canada. The total list of the pesticides that were analyzed for can been seen in Table A2 in the Appendix Two. Briefly, the biomixture samples were stored at -20˚C then freeze dried for 48 hours, and subsequently ground using a mortar and pestle. The freeze-dried samples were

kept frozen and shipped in an insulated cooler with ice to Lethbridge Research and Development Centre where the analysis was completed. First, 10 ml acetonitrile with 0.1% formic acid was added to the sample followed by sonication for 10 minutes then centrifuging at 3000 rpm for 10 minutes. The solvent was decanted into a separate test tube, and the extraction was repeated twice more. Each 10 mL extract was combined into a single test tube (approx. 30 mL), evaporated under nitrogen (38˚C water bath) and reconstituted in dichloromethane. Subsequently, diazomethane followed by 40 ml of hexane was added to the solution for methylation of the target compounds. After 20 minutes the residual diazomethane was blown off using a stream of nitrogen gas. The volume of the extracts was then reduced until 2 mL using a rotary evaporator and then reconstituted to exactly 10 mL of hexane. Extracts were analyzed using a 7890B gas chromatograph with a 7000C QQQ mass selective detector as described above. Detections below the LOQ were considered non-detects. The LOQ ranged from 0.48 ng/g to 1030 ng/g for the chemicals tested with the median LOQ of 10.1 ng/g.

3.5 Statistical Analysis

All statistical procedures were conducted using SAS© Studio. Normal distributions of the greenhouse gas dataset were tested using the Shapiro-Wilke test with normal distributions determined for CH_4, NO_2, N_2O and NH_3. PROC FMM was used to determine the lognormal distribution of CO_2 flux data. A t-test comparison at an alpha of 0.05 was conducted using PROC TTEST to compare the gas fluxes between the biobed cells. Pesticide concentration data from 2020 in both plastic and glass bottles were tested for normal distribution using the Shapiro-Wilke test then a paired t-test at an alpha of 0.05 was conducted. PROC FMM was used to determine an exponential distribution for pesticide concentration data. Pair-wise comparisons of least square

means (LSM) were made for the three sampling locations (Influent, Effluent One, and Effluent Two) using Bonferroni correction due to unequal variances at an alpha of 0.05 for the 17 pesticides detected using the multi-residue analysis. Pair-wise comparisons of LSM were made for the three sampling locations (Influent, Effluent One, and Effluent Two) using tukey method at an alpha of 0.05 for glyphosate, glufosinate, AMPA, and the 17 pesticides detected using the multi-residue analysis. The two pairwise comparisons were calculated using PROC GLIMMIX.

3.6 Results and Discussion

3.6.1 Inorganic Chemical Characteristics of Influent and Effluents

Table 3.1 summarizes the turbidity, free and total chlorine, dissolved oxygen (%), electroconductivity (µS) and pH of the Influent, Effluent One and Effluent Two for the period during which the biobed system was operational in both 2020 and 2021, i.e., when pesticide rinsate was added to the storage tank. The turbidity median was substantially greater in the Influent (305 NTU) than in Effluent One (8.2 NTU) > Effluent (1.63 NTU) (Table 3.1). The reduction in turbidity, which is visible in Figure 3.3, reflects the biobed rinsate getting cleaner as it flowed to the biobed system due to the degradation/mineralization and/or sorption of pesticide active ingredients in the biomatrix of the dual cell biobed, and possibly other ingredients in the products applied such as adjuvants. As such, the use of turbidity measurements may be used as a cost-effective tool for farmers to show that the biobed system is degrading pesticides.

During the growing seasons when pesticides were actively applied, turbidity reached its peak of 1,144 NTU in late June 2021, which is likely related to a full sprayer tank of glufosinate ammonium was deposited in the collection through and hence the rinsate storage tank during this

period, as reported in the biobed logbook by a pesticide applicator. The full sprayer tank could not be applied to the field due to a timing error so that the prepared solution could not be applied to the intended field crop.

Total and free chlorine ranged from 0 mg/L to 1.4 mg/L and decreased in the order of Influent > Effluent One > Effluent Two (Table 3.1). It is likely that this decrease occurred because chlorine continued to be volatilized while rinsate moved through the biobed system. Dissolved oxygen ranged from 25% to 134% and showed an increase in the order of Influent < Effluent One < Effluent Two (Table 3.1). This likely reflects the large size and depth of the rinsate storage tank (smaller surface area to depth ratio) versus the storage tanks that held the effluents (larger surface area to depth ratio). Electroconductivity, which ranged from 282 µS to 1390 µS, also increased in the order of Influent < Effluent One < Effluent Two, possibly because of the release and transport of ions from the biomixture into the effluent (also see Chapter 2, Table 2.1). The pH of the pesticide rinsate and effluents were relatively similar in range, overall ranging from moderately acidic, with a lowest observation of 6.02, to moderately alkaline, with a highest observation of 8.30 (Table 3.1).

Table 3.1 Field chemical characteristics of Rinsate Storage, Effluent One and Effluent Two during biobed operational period as measured by portable meters across two years.

		Influent			**Effluent One**			**Effluent Two**		
		Min	Median	Max	Min	Median	Max	Min	Median	Max
Turbidity	*NTU*	0.55	305	1,144	1.45	8.18	24.2	0.26	1.63	7.98
Chlorine (Free)	*mg/L*	0	0.1	1.2	0	0.035	0.3	0	0.005	0.06
Chlorine (Total)	*mg/L*	0	0.1	1.41	0	0.13	0.33	0	0.07	0.18
Dissolved Oxygen	*%*	24.7	38.2	96.6	50.2	85.7	126	72.1	104	134
Electroconductivity	*µS*	282	548	666	357	790	1090	511	1060	1,390
pH	*pH*	6.43	6.98	7.82	6.02	7.46	8.1	7.38	7.77	8.3

Figure 3.3 Image of influent and effluent liquids observed in the field on June 20, 2021. From left to right: Influent (rinsate storage), Effluent One, and Effluent Two.

3.6.2 Greenhouse Gas Flux Measurements.

Figure 3.4 shows the greenhouse gas fluxes for CO_2 (carbon dioxide), CH_4 (methane), NH_3 (ammonia), NO_2 (nitrogen dioxide), and N_2O (nitrous oxide) from the samples analyzed between May 4th to Sept 7th, 2021, that had readings for both biobeds. The fluxes demonstrate that NH_3, NO_2, and N_2O, most often acted as a source for greenhouse gasses, with maximum fluxes of 0.010 $gC/d/m^2$, 0.098 $gC/d/m^2$ and 0.012 $gC/d/m^2$ respectively, but did also act as a sink, with minimum flux values of -0.0090 $gC/d/m^2$, -0.017 $gC/d/m^2$ and -0.0069 $gC/d/m^2$ respectively (Figure 3.4). The biobed cells always acted as a source for greenhouse gasses in the case of CO2 and CH4, except for one flux of CH4 which was -0.0024 gC/d/m2. CH_4 had a maximum flux of 0.030 $gC/d/m^2$.

Mean fluxes of CO_2 were 5.5 $gC/d/m^2$ and 3.6 $gC/d/m^2$ in biobed cell one and biobed cell two respectively and were found to be significantly different. The quantity of liquid applied though drip irrigation to the first biobed was larger than the second biobed cell because only the effluent of biobed one was applied to the latter cell. This application difference may have contributed to

the difference in CO_2 fluxes found between the two biobed cells. Irrigation has been shown to increase CO_2 soil fluxes in a study in North Dakota (Sainju et al. 2008).

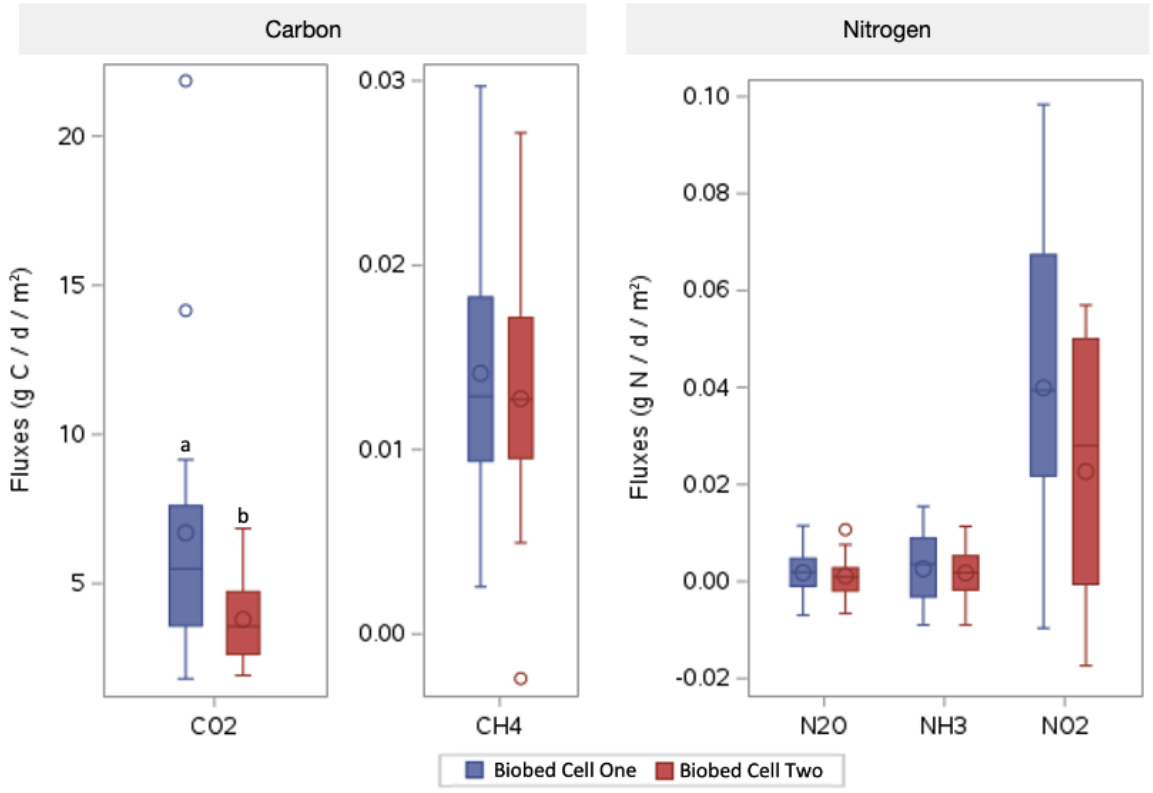

Figure 3.4 Gas fluxes (g C d^{-1} m^{-2}) of CO_2 and CH_4, and gas fluxes (g N d^{-1} m^{-2}) of N_2O, NH_3 and NO_2 from biobed cells From May 4th, 2021, to Sept 7th, 2021. Mean indicated with circle and median indicated with line. Significance from a paired T-Test at an α=0.05 indicated with letters.

