QUANTIFYING THE CONCENTRATION AND LOADS OF DISSOLVED ORGANIC CONTAMINANTS IN SNOWMELT RUNOFF FROM MANURE-AMENDED AGRICULTURAL FIELDS.

By

Haven S.J.S. Soto

A thesis submitted to the Faculty of Graduate Studies in partial fulfilment of the requirements for the Master of Science degree.

Department of Graduate Studies

Master of Science in Environmental and Social Change

The University of Winnipeg

Winnipeg, Manitoba, Canada

May, 2023

ABSTRACT

Agriculture is an integral part of Canada's economy, making 1.6% of the country's GDP. In Manitoba, swine production contributed nearly 1.4 billion dollars in revenue from 8.4 million pigs sold to the market. Liquid swine manure is widely used as fertilizer in the province. However, there are risks with the use of liquid swine manure as fertilizer because liquid swine manure can contain organic contaminants, such as antibiotics and steroidal hormones. Globally, there is evidence of increased soil and freshwater contamination from antibiotics and estrogens, resulting in the increased presence of antibiotic-resistant genes and estrogen-related physiological disruptions, respectively. In the Canadian Prairies, a majority of the annual runoff occurs during the brief snowmelt period, when runoff occurs over frozen soils. Temporal changes in the transport of antibiotics and estrogens during this important hydrological period are not well understood but are critical to understanding the fate of these organic contaminants.

This thesis quantified the dissolved concentration and load of a 1) steroidal hormone, 17β-estradiol, and 2) antibiotic, sulfamethoxazole, in snowmelt from an agricultural field with a history of manure application under different manure management practices (i.e., no manure applied, manure applied on the sub-surface, and manure applied on the surface) over the snowmelt period. Research experiments in chapter 2 used two components (a field study during snowmelt and a laboratory simulation with flooded intact soil cores collected from the manured field) to quantify the dissolved 17β-estradiol in flood water and pore water for the laboratory simulation and snowmelt for the field study. Chapter 3 quantified the dissolved sulfamethoxazole in snowmelt in the same field study as chapter 2.

17β-estradiol (mean laboratory pore water concentration = 1.65 ± 1.2 μg/L; mean laboratory flood water concentration = 0.488 ± 0.58 μg/L; and mean field snowmelt concentration = 0.0619 ± 0.048 μg/L) and sulfamethoxazole (0.0345 ± 0.066 μg/L) were detected in all water samples, although there were no significant differences in the concentrations measured among the different manure application methods. 17β-estradiol concentrations varied between the laboratory and the field, with higher concentrations measured in the laboratory simulation. Pore water concentrations of 17β-estradiol from the laboratory study significantly increased over time, corresponding with changes in pH. In contrast, there was no significant change in the field snowmelt concentrations measured over time for both 17β-estradiol and sulfamethoxazole. The mean cumulative load of 17β-estradiol (6.91 ± 3.7 ng/m^2) and sulfamethoxazole (4.12 ± 3.6 ng/m^2) approximates the magnitude of 17β-estradiol and sulfamethoxazole that could be mobilized from manured fields during snowmelt. There was a significant increase in cumulative load over time for both 17β-estradiol and sulfamethoxazole, suggesting that the load is driven by the snowmelt volume rather than concentration. Furthermore, the 17β-estradiol load from plots with manure applied on the sub-surface was significantly larger than the surface application of manure and no manure application. This thesis provides preliminary insights to improve current manure management practices in the Canadian Prairies to include organic contaminants.

ACKNOWLEDGEMENTS

To my supervisors and the power duo, Dr. Inoka Amarakoon and Dr. Nora Casson, thank you for the continued support, guidance, and patience you have both given me. I consistently aim to improve myself as a researcher, an academic, and overall human because of you. Thank you for trusting me to work on a project so out of my comfort zone, and despite all of that, we produced something worthwhile. Thank you to my committee members, Dr. Darshani Kumaragamage and Dr. Henry Wilson, for providing me with quality feedback and challenging me to constantly improve my work.

Thank you to the dream team, Madelynn Perry and Viranga Weerasinghe, for being by my side and helping each other out throughout our master's programs. I am so grateful we crossed paths and were able to work together through long days collecting samples, comparing results, and sharing ideas. To the folks in the multiple lab groups that I was able to work with, Emily Van, Claire Signatovich, Richard La, Charitha Hansima, and Karl Friesen-Hughes, I appreciate your friendship and support on long days. Especially when we collected samples or watched water drip for hours on end.

I am grateful to my family and friends for continuously supporting me throughout my academic and life endeavours. The choices I make often seem random, but you provide me with comfort and confidence to keep pursuing the things that are important to me. Special thanks to my parents, Vilma and Juan Soto, for supporting and loving me dearly; to my sister, Jossa Kumaragamage, for passionately talking about math and stats with me over coffee; to my brother-in-law, Sashika Kumaragamage, for the countless number of cups of tea and science conversations; to my cousin, Khloey Santos, for her stern moral support and food when I did not have time to cook; and to my brother, L'Jo Soto, for reminding me it is okay to relax and take care of myself.

Finally, a very special thanks to my cats, Mrs. Pants and Kit, for being the best stress relievers and practice audience for my presentations. And thanks to Kyubi (my sister's cat) for the cuddles when Mrs. Pants and Kit do not want to be near me.

An NSERC Discovery Grant provided financial support for this research to Inoka Amarakoon and Nora Casson. I received personal financial support from the University of Winnipeg Graduate Student Scholarship.

CO-AUTHORSHIP STATEMENT

This thesis is based on two manuscripts that will be submitted for publication (Chapters 2 and 3). Haven Soto, who led the definition of the research problem, formulation of the study design, sample collection and analysis, data analysis, interpretation of results, and writing of manuscripts, will be the lead author on each of the two manuscripts. Inoka Amarakoon, Nora Casson, and Darshani Kumaragamage, who contributed to the definition of the research problem, formulation of the study design, data analysis approach, interpretation of results and provided editorial guidance on the writing, will be a co-author on both manuscripts. Henry Wilson, who contributed to interpretation of results and provided editorial guidance, will be a co-author on both manuscripts. Chapter 2 is being prepared for submission to Science of the Total Environment. Chapter 3 has been published in the Canadian Journal of Soil Science as:

Haven S.J.S. Soto, Inoka D. Amarakoon, Nora J. Casson, Darshani Kumaragamage, and Henry F. Wilson. 2023. The fate of dissolved sulfamethoxazole during spring-thaw snowmelt in a field with a history of manure application. Canadian Journal of Soil Science. https://doi.org/10.1139/cjss-2023-0006

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: General Introduction

1.1 Agriculture in the Canadian Prairies

Agriculture is one of the largest industries in Canada, making 1.6% of the country's GDP in 2021 (Government of Canada, 2022). In Manitoba, livestock production, and in particular swine operations, are increasingly important components of the agriculture industry, with nearly 8.4 million pigs sold for close to $1.4 billion in 2021 (Government of Manitoba, 2022). Veterinary pharmaceuticals, such as steroids and antibiotics, are frequently used to improve the efficiency and overall health of livestock. Steroidal hormones are naturally occurring biological compounds, but synthetic steroidal hormones can be added to feeds to improve growth rates of animals (Adeel et al., 2017). Antibiotics are critical for preventing and treating bacterial infections (Sarmah et al., 2006), but they are also found to help improve animal growth rates (Cromwell, 2002; Holman and Chénier, 2015). Steroidal hormones and antibiotics are often detected in manure, as well as surface waters and soils (Kumar et al., 2005; Khanal et al., 2006; Kim et al., 2011). It is estimated that nearly 90% of the total worldwide estrogen found in surface waters originated from livestock manure (Khanal et al, 2006). Antibiotics are not fully metabolized when administered to animals and up to 90% of the antibiotics detected in manure are excreted in their non-metabolized form (Kumar et al., 2005; Kim et al., 2011). With the potential for high levels of contaminants being released into the environment, the use of steroidal hormones and antibiotics should be carefully monitored to prevent environmental contamination. Furthermore, understanding the fate of veterinary pharmaceuticals is critical for protecting ecosystem and human health.

Manure is frequently used to improve soil fertility by recycling nutrients and building organic matter on agricultural fields (Kumar et al., 2005). However, long-term manure use is frequently related to the release of excess nutrients and organic contaminants into the environment (Dolliver and Gupta, 2008; Kuchta et al., 2009; Conde-Cid et al., 2020; Havens et al., 2020). With a large swine population in Manitoba, nearly 2.1 kg of liquid swine manure is produced annually (Hofmann, 2001). Manitoba frequently uses liquid swine manure as fertilizer on agricultural fields, but liquid swine manure is known to be one of the major sources of estrogens in the environment (Odinga et al., 2022). Additionally, the prolonged use of liquid swine manure is more likely to result in higher levels of antibiotic-resistant genes found in soils relative to other types of manures (Wu et al., 2022).