The fluxes of CO2, N2O, and CH4 from the biobed are comparable to fluxes from studies of soils in south central Manitoba (Tenuta et al. 2010; Gao et al. 2014; Amiro et al. 2017). The fluxes were also similar studies in other regions of the world. For example, NH3 fluxes were comparable to the emissions measured for a soil in a river delta in China (Li 2013).

3.6.3 Multi-Residue Pesticide Residue Analyses in 2020

The only pesticides that were detected above the limit of quantification (LOQ) in 2020 were herbicides bromoxynil and metolachlor, and insecticide deltamethrin. Although bromoxynil, metolachlor, and deltamethrin were detected in the Influent, there were no detections of pesticides in Effluent One or Effluent Two (Table 3.2, Figure 3.5). Metolachlor was the most frequently detected pesticide active ingredient in Influent, as shown by both glass bottles (100% detection frequency) and plastic bottles (83% detection frequency) (Table 3.2). Metolachlor also had a numerically greater mean concentration, relatively to deltamethrin > bromoxynil (Table 3.2, Figure 3.5). However, deltamethrin was detected in the highest concentration at 28.9 µg/L in a plastic bottle on Sept 28th, 2020 (glass = 23.5 µg/L). For the other two pesticides, the maximum concentrations were 14.9 µg/L for metolachlor in a plastic sample bottle on August 31 (no data from glass bottle due to breakage), and 5.1 µg/L for bromoxynil in a plastic bottle sample on Aug 31st in 2020 (no data available from glass bottles due to bottle breakage).

During the 2020 season, deltamethrin had been documented in the rinsing logs for the biobed site which explains its presence in the biobed. However, bromoxynil and metolachlor had not. A full list of pesticides documented in the logbooks can be found in Table A3 in Appendix Two. It is possible that farm personnel may have omitted to fill the logbook after rinsing with a bromoxynil containing product as it was present in a chemical inventory of the farm. Metolachlor is a pesticide that is registered for use as presented in the Guide to Field Crop Protection by Manitoba Agriculture (2021), so it is also possible that this pesticide may have been used but not documented in the rinsate logbook situated at the biobed system site.

Table 3.2 Influent (Rinsate Storage Tank) pesticide detections above LOQ for samples collected in both plastic and glass sample bottles during 2020 (GUS=Groundwater Ubiquity Score, LOQ=Limit of Quantification, H=Herbicide, I=Insecticide). No detections in Effluent One or Effluent Two. K_{oc}, Soil DT_{50}, and GUS values were obtained from Lewis et al. (2016).

Active Ingredient	K_{oc}	Soil DT_{50}	GUS	LOQ	Number of Detections	Detection Frequency	Avg. Conc.	Min. Conc.	Max. Conc.
	L kg^{-1}	*d*				*%*	*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*
Glass (n= 4)									
Bromoxynil (H)	302	1.04 to 8	1.71	2.65	2	50	1.61	3.04	3.39
Deltamethrin (I)	10,240,000	21 to 58.2	-3.98	6.26	2	50	8.76	11.59	23.48
Metolachlor (H)	120	15 to 90	2.36	2.61	4	100	10.64	9.21	12.66
Plastic (n= 6)									
Bromoxynil (H)	302	1.04 to 8	1.71	2.65	4	66	3.26	4.53	5.22
Deltamethrin (I)	10,240,000	21 to 58.2	-3.98	6.26	2	33	8.35	21.22	28.90
Metolachlor (H)	120	15 to 90	2.36	2.61	5	83	11.53	12.74	14.87

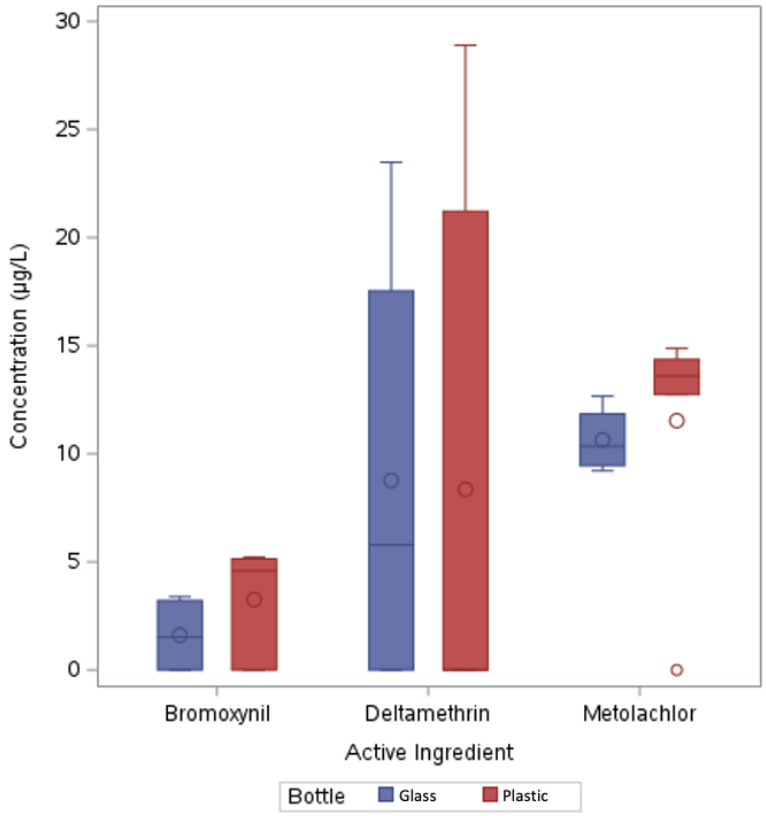

Figure 3.5 Concentrations of bromoxynil, deltamethrin, and metolachlor detected above LOQ in Influent samples from 2020 collected in plastic and glass bottles. Mean indicated with circle and median indicated with line. No pesticides were detected in Effluent One or Effluent Two above the LOQ.

Despite the efforts to reduce glass breakage, five glass samples either broke during freezing or transport of the samples to the laboratory for analysis. Glass bottles broke on August 17, August 31, and Sept 21, 2020, for the Influent samples, on August 31, 2020, for Effluent One samples, and on August 17, 2020, for Effluent Two samples. Consequently, pesticide concentration data for both plastic and glass bottles were only available on the following collection dates: August 24, and September 8, 14, and 28, 2020 (i.e., no broken glass bottles on these dates). As such, these dates were used in the statistical analyses to compare the pesticide concentrations quantified in glass versus plastic bottles (Figure 3.6, Figure 3.7).

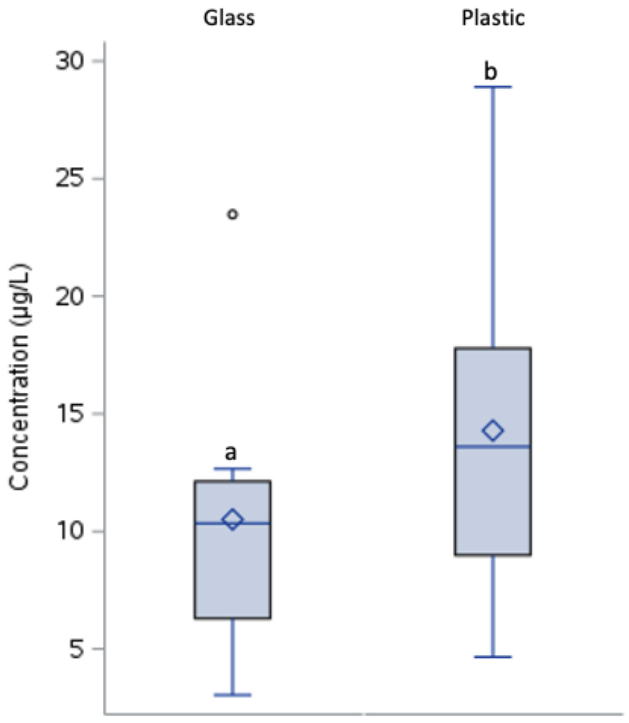

Figure 3.6 Concentration of all active ingredients detected above LOQ in Influent samples from 2020 collected in plastic and glass (amber) bottles (paired). No pesticides were detected in Effluent One or Effluent Two above the LOQ. Mean is indicated with a diamond and median with a line. A significance difference was determined at an $\alpha=0.05$.

Pesticide concentrations of each detected pesticide were numerically greater for samples collected in the plastic bottles versus samples collected in glass bottles. A paired t-test showed the total pesticide concentrations in the plastic bottles were significantly different than the glass at an $\alpha=0.05$ (Figure 3.6), which is also demonstrated when comparing in the line of regression to the 1:1 reference line in Figure 3.7. This suggests the possibility of a greater sorption of pesticide molecules by glass than plastic. However, more likely, it reflected the fact a headspace was required for the amber glass bottles to allow for expansion during the freezing process (to prevent glass breakage) which would have resulted in an increase pesticide volatilization relatively to a bottle that was filled to the rim.

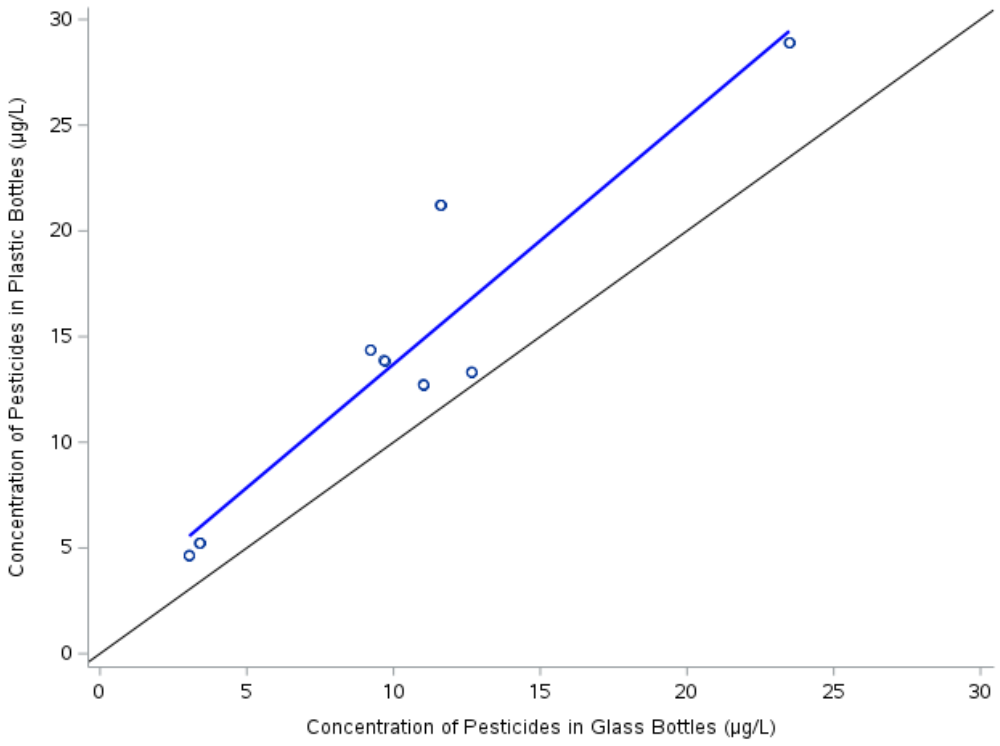

Figure 3.7 Concentration of active ingredients (µg/L) detected above the LOQ in influent and effluent in the glass samples bottles compared to plastic sample bottles during 2020. A 1:1 reference line is indicated in black.