To mitigate some of the environmental impacts of manure use on agricultural fields, manure management practices have been put in place. In most of Canada, manure application occurs during the fall before the winter months, to prepare for seeding that occurs in the spring. The application of manure in the period just before the growing season would be ideal to reduce the loss of steroidal hormones and pharmaceuticals in snowmelt runoff compared to application just before winter (Song et al., 2010). However, fall application is ideal for many farms because there are limited options for overwinter storage of manure and there is a narrow window that farmers can apply manure onto their fields (Liu et al., 2018). The application of manure after the snowmelt flooding would also delay the seeding of crops, increasing the chances of damages to crops caused by insects, heat, and/ or weed competition (Government of Manitoba, 2023). Soil compaction may also occur due to the use of heavy machinery (i.e., manure application) on wet soils, causing more damage to the field and decreasing farming efficiency (The Prairie Province's Committee on Livestock Development and Manure Management, 2004).

The method of manure application is critical for mitigating the losses of contaminants into the environment. Sub-surface application of manure can significantly reduce concentrations of contaminants lost into the environment. A study comparing chisel plowing and surface application of liquid swine manure on silt-loam soil showed there was a significant difference in the concentration of chlortetracycline and tylosin released into the environment after snow and rain runoff events (Dolliver and Gupta, 2008). In another study comparing the difference between surface application and incorporation of solid cattle manure on the Prairies, higher sulfamethazine concentrations were detected in runoff collected from plots with manure applied on the surface (Amarakoon et al., 2014). Furthermore, sub-surface manure application is seen to be better suited in reducing the odour for both solid and liquid manure (Agnew et al., 2010) and in reducing the volatilization of ammonia (Huijsmans et al., 2001) compared to the surface application of manure. Although the sub-surface application of manure has many positive aspects, studies also show that sub-surface application of manure tends to result in higher rates of soil erosion due to the disturbance of the top layer of agricultural soils, compared to surface application of manure (Maguire et al., 2011). In the context of mitigating contaminant losses into the environment, sub-surface application of manure would be the preferred method, especially during runoff events.

1.2 Snowmelt

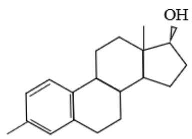
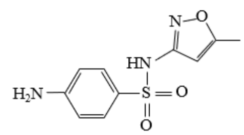
The Canadian Prairies are cold and frozen for many months of the year, which introduces unique challenges when examining the introduction of contaminants into the environment. In the Steinbach region of Manitoba, the annual precipitation is 580.5 mm of which approximately 107.1 mm falls as snow (based on 1981 – 2010 climate normals; Environment Canada, 2023). Although the relative proportion of snow to rain is relatively small, snowmelt contributes up to 80% of the annual water runoff (Liu et al., 2019a). The spring snowmelt period typically lasts approximately 2 weeks, during which there is an elevated loss of contaminants (Dolliver and Gupta, 2008; Kuchta et al., 2009; Liu et al., 2019b; Havens et al., 2020). Both antibiotics and hormones degrade slowly during winter months, due to colder temperatures (Amarakoon et al., 2016; Dolliver and Gupta, 2008; Havens et al., 2020). This is concerning because the breakdown of some organic contaminants can reduce the toxicity of the compounds (Khanal et al., 2006), and if these contaminants are persisting in soils, any dispersal into the environment and accumulation could be detrimental. With the delayed degradation that may be occurring in the winter, the fall application of manure may be increasing the likelihood of soils acting as reservoirs for organic contaminants.

Current manure management practices, which are effective in reducing water and soil contamination with nutrients (nitrogen and phosphorus) may not have the same effect on a wider range of organic contaminants. Therefore, understanding the transport potential of organic contaminants over the snowmelt period when there is an elevated release of contaminants would be critical in creating changes and/or additions to current agricultural practices to mitigate the losses of contaminants into the environment.

1.3 Steroidal Hormones and Antibiotics from Livestock practices

The persistence of organic contaminants (e.g., steroidal hormones and antibiotics) in soils is related to the soil pH, soil type and structure, and soil organic matter content (Conde-Cit et al., 2020). The contaminant's physico-chemical properties, such as the contaminant's solubility in water (Conde-Cid et al., 2020), polarity, and molecular structure (Semple et al., 2003) are other important factors to consider when understanding the persistence of organic contaminants in soils. This thesis focuses on one steroidal hormone (17β-estradiol) and one antibiotic (sulfamethoxazole), and their physico-chemical properties are summarized in Table 1.1.

Table 1.1: Physico-chemical properties of 17β-estradiol and sulfamethoxazole.

	17β-estradiol	Sulfamethoxazole
Structure		
Molecular Formula	$C_{18}H_{24}O_2$	$C_{10}H_{11}N_3O_3S$
Molecular Weight (g/mol)	270.4	253.3
K_{oc} (mL/g)	3 621[a]	219[c]
$logK_{ow}$	3.94[b]	0.89[d]
pK_a	10.6[b]	1.6, 5.7[e]
References	[a]Hildebrand et al., 2006 [b]Adeel et al., 2017	[c]Barron et al., 2009 [d]Lin and Gan, 2011 [e]Adesanya et al., 2021

1.3.1 17β-estradiol

There is a specific concern about the environmental fate of 17β-estradiol due to its relative abundance in livestock manure (Raman et al., 2004), its relative persistence in soils (Colucci et al., 2001), and its relative potency compared to other types of estrogens (Khanal et al., 2006). 17β-estradiol is a naturally occurring estrogen that is released by males and females but is predominantly used in the growth and development of females (Adeel et al., 2017). It is relatively small (270.4 g/mol), hydrophobic, and non-volatile (Hanselman et al., 2003). Under environmental pH (7 – 8), 17β-estradiol acts as a weak acid, with a pK_a of 10.6 (Hanselman et al., 2003; Adeel et al., 2017). 17β-estradiol is neutral in acidic and neutral environments and negatively charged in alkaline environments (Adeel et al., 2017). Furthermore, 17β-estradiol has a high affinity for soil particles rather than the aqueous phase, especially in soils with high humic content due to its relatively high organic-carbon partition coefficient, K_{oc} (Hildebrand et al., 2006; Sun et al., 2006).

Estrogens are excreted in livestock waste in high concentrations, which can end up in surface waters. High levels of estrogens have been shown to detrimentally affect aquatic species and humans (Adeel et al., 2017; Odinga et al., 2022). Fish are particularly sensitive to any exposure to estrogens, and there is evidence of the feminization of fish (e.g., *Danio rerio* and *Oryzias latipes*) and reduced spawning (e.g., *Pimephales promelas*; Ojoghoro et al., 2021). Human studies show that chronic and elevated exposure to exogenous estrogens and endocrine-disrupting compounds have been linked to prostate cancer (Prins, 2008; Prins et al., 2014) and breast cancer (Liang and Shang, 2013; Lecomte et al., 2017).

1.3.2 Sulfamethoxazole

Sulfamethoxazole is also a contaminant of concern because of its relative use and administration to livestock in the province of Manitoba. It is administered to livestock in tandem with trimethoprim as a synergistic pair of antibiotics for swine to treat urinary tract, respiratory, and gastrointestinal tract infections (Straub et al., 2016). However, this thesis will focus on sulfamethoxazole because of its relative concentration in sulfamethoxazole: trimethoprim (i.e., 5:1). Sulfamethoxazole was also more frequently detected in liquid swine manure more than any type of manure, such as cattle, poultry, or mixed manure (Wu et al., 2022). Sulfamethoxazole is part of the sulfonamide family of bacteriostatic antibiotics (Sarmah et al., 2006). Sulfonamides are a wide-spectrum antibiotic, targeting a majority of gram-positive and many gram-negative organisms. Sulfonamides act as a competitive inhibitor of the *p*-aminobenzoic acid in the folic acid metabolism cycle, which inhibits the multiplication of bacteria (Sarmah et al., 2006; Conde-Cid et al., 2020). This makes sulfonamides effective in preventing bacterial infections among livestock herds. Sulfamethoxazole is relatively small (253.3 g/mol) and relatively hydrophobic; thus, it has a higher affinity for the soil organic matter fraction (Barron et al., 2009). However, it is relatively mobile because of its small size and polarity in environmental pH's (Sarmah et al., 2006; Adesanya et al., 2021).

Antibiotics (including sulfamethoxazole) detected in the environment are linked to the increased presence of antibiotic-resistant genes (Di Cesare et al., 2015; Yue et al., 2021; Wu et al., 2022). This has resulted in decreased efficacy of antibiotic therapy in humans and animals, which reduces the possible treatments applicable for bacterial infections (Kumar et al., 2005; CCA, 2019). In Canada, an estimated 26 % of bacterial infections were resistant to first-line antibiotics in 2019, which could increase up to 40 % by 2050 (CCA, 2019). At that rate, it could reduce Canada's GDP by up to 396 billion dollars and a total of nearly 396 000 lives would be lost in 2050 (CCA, 2019). Therefore, it is critical to mitigate the dispersal of antibiotics into the environment to reduce the rise of antibiotic resistance in pathogenic bacteria.