The biomixture within each biobed cell was sampled on August 17, 2020 (See Chapter 2, Table 2.2, Figure 2.13), which was prior to any pesticide rinsate application, as well as on October 5, 2020, which was after the first season of rinsate had been processed. Pesticides detected in the biomixture through multi-residue analyses for August 17, 2020, is shown in Chapter 2, Table 2.2 and Figure 2.13. No pesticides were detected in the end of season samples from October 5, 2020, suggesting that the biobed was effective in degrading the three compounds that had been present in the Biomixture (see Chapter 2), as well as the three pesticides that were added as rinsate, i.e., herbicide bromoxynil and insecticides deltamethrin and metolachlor (Chapter 2).

3.6.4 Glyphosate, Glufosinate and AMPA Detections in 2020

Glyphosate was detected in the Influent with a mean concentration of 44,200 µg/L but was not detected in Effluent One or Effluent Two (Table 3.3 Figure 3.8). Aminomethylphosphonic acid (AMPA) was detected in the Influent with a mean concentration of 550 µg/L, at a much lesser mean concentration in the Effluent One (19 µg/L) and was not detected in Effluent Two (Figure 3.8). Glufosinate was not detected above the LOQ in any of the samples in 2020. Glyphosate being the highest detected pesticide active ingredient aligns with the fact that it accounted for 66% of rinsing logs entries in 2020. AMPA is the primary degradation product of glyphosate, so its presence in the Influent and the Effluent One is indicative of glyphosate degrading within the biobed. The presence of these three compounds in biomixture was not quantified due to a lack of method development to achieve such quantifications.

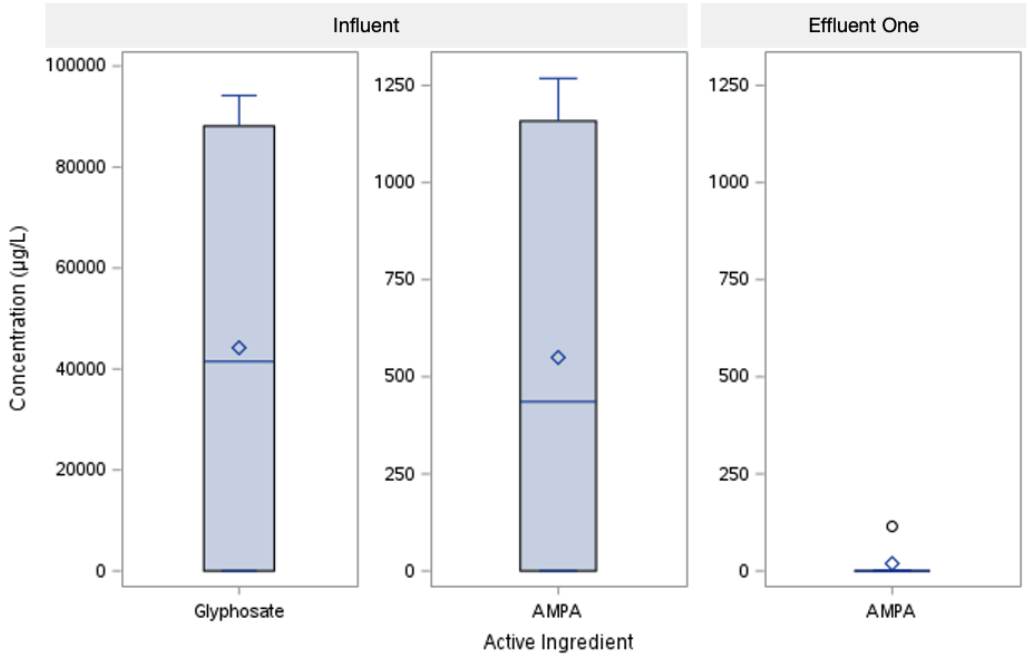

Figure 3.8 Concentration of glyphosate and AMPA (µg/L) detected above LOQ in influent and effluent one sampled in falcon tubes during 2020. There were no detections above the LOQ in Effluent Two. Mean is indicated with a diamond and median with a line.

Table 3.3 Influent and effluent detections of glyphosate, glufosinate and AMPA above LOQ for samples collected 2020 (GUS=Groundwater Ubiquity Score, LOQ=Limit of Quantification, H=Herbicide, I=Insecticide). K_{oc}, Soil DT_{50}, and GUS values were obtained from Lewis et al. (2016).

Active Ingredient	**K_{oc}** *L kg^{-1}*	**Soil DT_{50}** *d*	**GUS**	**LOQ**	**Number of Detections**	**Detection Frequency** *%*	**Avg. Conc.** *ng g^{-1}*	**Min. Conc.** *ng g^{-1}*	**Max. Conc.** *ng g^{-1}*
Influent (n=6)									
Glyphosate (H)	1,424	6.45 to 16.11	0.21	4.4	3	50	44209	82964	94155
AMPA	2,002	234 to 419	0.04	4.5	3	50	550	872	1268
Effluent One (n=6)									
AMPA	2,002	234 to 419	0.04	4.5	1	17	19.1	115	115

3.6.5 Multi-Residue Pesticide Residue Analyses in 2021

There were three glass sample bottles that broke due to the shipping in 2021: the Effluent One sample from July 12th, 2021, Effluent Two sample from June 14th, 2021, and Effluent Two sample from June 28th, 2021.

In total, 17 pesticides were detected in 2021 (Table 3.4), which included the three pesticides detected in 2020 (i.e, bromoxynil, deltamethrin, and metolachlor) (Table 3.2). Of the 17 pesticides detected, herbicides accounted for 71%, with the remaining 29% of detections were insecticides (Table 3.4). This is likely an accurate reflection of the pesticide products used on the farm as the 2021 biobed logbook indicated that no fungicide products were rinsed at the site. As well, the biobed logbook reported that 85% of the rinsing events at the biobed were done after herbicide applications, and the remaining portion were done after insecticides applications.

Pesticide active ingredients were more frequently detected and at greater concentrations in Influent than in Effluent One or Effluent Two (Table 3.4, Figure 3.9, Figure 3.10). Only seven pesticide active ingredients (2-4D-methyl, bentazone, bromoxynil, cyhalothrin, dicamba, dimethachlor, and mecoprop) moved into Effluent One, and three pesticide active ingredients (bentazone, cyhalothrin and dicamba) moved into Effluent Two (Table 3.4).

Out of the 27 total samples collected, the herbicide dicamba was the most frequently detected pesticide (63% of total samples) followed by the insecticide cyhalothrin (48%) and then the herbicide trifluralin (40.7%) (Table 3.4). In the Influent, dicamba was the active ingredient detected at the highest maximum concentration (1,532 µg/L) with other pesticides showing substantial smaller maximum concentrations ranging from ~ 141 µg/L for the herbicide clomazone to ~ 3.7 µg/L for the herbicide benzoylprop (Figure 3.9, Figure 3.10). Pesticides least detected were the insecticides deltamethrin (7%), and etrimfos (4%), and herbicides MCPB (7%) and benzoylprop (4%).

Table 3.4 Influent and effluent pesticide detections above LOQ for samples collected in glass sample bottles during 2021 (GUS=Groundwater Ubiquity Score, LOQ=Limit of Quantification, H=Herbicide, I=Insecticide). K_{oc}, Soil DT_{50}, and GUS values were obtained from Lewis et al. (2016).

Active Ingredient	K_{oc}	Soil DT_{50}	GUS	LOQ	Number of Detections	Detection Frequency	Avg. Conc.	Min. Conc.	Max. Conc.
	L kg^{-1}	*d*				*%*	*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*
Influent (n= 10)									
2,4-DB (H)	224	2.87 to 15.6	1.68	2.70	4	40	13.6	10.80	55.35
2,4-D-Methyl (H)	39.3	4.4 to 28.8	3.82	2.70	2	20	2.73	2.77	19.12
Bentazone (H)	55.3	7.5 to 20	1.95	2.67	4	40	6.78	3.91	24.84
Benzoylprop (H)				2.79	1	10	0.37	3.66	3.66
Bromoxynil (H)	302	1.04 to 8	1.71	2.65	3	30	4.01	3.30	27.43
Clomazone (H)	300	22.6 to 27.3	2.72	2.65	6	60	29.34	17.22	140.85
Cyhalothrin (I)	180,000	57	-2.20	2.70	7	70	7.45	2.77	18.68
Deltamethrin (I)	10,240,000	21 to 58.2	-3.98	6.26	2	20	5.18	9.49	42.35
Dicamba (H)		3.9 to 9.62	1.94	2.56	9	90	580.24	3.74	1532.34
Dimethachlor (H)		3.2 to 7	1.09	2.77	2	20	0.69	3.37	3.56
Etrimfos (I)	70	12.5	2.36	7.8	1	10	0.90	8.98	8.98
Flumetralin (H)	10,000	727	-1.63	2.67	6	60	3.93	3.02	13.17
MCPB (H)	104.7	3.65	1.12	2.68	2	20	0.87	4.15	4.53
Mecoprop (H)	47	8.2	2.29	2.70	6	60	3.35	2.92	9.22
Metolachlor (I)	120	15 to 90	2.36	2.61	6	60	2.34	2.64	7.55
Tetramethrin (I)	1423	0.32 to 3	-0.42	2.58	3	30	1.06	2.90	4.79
Trifluralin (H)	15,800	133.7 to 170	0.13	2.65	10	100	16.09	5.08	53.82
Effluent One (n= 9)									
2,4-D-Methyl (H)	39.3	4.4 to 28.8	3.82	2.70	1	11	0.31	2.77	2.77
Bentazone (H)	55.3	7.5 to 20	1.95	2.67	1	11	0.54	4.89	4.89
Bromoxynil (H)	302	1.04 to 8	1.71	2.65	1	11	2.68	24.15	24.15
Cyhalothrin (I)	180,000	57	-2.20	2.70	4	44	2.01	2.94	6.63
Dicamba (H)		3.9 to 9.62	1.94	2.56	6	66	116.02	5.62	515.26
Dimethachlor (H)		3.2 to 7	1.09	2.77	1	11	0.39	3.54	3.54
Mecoprop (H)	47	8.2	2.29	2.70	1	11	0.32	2.92	2.92
Trifluralin (H)	15,800	133.7 to 170	0.13	2.65	1	11	0.65	5.87	5.87
Effluent Two (n= 8)									
Bentazone (H)	55.3	7.5 to 20	1.95	2.67	4	50	3.63	6.25	8.76
Cyhalothrin (I)	180,000	57	-2.20	2.70	2	25	0.86	2.77	4.12
Dicamba (H)		3.9 to 9.62	1.94	2.56	2	25	25.30	7.18	195.24

The groundwater ubiquity score (GUS) is a tool used to predict the comparative ranking of pesticides moving towards groundwater, and is determined by using the equation [log(DT50]×[4 – log(Koc)], where DT_{50} is the half-life of the pesticide in soil and K_{oc} is the normalized organic carbon sorption coefficient of the pesticide (Gustafson 1989). The 17 pesticides detected in the rinsate had GUS values ranging from -3.98 (insecticide deltamethrin) to 3.82 (herbicide 2,4-D-methyl). GUS values ranged from -2.20 (insecticide cyhalothrin) to 3.82 (2,4-D-methyl) for Effluent One and only three pesticides were detected in Effluent Two: cyhalothrin (GUS = -2.20), dicamba (GUS=1.94), and bentazone (GUS = 1.95) (Table 3.4). For a study that included two dual-cell biobeds in Saskatchewan, bentazone (71%) was among the most frequently detected pesticides in the final effluent (Fatema et al. 2022). Another study found that approximately 90% of cyhalothrin was removed in a biobed study in France (de Roffignac et al. 2008). Despite cyhalothrin having an extremely low GUS value, the half-life for this pesticide is larger than most of the other detected pesticides, which might also help to explain its presence in the Effluent Two.

Overall pesticide concentrations between the three sampling locations at the biobed system (i.e. Influent, Effluent One, and Effluent Two) were compared, and the effect of the sampling locations was found significant (Table 3.5). Further investigation found that concentrations were significantly different between the Influent, Effluent One and Effluent Two (Table 3.5). This shows that dual-celled biobeds provided a significant improvement in pesticide reduction compared to single-celled biobeds. When glyphosate, glufosinate and AMPA (See Section 3.6.3) were added to the comparison, which followed a different sample extraction methodology than the multi-residue), significance was still found for the effect of sampling location with further

investigation also finding that concentrations were significantly different between Influent, Effluent One and Effluent Two (Table 3.6).

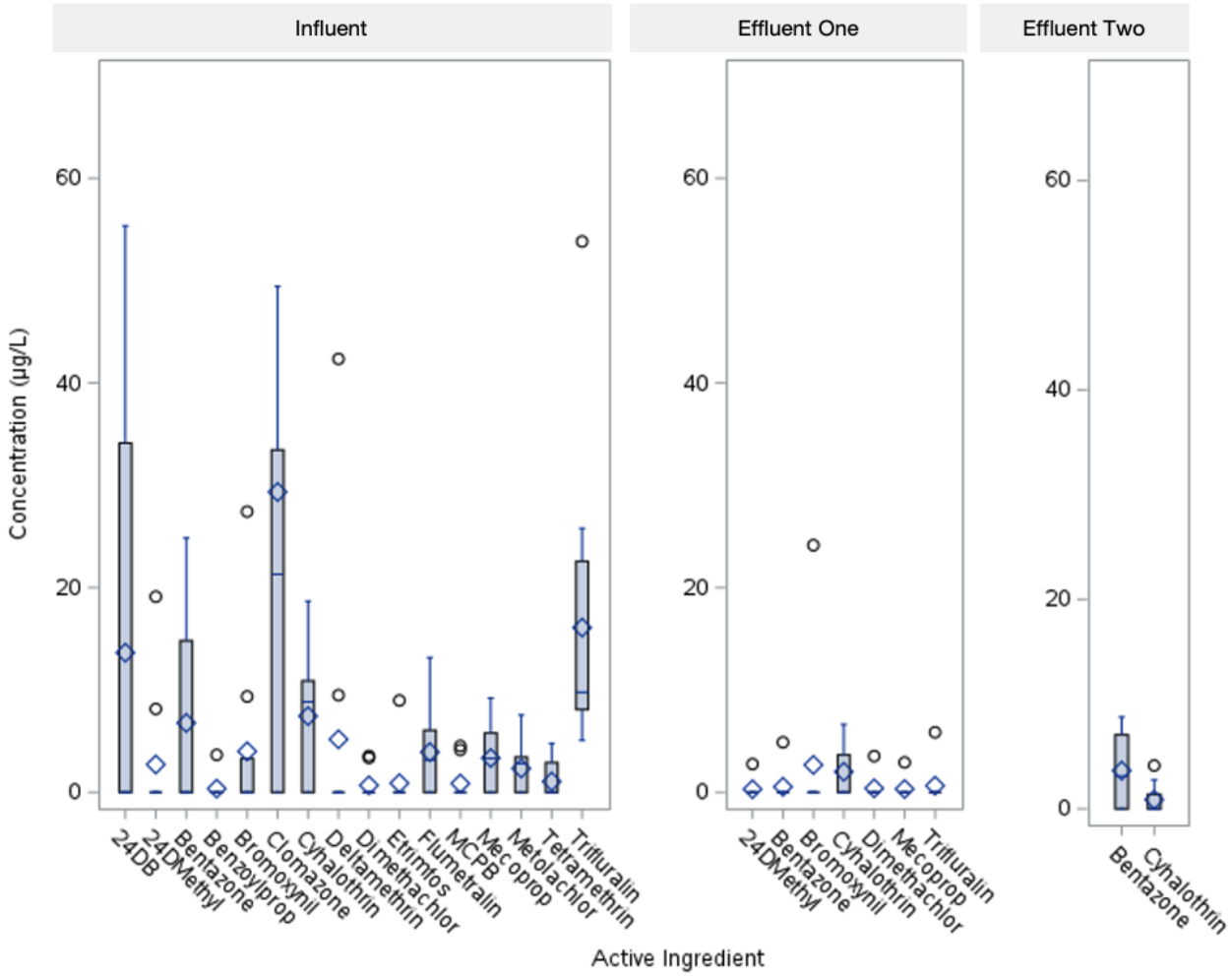

Figure 3.9 Concentration of active ingredients (µg/L) detected above LOQ, except dicamba, in influent and effluent sampled in glass bottles during 2021. Dicamba concentrations are shown is Figure 3.10. Mean is indicated with a diamond and median with a line. One concentration for Clomazone, of 140.85 µg/L, was removed for better visualization for all active ingredients.

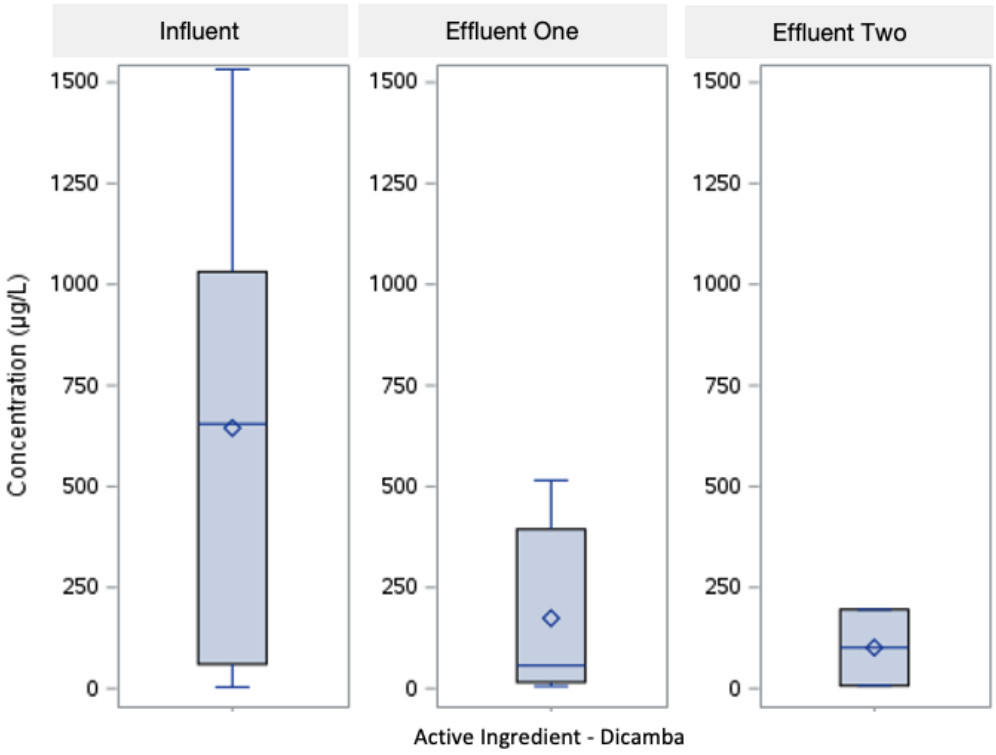

Figure 3.10 Concentration of dicamba (µg/L) detected above LOQ in influent and effluent sampled in glass bottles during 2021. Mean is indicated with a diamond and median with a line.

Table 3.5 Effect of sampling location on pesticide concentrations in 2021 analyzed through the multi-residue pesticide analysis.

Effect	Pesticide Concentration
	µg/L
Sample Location	
Influent	3.69**a**
Effluent One	1.98**b**
Effluent Two	0.56**c**
	P-value
Sampling Location	**<0.0001**
Effluent One x Effluent Two	**<0.0001**
Effluent One x Influent	**<0.0001**
Effluent Two x Influent	**<0.0001**

Table 3.6 Effect of sampling location on concentrations of glyphosate, glufosinate, AMPA, and the pesticides analyzed through the multi-residue pesticide analysis.

Effect	Pesticide Concentration
	µg/L
Sample Location	
Influent	8.56**a**
Effluent One	8.28**b**
Effluent Two	7.93**c**
	P-value
Sampling Location	**<0.0001**
Effluent One x Effluent Two	**0.0015**
Effluent One x Influent	**0.0054**
Effluent Two x Influent	**<0.0001**

3.6.6 Glyphosate, Glufosinate and AMPA Detections in 2021

The samples collected in falcon bottles in 2021 were analyzed for glyphosate, glufosinate and AMPA. Table 3.7 and Figure 3.11 shows the concentration data that was above the LOQ for glyphosate and glufosinate while Figure 3.12 shows the concentration data for AMPA. It should be noted that glufosinate ammonium was rinsed a total of eight times at the biobed site, of which one rinsing event included a full sprayer tank (680L) containing intended for field application, which was thirty-eight times larger than the average of the remaining self-reported (i.e., reported by field technician, or student, etc.) volume of rinsate left in sprayer tanks at the biobed in 2021 (18.2 L).