1.4 Objectives

The overall objective of this study was to better understand the temporal changes of concentrations and loads of organic contaminants in snowmelt conditions on the Canadian Prairies. The specific objective was to quantify the dissolved concentration and load of (i) 17β-estradiol in a laboratory simulation and a field study (**Chapter 2**) and (ii) sulfamethoxazole in a field study (**Chapter 3**) in snowmelt from agricultural fields that have a history of manure application under three manure application methods (i.e., no manure applied, manure applied on the sub-surface, and manure applied on the surface).

1.5 Thesis Outline

This thesis is prepared in a manuscript style in accordance with the University of Winnipeg Graduate Studies guidelines. There are two standalone manuscripts (chapters 2 and 3):

Chapter 2: The fate of 17β-estradiol in snowmelt from a field with a history of manure application: a laboratory simulation and field study; and

Chapter 3: The fate of dissolved sulfamethoxazole during spring-thaw snowmelt in a field with a history of manure application.

1.6 References

Adeel, M., Song, X., Wang, Y., Francis, D. and Yang., Y. 2017. Environmental impact of estrogens on human, animal, and plant life: a critical review. *Environ. Int.* **99**: 107 – 119. doi: 10.1016/j.envint.2016.12.010

Adesanya, T., Zvomuya, F., Sultana, T., Metcalfe, C. and Farenhorst, A. 2021. Dissipation of sulfamethoxazole and trimethoprim during temporary storage of biosolids: a microcosm study. *Chemosphere*. **269.** doi: 10.1016/j.chemosphere.2020.128729

Agnew, J., Laugë, C., Schoenau, J., Feddes, J. and Guo, H. 2010. Effect of manure type, application rate, and application method on odours from manure spreading. *Can. Agric. Eng.* **52**: 6.19 – 6.29.

Amarakoon, I.D., Zvomuya, F., Cessna, A.J., Degenhardt, D., Larney, F.J. and McAllister, T.A. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. *J. Environ. Qual.* **43**: 547 – 557.

Amarakoon, I.D., Zvomuya, F., Sura, S., Larney, F.J., Cessna, A.J., Xu, S. and McAllister, T.A. 2016. Dissipation of antimicrobials in feedlot manure compost after oral administration versus fortification after excretion. *J. Environ. Qual.* **45**: 503 – 510. doi: 10.2134/jeq2015.07.0408

Barron, L., Havel, J., Purcell, M., Szpak, M., Kelleher, B. and Paull, B. 2009. Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks. *Analyst*. **134**: 663–670. doi: 10.1039/B817822D.

Bavumiragira, J.P., Ge, J. and Yin, H. 2022. Fate and transport of pharmaceuticals in water systems: A process review. *Sci. Total Environ.* **823.** doi: 10.1016/j.scitotenv.2022.153635

Colucci, M.S., Bork, H. and Topp, E. 2001. Persistence of estrogenic hormones in agricultural soils: I.17β-estradiol and estrone. *J. Environ. Qual.* **30**: 2070 – 2076. doi: 10.2134/jeq2001.2070

Conde-Cid, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Fernández-Calviño, D. and Arias-Estévez, M. 2020. Tetracycline and sulfonamide antibiotics in soils: presence, fate and environmental risks. *Processes.* **8**. doi: doi:10.3390/pr8111479

Cromwell, G.L. 2002. Why and how antibiotics are used in swine production. *Anim. Biotechnol*. **13**: 7 – 27. doi: 10.1081/ABIO-120005767

Council of Canadian Academies (CCA), 2019. Forecasting the future of antimicrobial resistance (AMR) in Canada. Retrieved from: https://cca-reports.ca/forecasting-the-future-of-amr/ (accessed 01 April 2023).

Di Cesare, A., Eckert, E.M., Teruggi, A., Fontaneto, D., Bertoni, R., Callieri, C. and Corno, G. 2015. Constitutive presence of antibiotic resistance genes within the bacterial community of a large subalpine lake. *Molec. Ecol.* **24:** 3888 – 3900. doi: 10.1111/mec.13293

Dolliver, H. and Gupta, S. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *J. Environ. Qual.* **37**: 1227 – 1237. doi: 10.2134/jeq2007.0392

Environment Canada. 2023, January. Canadian climate normals 1981 – 2010 station data. Retrieved from: https://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?searchType=stnProv&lstProvince=MB&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=3675&dispBack=0 (accessed 04 April 2023)

Government of Canada. 2022, August. Overview of Canada's agriculture and agri-food sector. Retrieved from: https://agriculture.canada.ca/en/sector/overview (accessed 30 March 2023)

Government of Manitoba. 2022, April. *Sector Profile at a Glance: Hog Highlights*. Gov.mb. https://www.gov.mb.ca/agriculture/markets-and-statistics/livestock-statistics/pubs/hog-sector-profile.pdf

Government of Manitoba. 2023. *Rewards Versus Risk: Seeding Early in Manitoba*. Gov.mb. https://www.gov.mb.ca/agriculture/crops/crop-management/seeding-early-manitoba.html

Hanselman, T.A., Graetz, D.A. and Wilkie, A.C. 2003. Manure-borne estrogens as potential environmental contaminants: a review. *Environ. Sci. Technol.* **37**: 5471 – 5478. doi: 10.1021/es034410+

Havens, S.M., Hedman, C.J., Hemming, J.D.C., Mieritz, M.G. Shafer, M.M. and Schauer, J.J. 2020. Occurrence of estrogens, androgens and progestogens and estrogenic activity in surface water runoff from beef and dairy manure amended crop fields. *Sci. Total Environ.* **710**. doi: 10.1016/j.scitotenv.2019.136247

Hildebrand, C., Londry, K.L. and Farenhorst, A. 2006. Sorption and desorption of three endocrine disrupters in soil. *J. Environ. Sci. Health B.* **41**: 907 – 921. doi: 10.1080/03601230600806020

Hofmann, N.A. 2001. Geographical profile of manure production in Canada, Canadian 945 Electronic Library. Retrieved from: https://www150.statcan.gc.ca/n1/pub/16f0025x/manure-fumier/4058921-eng.htm on 14 Mar 2023

Holman, D.B. and Chénier, M.R. 2015. Antimicrobial use in swine production and its effect on the swine gut microbiota and antimicrobial resistance. *Can. J. Microbio.* **61:** 785 – 798.

Huijsmans, J.F.M., Hol, J.M.G. and Hendricks, M.M.W.B. 2001. Effect of application technique, manure characteristics, weather and field conditions on ammonia volatilization from manure applied to grassland. *Neth. J. Agric. Sci.* **49**: 323 – 342.

Khanal, S.K., Xie, B., Thompson, M.L., Sung, S., Ong, S. and Hans Van Leeuwen, J. 2006. Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. *Environ. Sci. Technol.* **40**: 6537 – 6546. doi: 10.1021/es0607739

Kim, K.R., Owens, G., Kwon, S.I., So, K.H., Lee, D.B. and Ok, Y.S. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water Air Soil Pollut*. **214**: 163 – 174.

Kuchta, S.L. Cessna, A.J., Elliot, J.A., Peru, K.M. and Headly, J.V. 2009. Transport of lincomycin to surface and ground water from manure-amended cropland. *J. Environ. Qual.* **38**: 1719 – 1727. doi: 10.2134/jeq2008.0365

Kumar, K., Gupta, S.C., Chander, Y. and Singh, A.K. 2005. Antibiotic use in agriculture and its impact on the terrestrial environment. *Adv. Agron*. doi: 10.1016/S0065-2113(05)87001-4

Lecomte, S., Habauzit, D., Charlier, T.D. and Pakdel, F. 2017. Emerging estrogen pollutants in the aquatic environment and breast cancer. *Genes*. **8**. doi: 10.3390/genes8090229

Liang, J. and Shang, Y. 2013. Estrogen and cancer. *Annu. Rev. Physiol.* **75:** 225 – 240. doi: 10.1146/annurev-physiol-030212-183708

Lin, K. and Gan, J. 2011. Sorption and degradation of waste-water associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere*. **83**: 240 – 246. doi: 10.1016/j.chemosphere.2010.12.083

Liu, J., Kleinman, P.J.A., Aronsson, H., Flaten, D., McDowell, R.W., Bechmann, M., Beegle, D.B., Robinson, T.P., Bryant, R.B., Liu, H., Sharpley, A.N. and Veith, T.L. 2018. A review of regulations and guidelines related to winter manure application. *Ambio*. **47:** 657 – 670. doi: 10.1007/s13280-018-1012-4

Liu, J., Baulch, H.M., Macrae, M.L., Wilson, H.F., Elliot, J.A., Bergstrom, L., Glenn, A.J. and Vadas, P.A. 2019a. Agricultural water quality in cold climates: processes, drivers, management options, and research needs. *J. Environ. Qual.* doi: 10.2134/jeq2019.05.0220

Liu, J., Elliot, J.A., Wilson, H.F. and Baulch, H.M. 2019b. Impacts of soil phosphorous drawdown on snowmelt and rainfall runoff water quality. *J. Environ. Qual.* doi: 10.2134/jeq2018.12.0437

Maguire, R.O., Kleinman, P.J.A., Dell, C.J., Beegle, D.B., Brandt, R.C., McGrath, J.M. and Ketterings, Q.M. 2011. Manure application technology in reduced tillage and forage systems: a review. *J. Environ. Qual*. **40**: 292 – 301. doi: 10.2134/jeq2009.0228

Odinga, E.S., Zhou, X., Mbao, E.O., Ali, Q., Waigi, M.G., Shiraku, M.L. and Ling, W. 2022. Distribution, ecological fate, and risks of steroid estrogens in environmental matrices. *Chemosphere*. **308**. doi: 10.1016/j.chemosphere.2022.136370

Ojoghoro, J.O., Scrimshaw, M.D. and Sumpter, J.P. 2021. Steroid hormones in the aquatic environment. *Environ. Sci. Technol.* **792**. doi: 10.1016/j.scitotenv.2021.148306

Prins, G.S. 2008. Endocrine disruptors and prostate cancer risk. *Endocr.-Rel. Cancer*. **15**: 649 – 656.