Glyphosate also accounted for 39.2% of logbook entries and glufosinate accounted for 15.7% of entries, which were two of the three most frequently rinsed pesticides at the biobed. Glyphosate had the highest detections in the Influent, with a median of 29,600 µg/L, but decreased substantially to 3,360 µg/L in Effluent One, then to 3,050 µg/L in Effluent Two (Figure 3.11).

Glufosinate slightly increased from Influent to Effluent One to Effluent Two where the median concentrations were 21,800 µg/L, 36,000 µg/L, and 48,100 µg/L respectively (Figure 3.11). The large dose of approximately 680 L of prepared spraying liquid containing glufosinate likely is the reason for the presence of glufosinate at such high concentrations in the effluents. A previous study looking at degradation in biomixtures found that degradation rate decreased with increasing concentration (Fogg et al. 2004). The mean concentrations for AMPA in the Influent, Effluent One and Effluent Two were 1,861 µg/L, 1,656 µg/L, and 1,430 µg/L respectively (Figure 3.12). The smaller decrease in concentration of AMPA may be a result of Glyphosate being transformed into AMPA and the longer half-life of AMPA compared to glyphosate.

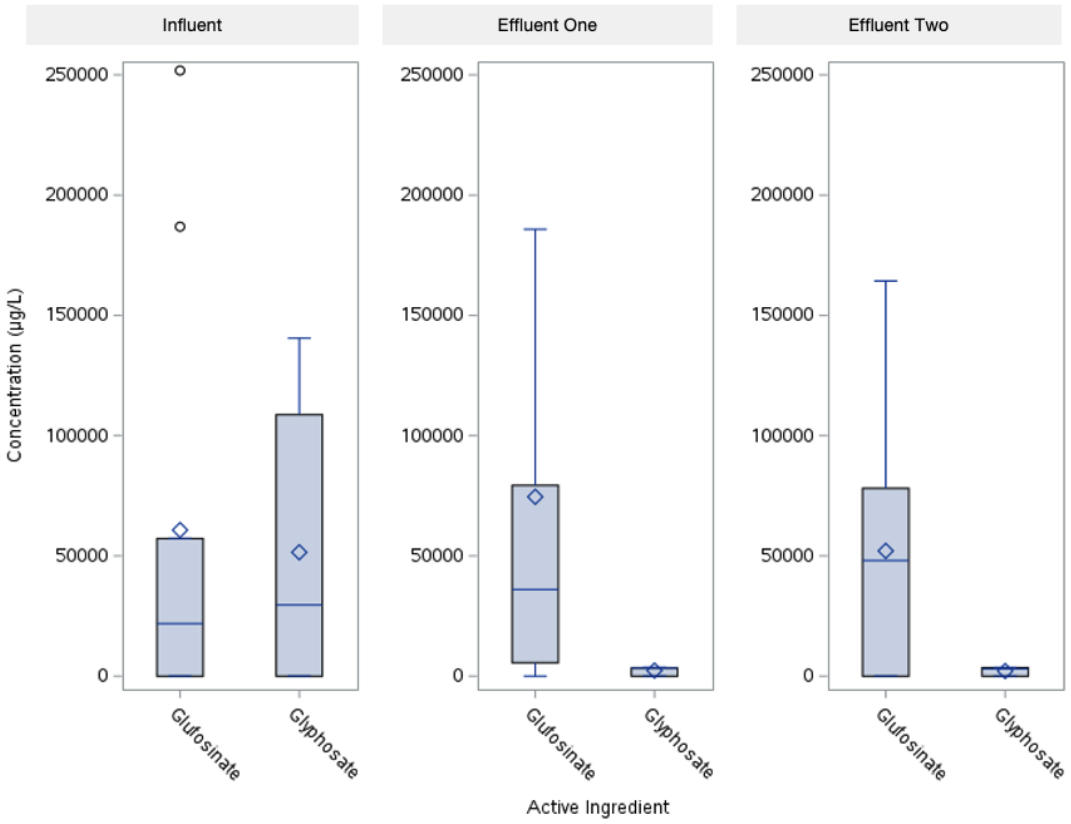

Figure 3.11 Concentration of glyphosate and glufosinate (µg/L) detected above LOQ in influent and effluent sampled in Falcon bottles during 2021. AMPA concentrations are shown in Figure 3.12. Mean is indicated with a diamond and median with a line.

Table 3.7 Influent and effluent detections of glyphosate, glufosinate and AMPA above LOQ for samples collected 2021 (GUS=Groundwater Ubiquity Score, LOQ=Limit of Quantification, H=Herbicide, I=Insecticide). K_{oc}, Soil DT_{50}, and GUS values were obtained from Lewis et al. (2016).

Active Ingredient	K_{oc} *L kg^{-1}*	Soil DT_{50} *d*	GUS	LOQ	Number of Detections	Detection Frequency *%*	Avg. Conc. *ng g^{-1}*	Min. Conc. *ng g^{-1}*	Max. Conc. *ng g^{-1}*
Influent (n=9)									
Glyphosate (H)	1,424	6.45 to 16.11	0.21	4.4	6	67	51501	3332	140517
Glufosinate (H)				5.3	6	67	60736	4629	251822
AMPA	2,002	234 to 419	0.04	4.5	6	67	1862	784	3815
Effluent One (n=9)									
Glyphosate (H)	1,424	6.45 to 16.11	0.21	4.4	6	67	2316	3315	3675
Glufosinate (H)				5.3	7	78	74562	5551	271355
AMPA	2,002	234 to 419	0.04	4.5	7	78	1656	136	2680
Effluent Two (n=8)									
Glyphosate (H)	1,424	6.45 to 16.11	0.21	4.4	5	63	2106	2984	3636
Glufosinate (H)				5.3	5	63	52075	41104	164312
AMPA	2,002	234 to 419	0.04	4.5	5	63	1435	2218	2370

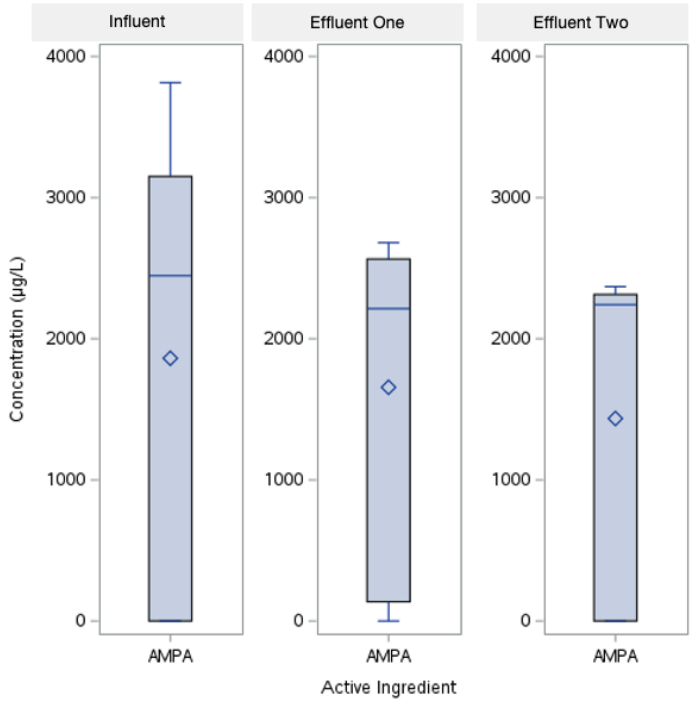

Figure 3.12 Concentration of AMPA (µg/L) detected above LOQ in influent and effluent sampled in Falcon bottles during 2021. Mean is indicated with a diamond and median with a line.

3.6.7 Pesticide Detections in the Biomixture

Similar to the year prior, the biomixture within each biobed cell was sampled prior to pesticide rinsate application, on June 3, 2021, and after the final rinsate had been processed, on October 14, 2021. Three pesticide active ingredients were detected at the beginning of the growing season (bifenthrin, diuron, and trifloxystrobin) and at 100% frequency (Table 3.8, Figure 3.13). Diuron was detected at much larger concentration that the other pesticides and diuron was the only pesticide detected at the end of the growing season (Table 3.8, Figure 3.13).

Diuron has a half-life of 147 to 229 days in soil which is longer than the half-lives reported for bifenthrin and trifloxystrobin and a GUS value of 2.65 which is higher than the other two pesticides (Table 3.8). Despite being detected in the end of the season, the mean concentration of diuron (657 ng/g) was lower than had been detected at the beginning of the season (3,790 ng/g), suggesting that diuron was degraded in the biobed system. The data suggests that the biobed was effective at degrading bifenthrin and trifloxystrobin because they were not detected at the end of the season. Importantly, the biobed was also effective at degrading the 12 of the 17 pesticides that were added through the rinsate since none of the chemicals were detected in the biomixture (Table 3.8). Bentazone, clomazone, etrimfos, metolachlor and trifluralin were not tested in the biomixture due to a lack of method development to achieve quantifications for these compounds. As such, these five pesticide active ingredients may be present in the biomixture as they could not be quantified in the biomixture analysis that was performed for this study.

Table 3.8 Pesticide detections above LOQ for biomixture samples collected in 2021 (GUS=Groundwater Ubiquity Score, LOQ=Limit of Quantification, H=Herbicide, F= Fungicide I=Insecticide). K_{oc}, Soil DT_{50}, and GUS values were obtained from Lewis et al. (2016).

Active Ingredient	K_{oc} *L kg^{-1}*	Soil DT_{50} *d*	GUS	LOQ	Number of Detections	Detection Frequency *%*	Avg. Conc. *ng g^{-1}*	Min. Conc. *ng g^{-1}*	Max. Conc. *ng g^{-1}*
Beginning of Season (n=12)									
Bifenthrin (I)	236,610	26 to 102.2	-2.66	9.50	12	100	98.26	61.91	150.6
Diuron (H)	680	146.6 to 229	2.65	20.73	12	100	3788	2325	6324
Trifloxystrobin (F)		0.34 to 1.69	0.15	11.45	12	100	130.5	78.95	195.8
End of Season (n=12)									
Diuron (H)	680	146.6 to 229	2.65	20.73	12	100	657.1	359.4	1003

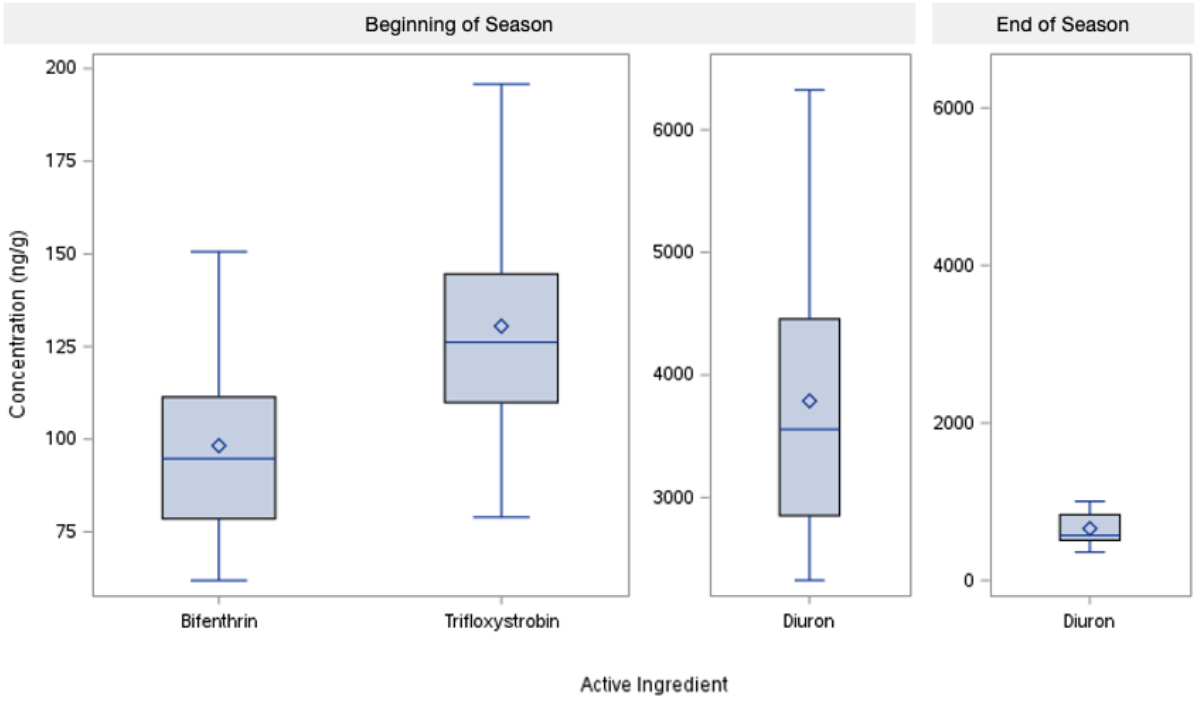

Figure 3.13 Concentration of Active Ingredients detected above LOQ in biomixture at the beginning of the season and end of season during 2021. Mean is indicated with a diamond and median with a line.

3.7 Conclusion

Pesticide detections in plastic bottles were significantly different than glass bottles with the frozen samples from 2020; however, a control was not utilized in this study so further studies are required to investigate such differences. Turbidity measurements may offer a cost-effective tool for farmers or other biobed users to indicate that the system is functioning. The biobed system did not pose a risk for greenhouse gas emissions when compared to studies of soils in the area. Differences in greenhouse gas fluxes between the biobed cells were likely due to biobed cell one receiving more liquid than the second cell. Thereby biobeds were an effective method for reducing point source pesticide contamination and that the dual cell biobeds provided additional benefit by improving pesticide removal. A significant difference was found between the effluent of the first biobed cell compared to the second which shows that dual-cell biobeds provided additional benefit in reducing pesticide contamination risk. Large deposits of pesticides can overburden the biobed system and result in lower pesticide removal rates. The pesticides detected in either the effluents or the biomixture at the end of the season tended to have either a higher GUS value or a longer half-life.

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4. OVERALL SYNTHESIS

4.1 Summary of Research

This thesis research first focused on the design and construction of a biobed system for pesticide rinsate management on a research farm in Carman, MB. Prior to this biobed the rinsate produced on the farm was deposited onto the soil surface in a small area on the farm. This can pose a risk to surface and ground water because high pesticide concentrations in the soil can result in slower pesticide degradation rates (Schoen and Winterlin 1987), and point-source contamination has been estimated to account for 90% of pesticide loading into water in European studies (Frede et al. 1998; Neumann et al. 2002). Many design features of the biobed were determined after observing three operational biobeds in Saskatchewan, Canada and speaking with the individuals who built and operated them. The design features influenced by this visit were having a roof cover to prevent precipitation from entering the system, having automated pumping so the system can operate without daily interventions, utilizing drip emitters as the mode for rinsate application, and incorporating water flow meters to monitor and quantify the liquid in the system. The biobed construction commenced September 2019 and was finished in August 2020 with the first pesticide rinsate being applied to the biobed on August 25th, 2020. The construction was successful due to the collaboration of the graduate student, farm personnel and summer students. Construction required numerous skills like welding (for building supports for the solar panels and trough), carpentry (for building roof structure and trough backsplash), plumbing (for rinsing collection area and drip irrigation), and wiring (for wiring pumps and probes).

Prior to pesticide rinsate being cycled through the biobed, weeds established on the biobed surface. A quadrat study was conducted to observe the weed species that had grown on the biobed. All of the weed species observed in the quadrat study were in the top 20 weed species for their relative abundance in Manitoba with 4 of them being in the top 10 (Leeson et al. 2017). The weeds that had established on the biobed surface seemed to be representative of the weeds in the area based on the Manitoba Weed Survey.

Sentek Drill & Drop Soil moisture probes were installed in the biomixture to monitor the temperature and moisture. Biomix temperature followed similar trends to the ambient air temperature recorded by the weather station that was at the biobed site. Temperature and moisture data appeared followed similar trends between the two years for the months that were collected in both years. Moisture data was unreliable when temperatures were below zero degrees Celsius where the readings were uncharacteristically low.

Greenhouse gas fluxes were analyzed for CO_2 (carbon dioxide), CH_4 (methane), NH_3 (ammonia), NO_2 (nitrogen dioxide), and N_2O (nitrous oxide) between May 4th and Sept 7th, 2021. The fluxes between the biobed cells were not significantly different except for CO_2. The higher fluxes of the first biobed cell is likely due to the larger volume of liquid being applied to the first cell as irrigation has been shown to increase CO_2 fluxes in soils (Sainju et al. 2008).

Influent, Effluent One and Effluent Two samples were taken in both years for multi-residue pesticide analyses. In conjunction with the sampling, probe measurements for turbidity, free and total chlorine, dissolved oxygen (%), electroconductivity (µS) and pH were taken of the liquids.

Turbidity measurements may be a cost-effective way for farmers or biobed users to indicate that the biobed system is degrading and/or retaining pesticides as it was greatest in the Influent > Effluent One > Effluent Two. Due to restrictions brought on by the COVID-19 pandemic, influent and effluent samples were taken in both plastic and glass bottles in 2020 because samples needed to be frozen until there was a capacity to analyze them. The multi-residue pesticide analyses were completed for both sample bottles and a significant but small difference in their concentrations was determined. There was no control in the study so future studies should investigate the basis for these differences. In 2020, three pesticides (bromoxynil, deltamethrin, and metolachlor) were detected in the Influent of the biobed, however, there were no detections in either effluent suggesting that they were degraded or sorbed in the biobed. Two additional pesticides (glyphosate, glufosinate), and one metabolite (aminomethylphosphonic acid (AMPA)) were also analyzed in the influent and effluent samples in 2020 using a different analysis. Glyphosate and AMPA were detected in the Influent and AMPA was detected in Effluent One but not Effluent Two, again suggesting that these chemicals were degraded or sorbed in the biobed. AMPA being present suggests that glyphosate was being degraded as it is a metabolite of glyphosate.

In 2021, the multi-residue analysis showed 17 pesticides being detected in the influent, eight in Effluent One and three in Effluent Two. A significant difference was found between the pesticide concentrations of the Influent, Effluent One and Effluent Two which proves that the inclusion of a second biobed cell significantly improved pesticide reduction. This significant difference between the concentrations between Effluent One and Effluent Two supports the hypothesis of this research that having a second biobed cell would be a better protection strategy

for surface and groundwater. The second analysis of glyphosate, glufosinate, and AMPA showed that all three were detected in the Influent, Effluent One and Effluent Two. When these concentration data were added to the previous set, a significant difference was found between Influent, Effluent One and Effluent Two, which again supports the hypothesis that having a second biobed cell would be a better protection strategy for surface and groundwater. Notably, glyphosate decreased substantially from Influent to Effluent One and the presence of AMPA indicated degradation of glyphosate. There was a large input of liquid containing glufosinate which likely overburdened the system and resulted in lower degradation rate. It has been shown that increasing concentration decreases degradation rate (Fogg et al. 2004). This lowered degradation rate contributed to the glufosinate detections in both effluents.

The biomixture was analyzed for 111 pesticides between the beginning and end of season in both 2020 and 2021. Because the sampling at the beginning of the season in 2020 occurred before any pesticide rinsate had entered the system, the pesticides detected at that time would have come from one of the Biomixture components. 2,4-DB, dicamba, and metrafenone were all detected in the initial sample in 2020 suggesting that those pesticides were present on either the straw, peat or soil used in the biomix. There were no detections at the end of 2020 which suggests the degradation of the pesticides. In 2021 trifloxystrobin, diuron and metrafenone were detected in the beginning sample of 2021. Of those, only Diuron was detected at the end of the season but at much lower concentrations suggesting the pesticide was partially degraded. Diuron had the highest GUS, and K_{oc} of the pesticides detected which contributed to its persistence. The lack of presences of the pesticides detected in the rinsate indicate the degradation of these compounds.

4.2 Practical Implications of the Research

Biobeds are still a relatively new management practice in Canada despite being more commonly adopted elsewhere in the world, such as in Latin America and Europe. This research confirms the effectiveness of using biobeds to protect surface and ground water from pesticide contamination within the Canadian Prairies. Specifically, this study showed a significant decrease in the concentrations and a reduction in the number of pesticides detected from the Influent (rinsate) of the biobed system to Effluent One and Effluent Two, thus demonstrating that biobed systems are a viable option to minimize contamination risks that are associated with the direct exposure of pesticide rinsate into the environment. The significant difference in pesticide concentrations and reduced number of pesticides detected between Effluent One and Effluent Two also prove that there is greater reduction in pesticides by constructing dual-celled biobed compared to a single-celled biobed. Pesticide applicators that could benefit from implementing biobed rinsate management systems could include those involved in pest control management on farms, research stations and golf courses, in land management applications in municipalities, and in parks or other recreational areas, and greenhouse operations.

Design features that were considered in this study are detailed in Appendix One and were based on biobed sites in Saskatchewan that were visited at the onset of this study in 2019. The biobed established for this graduate project was built with a primary focus on conducting research which added some complexity and costs to the system that may be excessive and/or undesirable for more general applications such as on farms (i.e. numerous moisture probes, roof structure over biobed surface, flow meters, container storage, etc.). In more practical designs, there will be options to address the particular needs or budgets of a party interested in implementing a biobed.

Costs for the biobed system could be reduced by having a less automized system and/or by upcycling existing materials available at a given site such as using chemical totes or unused tanks (Braul and Sheedy 2018). As another example, the biobed system established in this study is an above-ground system that requires large containers. The use of an in-ground biobed systems that does not require such containers and could potentially reduce development costs.