Prins, G.S., Hu, W., Shi, G., Hu, D., Majumdar, S., Li, G., Huang, K., Nelles, J.L., Ho, S., Walker, C.L., Kajdacsy-Balla, A., and van Breeman, R.B. 2014. Bisphenol a promotes human prostate stem-progenitor cell self-renewal and increases *in vivo* carcinogenesis in human prostate epithelium. *Endocrinology*. **155**: 805 – 817. doi: 10.1210/en.2013-1955

Raman, D.R., Williams, E.L., Layton, A.C., Burns, R.T., Easter, J.P., Daugherty, A.S., Mullen, M.D. and Sayler, G.S. 2004. Estrogen content of dairy and swine wastes. *Environ. Sci. Technol.* **38:** 3567- 3573. doi: 10.1021/es0353208

Sarmah, A.K., Meyer, M.T. and Boxall, A.B.A. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*. **65**:725 – 759.

Semple, K.T., Morriss, W.J. and Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *Eur. J. Soil Sci.* **54:** 809 – 818. doi: 10.1046/j.1351-0754.2003.0564.x

Song, W., Ding, Y., Chiou, C.T. and Li., H. 2010. Selected veterinary pharmaceuticals in agricultural water and soil from land application of animal manure. *J. Environ. Qual*. **39**: 1211 – 1217. doi: 10.2134/jeq2009.0090

Straub, J. O. 2016. Aquatic environmental risk assessment for human use of the old antibiotic sulfamethoxazole in Europe. *Environ. Toxicol. Chem*. **35**: 767 – 779.

Sun, W.L., Ni, J.R. and Liu, T.T. 2006. Effect of sediment humic substances on sorption of selected endocrine disruptors. *Water Air Soil Pollut.* **6**: 583 – 591. doi: 10.1007/s11267-006-9043-4

The Environment Act, Livestock Manure and Mortalities Management Regulation (1998, c. E125). Retrieved from the Government of Manitoba website: https://web2.gov.mb.ca/laws/regs/current/_pdf-regs.php?reg=42/98

The Prairie Province's Committee on Livestock Development and Manure Management. 2004. *Tri-Provincial Manure Application and Use Guidelines.* Gov.mb. https://www.gov.mb.ca/agriculture/crops/guides-and-publications/pubs/manure-application-and-use-guidelines.pdf

Wu, J., Wang, J., Li, W., Guo, S., Li, K., Xu, P., Ok, Y.S., Jones, D.L. and Zou, J. 2022. Antibiotics and antibiotic resistance genes in agricultural soils: a systematic analysis. *Crit. Rev. Environ. Sci. Technol.* doi: 10.1080/10643389.2022.2094693

Yue, Z., Zhang, J., Zhou, S., Ding, C., Wan, L., Liu, J., Chen, L. and Wang, X. 2021. Pollution characteristics of livestock faeces and the key driver of the spread of antibiotic resistance genes. *J. Hazard. Mater.* **409**. doi: 10.1016/j.jhazmat.2020.124957

Zhang, K., Zhang, Z., Hu, Z., Zeng, F. Chen, C., Yang, X. and Li, Y. 2020. Bacterial community composition and function succession under anaerobic conditions impacts the biodegradation of 17β-estradiol and its environmental risk. *Environ Poll*. doi: 10.1016/j.envpol.2020.115155

Chapter 2: The fate of 17β-estradiol in snowmelt from a field with a history of manure application: a laboratory simulation and field study

2.1 Abstract

Estrogens are naturally occurring biological compounds and can be detected in human and livestock waste. Liquid swine manure is widely used as fertilizer in the Canadian Prairies on agricultural fields. Studies have shown that most estrogens in the environment come from agricultural fields, resulting in a high risk of soil and freshwater contamination. In the Prairies, a majority of the annual runoff occurs during the brief snowmelt period, when soils are frozen. Controls on the transport of estrogens during this important hydrological period are not well understood but are critical to understanding the fate of estrogens. This study quantified the concentration and load of 17β-estradiol in snowmelt from an agricultural field with a long history of manure application under different manure management practices (i.e., no manure applied, manure applied on the sub-surface, and manure applied on the surface), using two components: a laboratory simulation with flooded intact soil cores and a field study during snowmelt. Concentrations of 17β-estradiol varied between the laboratory and the field, with higher concentrations measured in the laboratory simulation (mean laboratory pore water concentration = 1.65 ± 1.2 μg/L; mean laboratory flood water concentration = 0.488 ± 0.58 μg/L; and mean field snowmelt concentration = 0.0619 ± 0.048 μg/L). There were no significant differences among manure management practices. Laboratory pore water concentrations significantly increased over time, corresponding with changes in pH. In contrast, there was no significant change in the field snowmelt concentrations over time.

The mean cumulative load from the field study (6.91 ± 3.7 ng/m^2) approximates the magnitude of 17β-estradiol that could be mobilized from manured fields during snowmelt, and the load from plots with manure applied on the sub-surface was significantly larger than in other manure management practices. This study provides preliminary insights to improve current manure management practices in the Canadian Prairies.

2.2 Introduction

Estrogens are naturally occurring biological compounds that play an essential role in animal and human physiology. In recent years, studies have shown that estrogens can cause detrimental effects on humans and animals when exposed to an excess of estrogens (Liang and Shang, 2013; Arnold et al., 2014; Adeel et al., 2017; Lee Pow et al., 2017; Odinga et al., 2022). For example, the excess presence of natural and synthetic estrogens present in the environment has caused the feminization of male fish, resulting in low sperm count, and altering of the reproductive system (Arnold et al., 2014) or disrupting the formation of secondary sexual characteristics in fish (Lee Pow et al., 2017). In humans, increased exposure to estrogens has resulted in an increased formation of cancerous cells (Liang and Shang, 2013), including increased breast cancer risk in women (Moore et al., 2016). In agricultural systems, 17β-estradiol is a concern because it is an abundant contaminant in livestock manure (Raman et al., 2004) and is relatively persistent in soils (Colucci et al., 2001). While 17β-estradiol contamination can come from human waste (e.g., via the use of birth control; Adeel et al., 2017), the introduction of 17β-estradiol in the environment is dominated by agricultural practices, especially via the use of manure on agricultural fields (Combalbert and Hernandez-Raquet, 2010).

The concentrations and types of hormones of concern in agricultural areas are dependent on the dominant management practices in the region. In the province of Manitoba, Canada, swine production has dramatically increased in size and economic importance to an estimated 8.4 million pigs sold in 2021 (Government of Manitoba, 2022). As a result, the large production and use of manure on agricultural fields have caused a lot of interest in manure management to mitigate contaminant residues in soils and losses into waterbodies, resulting in harmful side effects. Current agricultural practices place an emphasis on the 4R of nutrient management (i.e., right source, right rate, right time, and right place), to improve the productivity and practicality of agricultural practices along with the efficiency and environmental protection (Johnston and Bruulsema, 2014). There are many ways that 4R can be implemented for a variety of nutrients (e.g., Government of Manitoba, 2007; Reid et al., 2019) and perhaps these recommendations and regulations could be applied in the context of organic contaminants.

The hydrological regime in the Canadian Prairies is dominated by spring snowmelt, and up to 80% of the annual runoff occurs during this time period and snowmelt can result in flooding (Liu et al., 2019). The prolonged snowmelt periods result in a) transport of nutrients and contaminants routed rapidly to streams via surface flow over frozen ground and b) a prolonged period of flooded soils (Meyer and Wania, 2008). There has been considerable work done on nutrient transport during the snowmelt period (e.g., Costa et al., 2018; Wilson et al., 2019) in which influxes of nutrients are released into the environment during the snowmelt period because of the high solubility in water and the dispersion of negatively charged soil particles increasing the mobilization (due to the repulsion) of small, negatively charged nutrients such as phosphorus and nitrate. Relatively less work has focused on organic contaminants (e.g., Dolliver and Gupta, 2008; Kuchta et al., 2009; Havens et al., 2020) and among these studies, two main findings emerged: 1) organic contaminants are detected in surface waters, and 2) similar to nutrients, elevated concentrations of organic contaminants are detected in surface water during the snowmelt period. However, the temporal changes in the organic contaminant concentrations and loadings to surface water during the snowmelt period are not well understood.