Collection area designs recommended by the AAFC in their most recent guideline on biobeds are collection pads comprised of either concrete or a polyethylene liner (Braul and Sheedy 2018). The current study demonstrated an alternate option for the collection of rinsate (i.e., a collection trough), thus expanding on the options available for designing a biobed system. The collection trough was constructed using modified tanks and a backsplash. This alternate option is for pesticide applicators to position their sprayer (nozzles) above the collection trough and release the rinsate directly into the trough, which is then pumped into a rinsate storage tank to accomplish controlled pesticide rinsate applications onto the biobed.

Another impact of biobed design is the effect on the environment. One such consideration in the greenhouse gas emissions related to such a system. Greenhouse gas fluxes were shown to be largely comparable to that of the soils (Tenuta et al. 2010; Gao et al. 2014; Amiro et al. 2017) in the area. This suggests that biobed systems can be used protect surface and groundwaters from point source pesticide contamination while not posing a large concern for their potential for greenhouse gas emissions.

The moisture probe readings for the three probes in each biobed reported similarly during this study which suggests that the temperature and moisture conditions of the biomixture remain consistent throughout the container. Biobed systems monitored by farmers or other pesticide users could save costs by monitoring biomixture conditions with a singular probe for each biobed cell.

4.3 Limitations of the Research and Recommendations for Future Studies

During this study, rinsing activity at the biobed site was tracked and documented using a physical logbook that users filled out the site during when they rinsed their equipment at the facility. It is possible that some rinsing activity at the biobed site was missed due to farm personnel unintentionally neglecting to fill out the logbook with their activity. Moreover, some of the entries in the logbook were incompletely filled out or had handwriting that was difficult to interpret. Future studies could incorporate cameras to observe the rinsing occurrences which could be used to validate their activity logs and potentially capture rinsing activity where a user could have forgotten to fill out the logbook. An electronic activity logging system could also be implemented using a QR code posted on site that users scan using a mobile device that opens a virtual survey for them to complete. This could help with any potential legibility issues from interpreting individual's handwriting and ensure that all the requested information is supplied for each entry by utilizing mandatory fields in the survey. It would also allow for easier access to feedback about potential operation issues or concerns for the persons managing the site if they can virtually monitor user feedback without having to visit the site. Additionally, having a virtual logbook would prevent the risk of the misplacement of logbooks or any loose pages. A potential limitation to using a virtual survey is that all personnel who utilize the rinsing at the site would

require a smart phone or tablet that has access to the internet at the site to fill it out. If personnel do not each have access to a device connected to the internet to fill out the survey, a wall-mounted tablet with access to the internet could be placed on site.

During this research, there was no capability for samples to be analyzed in proximity to the research site. This required samples to be collected and then shipped to a laboratory in the Province of Alberta with the ability to analyze the samples. Future studies in Manitoba will benefit from having access to the analytical services of the MASS Lab in the Faculty of Agriculture and Food Sciences at the University of Manitoba in Winnipeg that has opened subsequently to this research. This could reduce costs associated with shipping, potentially the time waiting for results, and prevent the risk of samples being damaged during shipping transit.

The development and construction of the biobed predominantly occurred during the COVID-19 pandemic. Following the rules and regulations associated with this period added a level of complexity in completing tasks for establishing the research site, such as social distancing and limiting the amount of people on site whenever possible. In addition, some of the materials and supplies that were ordered for the construction, such as some of the tanks, were also delayed due to supply issues caused by the pandemic. Additional time was spent planning and documenting all the activities on site to ensure that the execution of all the construction tasks were abiding by all the recommendations at the time.

Not all the active ingredients that were documented in the rinsing logs were able to be tested during this study. Future studies at this research farm should include the pesticide active

ingredients that are mentioned in the logbook (See Appendix Two) since their use is now documented. Additionally, not all the pesticides detected in the influents and the effluents were able to be tested in the biomix. Further studies that include testing the biomixture for all the pesticides detected in the influents and the effluents could give better indication of whether pesticides are being sorbed or degraded. Additionally future studies could include testing for more metabolites of pesticides.

In this study, the effluent of the biobed system was used as irrigation on an adjacent grassy parcel. Future studies could investigate a closed or circular biobed system where the final effluent is deposited back into the rinsate storage to be cycled through the system again. This may allow for more of the pesticide active ingredients and metabolites to be degraded, especially for the pesticides are less effectively removed by the system. Additionally perennial grasses planted on second cell could help to increase evapotranspiration and reduce the volume of liquid being added back through the system.

The biomixture for a biobed system requires a field soil component. Some farms, individuals or groups interested in establishing a biobed in Manitoba may not have access to the optimal soil types for use in a biomixture, i.e., loamy soils, and may use soils with more sand or clay. Future studies could investigate whether this change in soil type has any effect on biobed performance in Manitoba.

During the operation of the biobed system in this study, the drip emitters used for the application of the influent would occasionally plug and require maintenance or replacement. This was

planned to be mitigated at the biobed system in Carman, MB by using sediment filters placed throughout the system before all pumps. Since plugging of drip emitters still occurred during this research, future studies or biobed sites could utilize multiple filters in sequence that have an increasingly fine filter prior to the drip emitters to prevent them from plugging while also avoiding the filters from clogging from any sediment collected from the rinsate.

In this study, the frequency of influent and effluent sampling differed between the first and second year due to the closure and changing operational capacity of the laboratory used for analysis that was caused by the COVID-19 pandemic. A frequency of both weekly and bi-weekly sampling provided adequate results in this study. Future studies may opt for the bi-weekly sampling frequency for the benefit of reducing costs and the convenience of sampling less frequently.

Further studies should be conducted on the lifecycle of the biomixture and its effects on the performance of biobeds under Manitoba conditions, or more broadly for the Canadian Prairies. Studies could investigate the saturation of pesticides in the biomixture and its effects on the sorption of pesticides and the effect on microbial communities. Additionally, the optimal lifetime of the biomixture should be investigated to further support recommendations on how frequently the biomixture should be replaced within a biobed. Additional studies on the practices of disposing of previously used biomixture should be explored, with what pesticides remain and the optimal duration of composting the used biomixture being key considerations.

The pesticide concentrations from the frozen glass and plastic bottles were determined to be

significantly different in this study; however, a control for this was not used in this study. Future studies could better investigate this by adding a control group to the study the detections of plastic and glass bottles.

Subsamples of the biomixture samples taken prior to rinsate the biobed operational period and after the biobed operational season were collected and stored with the intent to look at microbial communities and potentially antibiotic resistant genes. DNA was extracted from these samples using a E.Z.N.A® Soil DNA Kit by Omega Bio-Tek. A future study using these samples will investigate the changes in microbial communities and antibiotic resistant genes after pesticide rinsate application to the biobed system.

4.4 References

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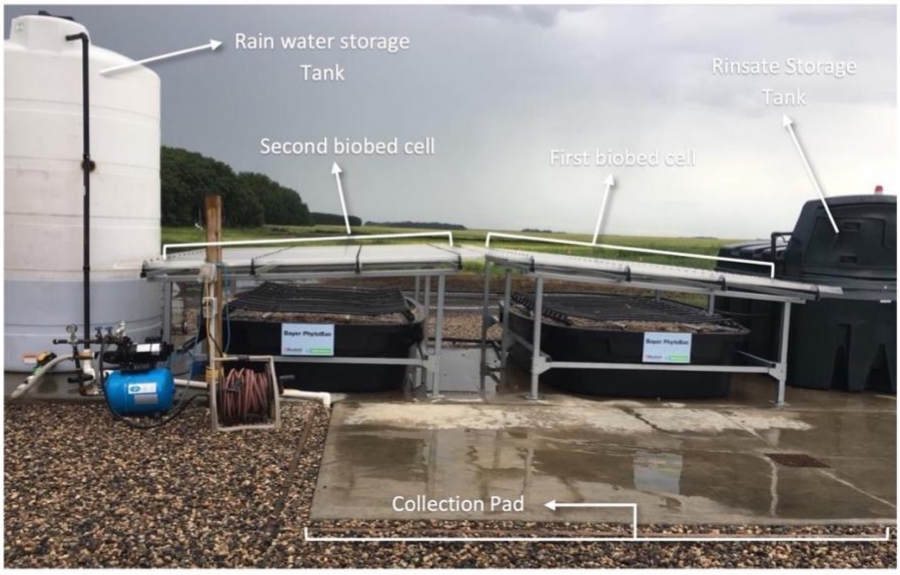
APPENDIX ONE

Biobed Tour Summary Document

Site 1: Rosthern, SK

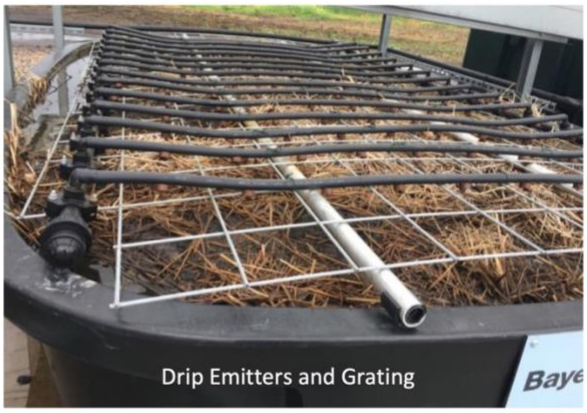


- Phytobac is a closed system where the liquid that is collected after travelling through the second cell is returned to the initial tank to run through the system again. The only way water leaves this system is through evaporation and there is no final product.
- Exact kit from Europe was used to build this biobed as it was an early prototype for the Phytobac in Canada.
- The concrete collection pad has a switch so that the water either goes to a rainwater storage tank or to the rinsate tank. The stored rainwater is used for cleaning or to supplement rinsate during summer to prevent bed from drying out.

Rain water or Rinsate Switch and Signage

1

- It has the capacity of processing 5 to 10 thousand liters.
- The pump for applying rinsate to bed is run on a timer, but Phytobac systems elsewhere can also be run from soil moisture.
- Pea gravel is bottom layer of biobed (part that will become saturated)
- The biobed cells have no heating and runs for 5 months.
- Drip emitters are attached to grating and placed over the biobed surface.
- All the pumps automatically run in this system.
- All plastic used is UV resistant, double lined, and chemical resistant.
- Biomixture is just straw and soil. There is no compost/peat portion.
- The main winterization step is to deal with the sprinklers.
- The system was being run aggressively and it was raining at the time of visit, so biobed cells were a bit waterlogged.