Therefore, to better understand the transport of 17β-estradiol in snowmelt from manure-amended agricultural fields into surface waters, this study aimed to quantify the concentration and load of 17β-estradiol in snowmelt from plots that have a history of manure application and freshly amended with different liquid swine manure application methods (i.e., no manure applied, manure applied on the surface, and manure applied in the sub-surface). The study's hypotheses are 1) the 17β-estradiol concentration and load in snowmelt will depend on the method of manure application, and 2) the concentration and load of 17β-estradiol will change over the snowmelt period.

2.3 Materials and Methods

The laboratory simulation and field study share similar methods; thus, Figure 2.1 is a flow chart that depicts the overall steps, including the shared and differing steps. In the fall of 2021, 12 plots were set up in an agricultural field in south-eastern Manitoba, Canada (49° 32′ 5″ N, 96° 51′5″ W), that has a history of manure application. The soil in this area is described as Osborne series and classified as Rego Humic Glysol. The soil texture is Clay, with a pH of 7.9 and organic matter content of 7.1 %. The plots were 3 m x 1 m in size with 0.6 m alleys between the plots. To minimize spatial variation, a randomized complete block design was used, with four replicate plots and three treatments applied within each block: liquid swine applied on the surface of the field (i.e., surface applied), liquid swine manure applied on the sub-surface of the field (i.e., 15 cm below the top of the soil), or no manure added (i.e., control; Figure 2.2). The manure (~2% solid content) was manually applied at an even distribution throughout the plot to ensure equal saturation for each plot at an application rate of 170 000 L/ha, following manure application rate practices from the producer.

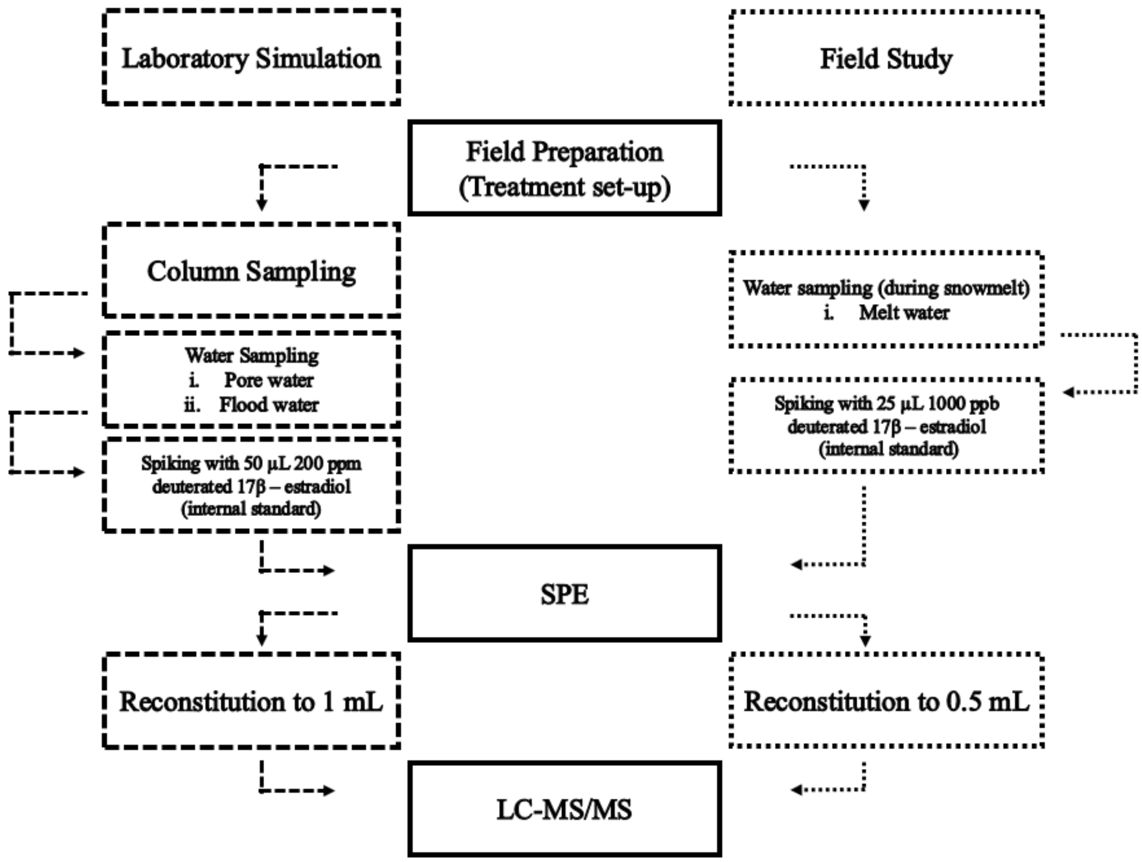


Figure 2.1: Flowchart of methods for the laboratory simulation and field study. The methods that are the same for the laboratory simulation and the field study are inside the solid box.

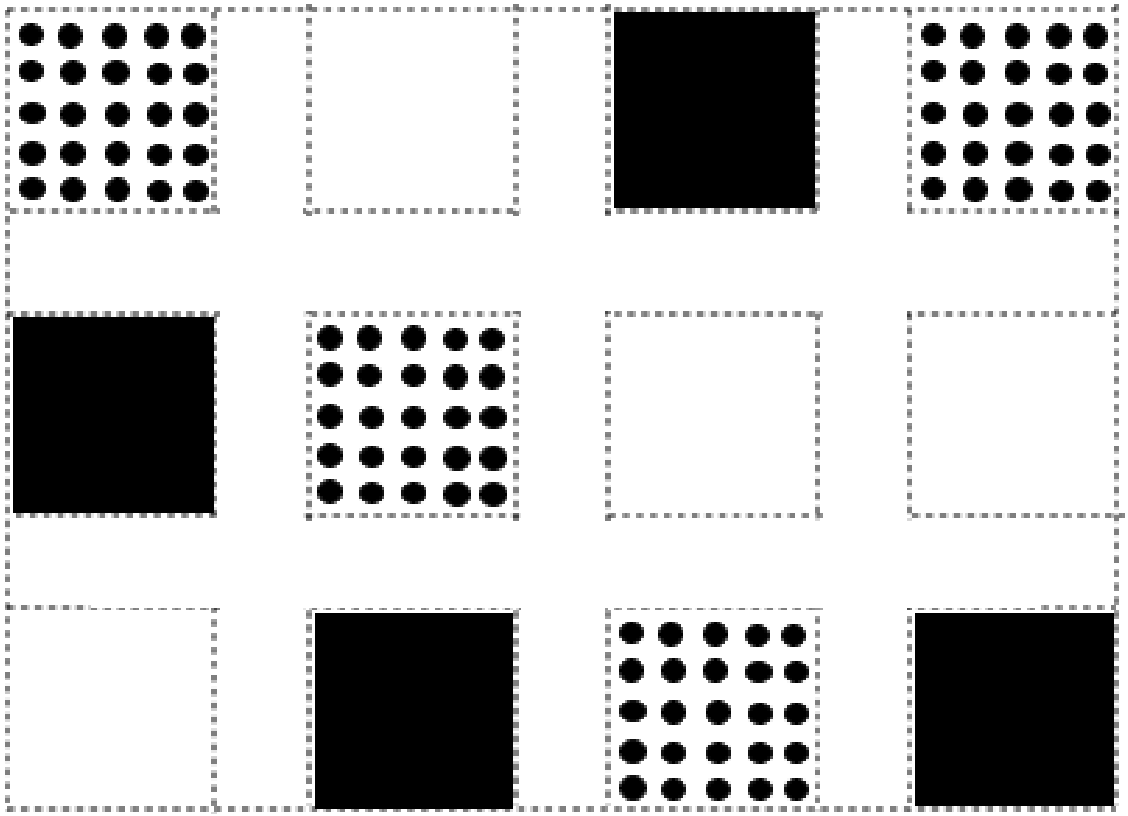


Figure 2.2: Schematic of the plot design in the field with the three treatments: sub-surface application (circles), surface application (black), no manure applied (white). Manure was applied at a rate of 170 000 L/ha on the surface and 15 cm into the sub-surface.

2.3.1 Laboratory simulation of snowmelt

2.3.1.1 Column sampling and preparation

Single intact soil cores (15 cm) were collected using 30 cm x 9.9 cm PVC pipes from within the boundaries of each plot and secured the bottom of the pipes with a lid. The columns were then transported to the University of Winnipeg immediately after collection and placed in a cooler at 4°C for the remainder of sampling to simulate snowmelt temperature conditions.

Two 0.15 µm Rhizon MOM samplers (Rhizosphere Research Products) were placed in the columns approximately 45 ° away from each other, 5 cm below the top of the soil through pre-drilled holes in the PVC pipes and were sealed using silicone (LePage Construction Adhesive) to prevent leakages. The base of the columns was also sealed using caps and silicone. The columns were kept at room temperature for 24 hrs to dry and cure the silicone, then promptly returned the columns into the cooler set at 4°C.

2.3.1.2 Water sampling

To simulate snowmelt flooding, approximately 700 mL Milli-Q water (Synergy® Water Purification System, Millipore Sigma; 18 MΩ cm) was added up to get a 10 cm water head above the soil column, which was re-filled after each sampling day to maintain a water head of 10 cm. The overlaying water on the soil surface (hereafter “flood water”) and the water from the soil pore (hereafter “pore water”) were collected every 3rd day, starting on the day of flooding (day 0) up to day 21 for a total of 7 sampling days. Approximately 30 mL of pore water was collected by pulling the syringe attached to the Rhizon MOM sampler about 16 hours before the sampling day to account for the slow flow rate. Flood water (30 mL) was collected with syringes and then filtered using a 0.45 μm syringe filters. The pH was measured for each water sample (Fisherbrand Accumet AB15 pH meter, Thermo Fisher Scientific). The water samples were kept in a cooler at 4°C until analysis.

2.3.2 Field study

Runoff boxes made with high-density polyethylene puckboards supported with wooden frames (1.2 m x 0.9 m x 0.6 m) were placed 15 cm into the soil surface in the fall of 2021 to collect the snow over the winter for each plot as described in Figure 2.2. From March 19 – April 5, 2022, snowmelt samples were collected every day (between 12 pm – 2 pm) when the temperature was above 0 °C and liquid snowmelt was present in the plots. The average maximum temperature on sampling days was 4.2 °C, and the total precipitation over this period was 10.3 mm, including rain on days 3 and 7. The snowmelt was collected using a pump (Mastercraft 12V Rubber Impeller Transfer Pump, LEO Group Pump, Co., LTD.) attached to a hose with one end covered with a screen filter to avoid collecting large particles. The total snowmelt volume from each plot was recorded, and a 250 mL subsample from each plot was collected. Over the period of the snowmelt, there were 10 days when liquid snowmelt was available for collection. The samples were stored in a cooler and immediately transported to the University of Winnipeg, where the samples were immediately filtered with 0.45 μm filter papers (Metricel® Membrane Filter, 47 mm, Pall Corporation) using a vacuum filtration system (Millipore Sigma, Sigma-Aldrich Co. LLC.). Samples over two sampling days were combined to ensure there was a sufficient mass of 17β-estradiol present for analysis.

2.3.3 Solid phase extraction and reconstitution

The methods for sample preparation and analysis were based on the methods from Amarakoon et al. (2014) and adapted to fit this research. Each pore water and flood water sample from the laboratory simulation was spiked with 50 µL of 200 ppm deuterated 17β-estradiol (Sigma-Aldrich Co. LLC.) as the internal standard to measure analytical method efficiency. Similarly, all the field study samples were spiked with 25 µL of 1000 ppb deuterated 17β-estradiol (Sigma-Aldrich Co. LLC.). All water samples were pre-concentrated using solid-phase extraction (SPE) using SPE Restek Hydrophilic Lipophilic Balance (HLB) cartridges (6 mg of sorbent, 3 mL; Oasis Waters). The HLB cartridges were conditioned with 3 ml MeOH followed by 3 mL of Milli-Q, and the samples were passed through a vacuum at a flow rate of 1 mL/ min. Once the samples were passed through, the cartridges were rinsed with 3 mL Milli-Q to remove excess salts and allowed to dry for 1 min under vacuum. The samples were stored at -20°C until they were transferred, 24 hr before elution, to a refrigerator at 4°C.

The samples were eluted using 3 mL of MeOH, allowing gravity to pull the samples through the HLB cartridges. The eluates were dried to completeness using a 45°C water bath and a nitrogen evaporator (OA-SYS Heating system and N-Evap 111, Organomation Associates, Inc.) using a gentle stream of Ultrapure N_2 (Praxiar Canada Inc.). For reconstitution, 1 mL of 50/50 (v/v) MeOH:Milli-Q was added to the laboratory simulation samples and used a vortex to reconstitute the samples. The field samples were reconstituted with 0.5 mL of 50/50 (v/v) MeOH:Milli-Q. All samples were syringe-filtered into 2 mL amber glass vials (Chromatographic Specialties Inc.). The samples were stored at -20 °C until analysis (approximately 1.5 weeks) to prevent possible decomposition during this period.

2.3.4 Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

A methanol stock solution of 17β-estradiol was made and used to create a calibration curve. The methanol stock solution was diluted to 0.1, 1, 5, 10, 50, and 100 µg/L in 50/50 (v/v) MeOH:Milli-Q as the calibration curve for quantitative assessments of the data.

Chromatography was performed with an Agilent 1260 Infinity II UHPLC (Agilent Technologies, Inc.). Separation was performed using an Agilent Eclipse Plus C_{18} column (2.1 mm x 50 mm, 1.8 µm dp) coupled to an Agilent Eclipse Plus C_{18} guard column (2.1 mm x 5 mm) at 42 ºC at 0.3 mL/min. The optimization injection volume was 2 µL and the injection volume during analysis was 10 µL. Mobile phase A was Milli-Q water, and mobile phase B was acetonitrile. Gradient elution was performed as follows: 0 - 2.00 min linear ramp from 5% to 95% B, 2.01 - 4.00 hold at 95% B, 4.01 - 5.00 linear ramp from 95% to 5% B, followed by re-equilibration from 5.01 - 8.00 min at 5% B. The columns were flushed with 5% B for 20 min upon completion of all analytical runs, followed by 25 min of 100% B for column storage.

Qualitative assessment and quantification were performed through multiple reaction monitoring on a mass spectrometer (Agilent 6470 triple quadrupole, Agilent Technologies, Inc.) in negative electrospray ionization mode, a capillary voltage of 4000 V, and a source temperature of 300°C. Nitrogen was used for desolvation, drying gas, and nebulization: desolvation and drying gas were set at a flow rate of 11 L/min, and nebulization at 15 psi. The collision gas was set to a flow rate of 16.8 L/min using ultrapure nitrogen. The MS1 and MS2 heaters were set at 100°C. The method limit of detection was 0.0274 µg/L, and the method limit of quantification was 0.0457 µg/L. The percent recovery was 101.2 ± 14.7 %. Linearity for 17β-estradiol throughout the 7-point calibration range was $R^2 > 0.99$.

2.3.5 Statistical analysis

The laboratory simulation and snowmelt field data were analyzed separately. Furthermore, the pore water and flood water from the simulation study were also analyzed separately. However, the following calculations and analyses were performed for both the laboratory simulation and snowmelt field data. A two-sample t-test was performed to test the difference between the pore water and flood water 17β-estradiol concentration. Repeated measures ANOVA was run to test the effects of liquid swine manure application method and sampling day on the concentrations of 17β-estradiol at a significance level of 0.05. The model had manure application method and sampling day as the fixed effects, with the plot (i.e., replicate) as the random effect, and the day as the repeating effect. The concentration was log transformed to meet normality assumptions for analysis.

The load was calculated by multiplying the 17β-estradiol concentration of the sample with the corresponding snowmelt volume of each plot. The cumulative load for each plot was then calculated by taking the sum of the load measured for each sampling day, summarized as:

$$Cumulative\ Load_i = \sum_{j=1}^{n}(VC)_j \qquad (1),$$

where V is the snowmelt volume, C is the concentration of 17β-estradiol, *i* is the plot, and *j* is the sampling day with *n* number of days. ANOVA was used to test the effects of the manure application method and sampling day on the load of 17β-estradiol. The data were log transformed to meet the normality assumptions for ANOVA. One-way ANOVA was used to test the effects of the manure application method on the final cumulative load of 17β-estradiol. The data was also log transformed to meet normality assumptions.

The weighted 17β-estradiol concentration $(C_W)_i$ from each plot of the field study was calculated by:

$$(C_{\mathrm{W}})_i = \frac{(cumulative\ load)_i}{\left(\sum_{j=1}^{n} V_j\right)_i} \quad (2),$$

where $(cumulative\ load)_i$ is the calculated cumulative load for each plot (equation 1) and $\left(\sum_{j=1}^{n} V_j\right)_i$ is the sum of the snowmelt volume over the entire sampling period (*n*) from each individual plot (*i*), where *j* is the sampling day. The weighted concentration was calculated to account for any effect of the snowmelt volume of each individual plot due to the high variability of the snowmelt volume. A Pearson correlation test was used to determine if there was a correlation between the weighted 17β-estradiol concentration and the total volume of water collected from each plot in order to eliminate the possibility of a dilution effect occurring on the 17β-estradiol concentration. One-way ANOVA was also used to test for the effects of the manure application methods on the weighted concentration of 17β-estradiol for the final day of sampling.