Drip Emitters and Grating


Connections from Rain Water Storage Tank


Sump


Rain Water Storage Tank


Connectors for Rinsate Tank and Automation Electronics

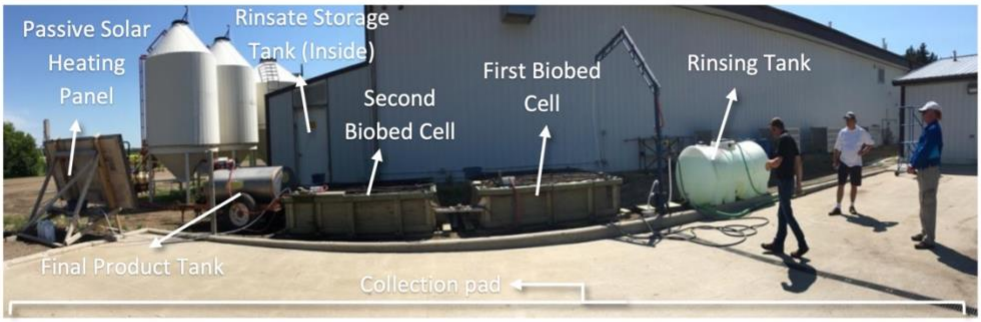
2

- Each biobed cell has a plastic roof above it to prevent rain water from entering the biobed surface that is clear to allow sunlight in to encourage evaporation.
- There is weeping tile at the bottom of biobed cells so that the water can be collected and run through the system again.

Site 2: Outlook, SK

- Large concrete collection pad (20ft x 60ft) that can accommodate the boom in front of biobed.
- The collection pad had a switch so that the water can be diverted if it is rainwater or feed into rinsate tank if it is rinsate. Rain water is not collected at this site.
- At time of visit biobed was estimated to have been running for 60 days and to have had 3000 gallons of rinsate enter the system.

3



- No roof cover or temporary cover for top of biobed, so rain events will add water to the biobed surfaces.
- Pea gravel is bottom layer of biobed (part that will become saturated)
- Biobed containers were constructed specifically for the biobed with wood frame and a liner.
- Unable to get grass to grow on first or second biobed despite no pre-emergence herbicides being put through biobed.
- Biomixture was made with wood instead of the straw component.
- Black drip emitters are rated for a gallon an hour. They replaced an old set that plugged.
- Outlook uses final product as dust control on their roads or just empties it onto gravel. They do not put any end product on vegetated areas.


Second Biobed Cell


First Biobed Cell


Final Product Tank

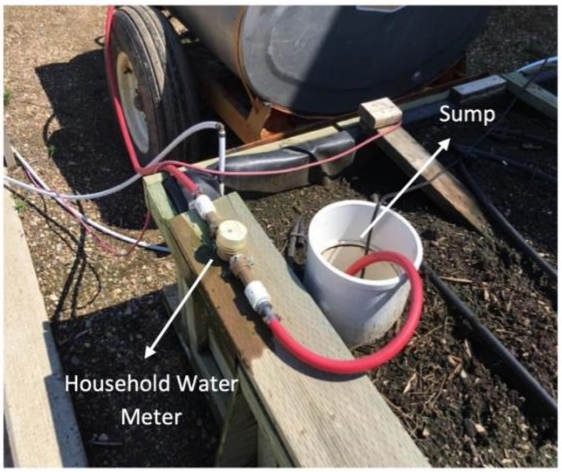
- 100V pumps are used for sumps.
- They usually start shutting the biobed down in September.
- Farm manager said that 3 or 4 days of running the heat tape can get the bed close to 30C.

- Each biobed cell is heated using different system. First biobed is heated using heat tape, and second biobed is heated using passive solar heating.
- Passive solar heating uses glycol as the liquid in the tubing.
- They have household water metres at various stages of the biobed to monitor the amount of water travelling through system.


Sump before Rinsate Storage


Rinsate Storage Tank


Pump




Passive Solar Heating Panel

Site 3: Saskatoon, SK

- This is a small system that was built for under $1000 by using a lot of recycled materials.
- The biobed containers are repurposed chemical totes
- No pumps are used for this biobed.
- Rinsate tank is above biobed cells so the rinsate moves through system through gravity.
- Pea gravel is bottom layer of biobed (part that will become saturated)


Hose with holes

- Drip system is a standard garden hose with holes punctured into it.
- The second biobed has native shrub species planted as a green cover. They chose shrubs due to their water usage and their deep roots.
- One thing they may have done differently is have a pump for the liquid coming out of the biobed cells so that it would automatically enter the rinsate tank again. They currently have to manually pour the liquid back into the storage tank.
- There is a temporary plastic cover that can be used to prevent rainwater from entering biobed surface that needs to be manually placed. It is clear to allow sunlight through.
- They run rinsate through the first biobed twice, and the second biobed once as an extra precaution

6

- They do not heat their biobed but they do not need to use it until mid-June so it melts in time.
- Same tank is used for the first and second biobed cells and there is a valve to change which it will feed into.

7

APPENDIX TWO

Chapter 3

Table A1: List of 142 Pesticide compounds that were analyzed for in the Influent, Effluent One and Effluent Two samples.

2,4-DB	Fenamidone
2,4-D-methyl (2,4-dichlorophenoxy acetate methyl)	Fenthion
2,6-Dichlorobenzonitrile, (dichlobenil)	Flamoxadone
Alachlor	Flamprop-isopropyl
Aldrin	Fluazifop-p-butyl
Allidochlor	Flumetralin
Atrazine	Flutriafol
Azinphos-methyl	Fonofos
Benalaxyl	Heptachlor
Benfluralin	Heptachlor exo-epoxide (isomer B)
Bentazone	Hexazinone
Benzoylprop-ethyl	Imazamethabenz
BHC-alpha (benzene hexachloride)	Iprodione
BHC-beta	Isofenphos
BHC-delta	Kresoxim-methyl
BHC-gamma (lindane, gamma HCH)	Malathion
Bifenthrin	MCPA-methyl
Boscalid	MCPB-methyl
Bromophos-ethyl	Mecoprop
Bromopropylate	Metalaxyl
Bromoxynil	Methoxychlor, p,p
Bupirimate	Metolachlor
Butachlor	Metrafenone
Butralin	Mirex
Butylate	Monolinuron
Captan	Myclobutanil
Carbaryl	Naled
Carbofuran	Napropamide
Carfentrazone-ethyl	Nitrapyrin
Chlordane-cis (alpha)	Oxyfluorfen
Chlordane-trans (gamma)	Pendimethalin (penoxaline)
Chlormephos	Permethrin I
Chloroneb	Permethrin II (trans)
Chlorpyrifos	Phorate
Chlorpyrifos-methyl	Phosmet
Chlorthiamid	Picoxystrobin

Clodinafop-propargyl	Piperonyl butoxide
Clomazone	Pirimicarb
Clopyralid	Pirimiphos-ethyl
Cycloate	Pirimiphos-methyl
Cyfluthrin I	Procymidone
Cyhalothrin (lambda)	Prometon
Cypermethrin I (zeta)	Prometryn
Cypermethrin III (beta)	Pronamide (propyzamide)
Cyprodinil	Propetamphos
DDD-o,p	Propham
DDD-p,p	Propoxur
DDE-o,p	Pyraclostrobin
DDE-p,p	Pyridaben
DDT-o,p	Pyrimethanil
DDT-p,p	Quinclorac
Deltamethrin	Quinoxyfen
Diazinon	Quintozene
Dicamba-methyl	Quizalofop-ethyl
Dichlofenthion	Ronnel (fenchlorphos)
Dichlorvos	Simazine
Dieldrin	Spirodiclofen
Dimethachlor	Spiromesifen
Dimethenamid-P	Spiroxamine
Dimethoate	Terbacil
Dimethomorph	Terbufos
Dioxathion	Terbutryn
Diphenamid	Tetradifon
Diuron	Tetramethrin
Endrin	Tetrasul
EPTC	Triallate
Ethalfluralin	Trifloxystrobin
Ethion	Trifluralin
Ethofumesate	Triticonazole
Etridiazole (terrazole, echlomezol)	Vinclozolin
Etrimfos	Zoxamide

Table A2: List of 111 pesticide compounds that were analyzed for in biomixture samples.

2,4-DB	Fluazifop-p-butyl
2,4-D-methyl (2,4-dichlorophenoxy acetate methyl)	Flumetralin
2,4-Dichlorophenol,	Flumioxazin
2,6-Dichlorobenzonitrile, (dichlobenil)	Flutriafol
Alachlor	Folpet
Aldrin	Fonofos
Allidochlor	Heptachlor
Azinphos-methyl	Heptachlor exo-epoxide (isomer B)
Benalaxyl	Hexazinone
Benfluralin	Imazamethabenz
Benzoylprop-ethyl	Iprodione
BHC-alpha (benzene hexachloride)	Isofenphos
BHC-delta	Kresoxim-methyl
BHC-gamma (lindane, gamma HCH)	Malathion
Bifenthrin	MCPA-methyl
Bromophos-ethyl	MCPB-methyl
Bromopropylate	Mecoprop
Bromoxynil	Metalaxyl
Bupirimate	Methoxychlor, p,p
Butralin	Metrafenone
Captan	Mirex
Carbaryl	Monolinuron
Chlordane-cis (alpha)	Myclobutanil
Chlordane-trans (gamma)	Naled
Chloroneb	Napropamide
Chlorpyrifos	Nitrapyrin
Chlorthiamid	Oxyfluorfen
Clodinafop-propargyl	Pendimethalin (penoxaline)
Cycloate	Permethrin I
Cyfluthrin I	Permethrin II (trans)
Cyhalothrin (lambda)	Phorate
Cypermethrin III (beta)	phosmet
Cyprodinil	Picoxystrobin
DDD-o,p	Piperonyl butoxide
DDE-o,p	Pirimiphos-ethyl
DDE-p,p	Pirimiphos-methyl
DDT-o,p	Procymidone
DDT-p,p	Prometon

Deltamethrin	Prometryn
Diazinon	Pronamide (propyzamide)
Dicamba-methyl	Propetamphos
Dichlofenthion	Propham
Dichlorvos	Propoxur
Dieldrin	Pyraclostrobin
Dimethachlor	Quinclorac
Dimethenamid-P	Quinoxyfen
Dimethoate	Ronnel (fenchlorphos)
Dimethomorph	Simazine
Dioxathion	Spirodiclofen
Diuron	Spiroxamine
Endrin	Terbufos
Ethalfluralin	Tetramethrin
Ethion	Trifloxystrobin
Ethofumesate	Triticonazole
Etridiazole (terrazole, echlomezol)	Zoxamide
Flamprop-isopropyl	

Table A3: List of pesticide active ingredients in entries of rinsing logbook in 2020 and 2021.

Year	Pesticide Active Ingredient
2020	Deltamethrin
	Glyphosate
2021	Tralkoxydim
	Pinoxaden
	Bromoxynil
	Clethodim
	Chlorantraniliprole
	Deltamethrin
	Saflufenacil
	Glufosinate
	MCPA
	Fenoxaprop
	Glyphosate
	Diquat ion
	Trifluralin