To account for any changes to pore water and flood water pH in the laboratory study, repeated measures analysis was used to test the effects of liquid swine manure application methods and sampling date on the pore water and flood water pH. The manure application method and sampling date are the fixed effects, the replicates are the random factor, and the day is the repeating factory. To test if there is a relationship between 17β-estradiol concentration and pH, a Pearson correlation test was performed. R (version 4.2.2, R Development Core Team, 2022) was used for all statistical analyses.

2.4 Results

2.4.1 Laboratory simulation of snowmelt

There was a significant difference between the 17β-estradiol concentrations in pore water compared to flood water ($t_{137;\ 0.05} = 7.294$, $p < 0.0001$; Figure 2.3). As such, further analysis of pore water and flood water was treated separately in subsequent analysis.

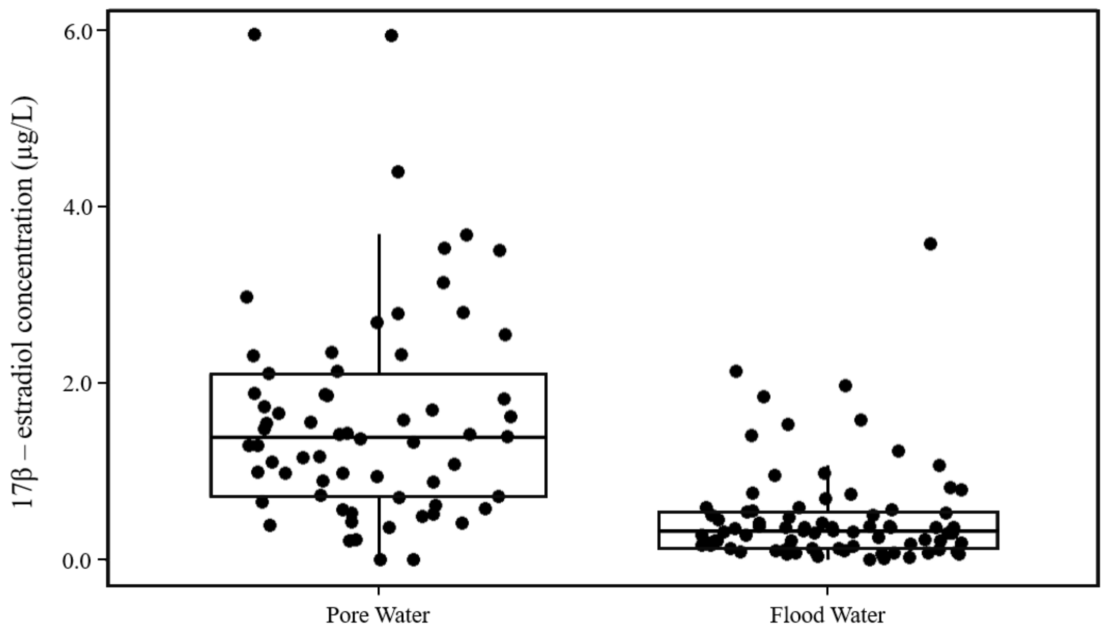


Figure 2.3: The concentrations of 17β-estradiol (µg/L) in pore water and flood water across the liquid swine manure application methods and over the entire sampling period.

The mean concentration of 17β-estradiol in pore water was 1.647 ± 1.22 μg/L. There was no significant effect of liquid swine manure application method on the 17β-estradiol concentrations in pore water ($F_{2;\,0.05}$ = 0.2752, *p* = 0.765, Table 2.1), but there was a significant difference in the 17β-estradiol concentrations over time ($F_{5;\,0.05}$ = 2.776, *p* = 0.0318, Table 2.1); the mean concentration generally increased over time, and day 21 had a significantly higher concentration compared with day 1. In the flood water, the mean concentration of 17β-estradiol was 0.488 ± 0.58 μg/L. Similar to pore water, there was no significant effect of manure application method on the 17β-estradiol concentration ($F_{2;\,0.05}$ = 2.581, *p* = 0.130, Table 2.1), but there was a significant difference in 17β-estradiol concentrations over time ($F_{6;\,0.05}$ = 4.150, *p* = 0.0020, Table 2.1). The concentrations increased to a maximum on day 10, and then decreased to a minimum on day 21, although the only significant difference in concentrations was between day 10 and day 21.

Table 2.1: The least-square means and standard error of the 17β-estradiol concentrations (µg/L) and pH in pore water and flood water for the treatments (i.e., control, sub-surface applied, and surface applied liquid swine manure (LSM)) and the sampling days (i.e., 0, 3, 7, 10, 14, 17, and 21) for the laboratory simulation of snowmelt. Significant results are bolded.

Treatment	**Concentration (µg/L)**		**pH**	
	Pore Water	**Flood Water**	**Pore Water**	**Flood Water**
Application method				
Control	1.21 ± 0.29	0.219 ± 0.071	7.74 ± 0.072	7.61 ± 0.14
Sub-Surface Applied LSM	1.06 ± 0.25	0.507 ± 0.16	7.64 ± 0.071	7.46 ± 0.14
Surface Applied LSM	1.35 ± 0.33	0.195 ± 0.064	7.58 ± 0.073	7.46 ± 0.14
Sampling day				
0	0.67 ± 0.23 **b**	0.190 ± 0.076 ab	7.37 ± 0.081 **b**	7.17 ± 0.11 **b**
3	1.09 ± 0.24 ab	0.327 ± 0.091 ab	7.75 ± 0.059 ab	7.96 ± 0.089 **a**
7	1.09 ± 0.24 ab	0.270 ± 0.081 ab	7.59 ± 0.059 ab	7.39 ± 0.091 ab
10	1.18 ± 0.25 ab	0.573 ± 0.16 **a**	7.59 ± 0.057 ab	7.41 ± 0.089 ab
14	1.63 ± 0.34 ab	0.416 ± 0.12 ab	7.86 ± 0.057 **a**	7.58 ± 0.089 ab
17	-	0.230 ± 0.064 ab	-	7.43 ± 0.089 ab
21	1.98 ± 0.42 **a**	0.141 ± 0.039 **b**	7.78 ± 0.057 ab	7.62 ± 0.089 ab
	p-value			
Application method	0.7656	0.13008	0.321	0.662
Sampling day	**0.0318**	**0.00204**	< **0.0001**	< **0.0001**
Application method: Sampling day	0.3502	0.40995	0.532	0.988

The mean cumulative load of 17β-estradiol was 0.154 ± 0.0032 mg/m^2 for the final day of 21-day snowmelt simulation period. There was no significant difference in 17β-estradiol concentrations based on the liquid swine manure application method ($F_{2;\,0.05} = 0.013$, $p = 0.987$).

There was a change in pH over time for pore water ($F_{5;\,0.05} = 21.16$, $p < 0.0001$; Table 2.1) and flood water ($F_{6;\,0.05} = 16.68$, $p < 0.0001$; Table 2.1), but there was no significant effect of liquid swine manure application method on the pH for pore water ($F_{2;\,0.05} = 1.289$, $p = 0.3217$; Table 2.1) and flood water ($F_{2;\,0.05} = 0.4643$, $p = 0.6429$; Table 2.1). There was a positive linear relationship between pH and 17β-estradiol concentration in pore water ($r_{60;\,0.05} = 0.5777$, $p < 0.0001$; Figure 2.4). In contrast, there was no relationship between pH and 17β-estradiol concentration in flood water ($r_{74;\,0.05} = -0.1957$, $p = 0.0924$; Figure 2.4).

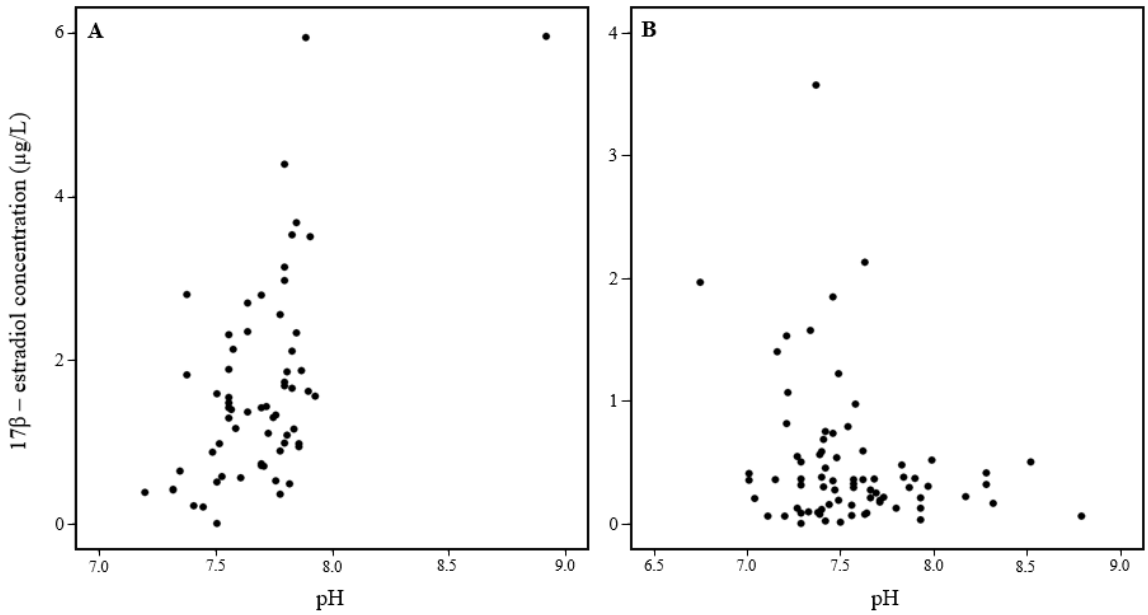


Figure 2.4: Scatterplot of 17β-estradiol concentrations (µg/L) in (A) pore water ($r_{60;\,0.05}$ = 0.5777, $p < 0.0001$) and (B) flood water ($r_{73;\,0.05}$ = -0.1957, $p = 0.0924$) compared to pH in a laboratory simulation.

2.4.2 Field study

The mean concentration of 17β-estradiol over the entire sampling period among the treatments was 0.0619 ± 0.048 μg/L and did not vary among the treatments (i.e., liquid swine manure application method; $F_{2;\ 0.05} = 0.791$, $p = 0.485$; Figure 2.5a) nor was there a significant difference in the concentrations among sampling days ($F_{4;\ 0.05} = 1.93$, $p = 0.131$; Figure 2.5b). The maximum concentration of 17β-estradiol observed was 0.191 μg/L.

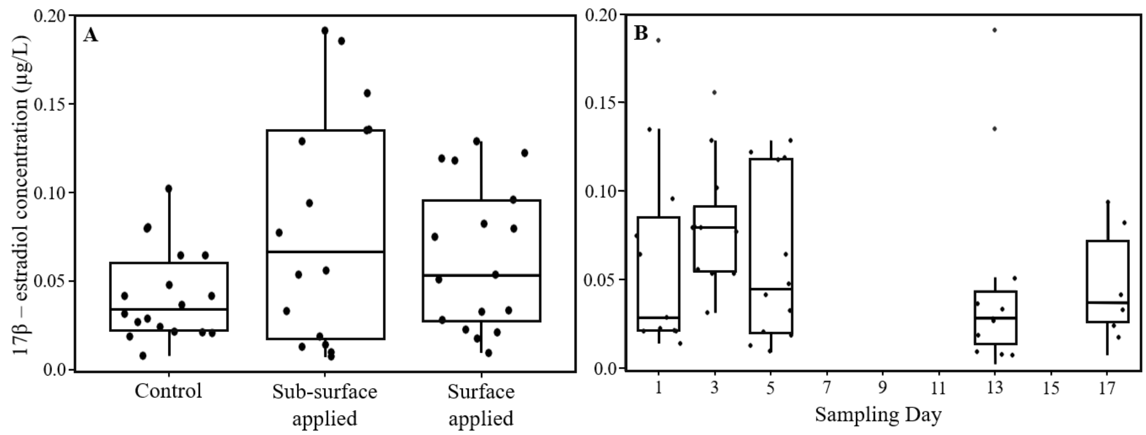


Figure 2.5: Boxplot of the concentrations (μg/L) of 17β-estradiol dissolved in snowmelt collected from field plots manured in the fall of 2021 among A) three treatments (i.e., control, sub-surface applied, and surface applied liquid swine manure) and B) over the sampling period collected from March to April 2022.

There was a steady increase in the load over time ($F_{4;0.05} = 12.29$, $p < 0.0001$), although the magnitude of the load introduced is not as large in the later dates. There is no significant relationship between the weighted 17β-estradiol concentration and the snowmelt volumes ($r_{9;0.05} = -0.0764$, $p = 0.8233$), nor was there a significant difference in the weighted concentration of 17β-estradiol ($F_{2;0.05} = 1.32$, $p = 0.319$; Figure 2.6a). The mean total cumulative load of 17β-estradiol was 6.91 ± 3.7 ng/m^2 for the snowmelt period, although the range was large [1.35 ng/m^2 – 14.9 ng/m^2], and there was a significant difference between 17β-estradiol cumulative load collected from plots with different liquid swine manure application methods ($F_{2; 0.05} = 6.35$, $p = 0.00304$; Figure 2.6b). Manure applied on the sub-surface of the plots showed a higher cumulative load compared to the plots without manure or manure applied on the surface.

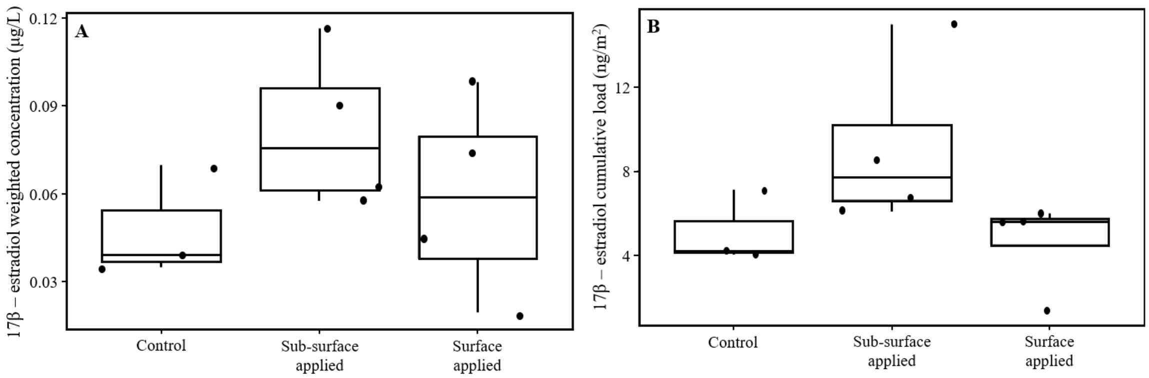


Figure 2.6: Boxplot of A) weighted 17β-estradiol concentration (µg/L) and B) 17β-estradiol cumulative load (ng/m^2) dissolved in snowmelt collected in March – April 2022, from field plots manured in the fall of 2021 with three different methods: no liquid swine manure applied (control), in the sub-surface, and on the surface.

2.5 Discussion

2.5.1 Transport and fate of 17β-estradiol

This study aimed to quantify the concentration and load of 17β-estradiol dissolved in snowmelt from a laboratory simulation and a field study. There were detectable concentrations of 17β-estradiol present in all treatments for the flood water and pore water in the laboratory simulation, as well as in the snowmelt collected from the field study. However, there was no significant difference between manure-applied treatments and no manure-applied control on the 17β-estradiol concentrations, suggesting that the study site had ample reservoirs of 17β-estradiol. The soil in the region that the snowmelt samples were taken from in this study belongs to the Osborne series and is classified as Rego Humic Gleysol (MAFRI, 2010), which is characterized by high clay and humic content. There is evidence that 17β-estradiol is sorbed onto soils that have high clay and humic materials present in the soil (Hildebrand et al., 2006; Sun et al., 2006) because of the strong π-π interactions between the phenolic rings on the 17β-estradiol with the organic matter in the soil (Yamamoto et al., 2003). Furthermore, 17β-estradiol has a relatively high K_d and K_{oc}, resulting in a higher affinity for the soil colloids compared to the aqueous phase due to its hydrophobicity (Bavumiragira et al., 2022). The reservoir of 17β-estradiol is likely from the history of manure application in this field, and therefore the fresh manure application during the study year did not change the background concentrations. However, with an estimated 21 billion kg of liquid swine manure produced annually in Manitoba (Hofmann, 2001), there is a large risk for 17β-estradiol transport off-site from manured agricultural lands.

The presence of 17β-estradiol in both the pore water and flood water indicates the possibility of groundwater and surface water contamination, respectively. It is especially important to focus on the 17β-estradiol present in the pore water because this laboratory study showed that the average 17β-estradiol concentration found in pore water was nearly 110% greater than the average 17β-estradiol concentration in flood water during the lab simulation. Contaminants that are sorbed onto the soil colloidal fractions have been shown to be released into the pore water first, followed by the diffusion of contaminants into the flood water (Christou et al., 2017), resulting in the large disparity between the pore water and flood water concentrations. Furthermore, the 17β-estradiol concentration found in pore water increased significantly over time, with the highest concentration measured on the final day of sampling. Hydrophobic contaminants are more likely to be released in bulk at the latter end of the snowmelt period due to increased snow density and improved capacity for particles of contaminants passing through the snowpack (i.e., filtering capacity) preventing the elution of hydrophobic contaminants into meltwater (Meyer and Wania, 2008), possibly explaining the peak of 17β-estradiol concentration in the pore water observed on the final day of sampling.

Another possible explanation for the mobilization of 17β-estradiol into surface waters can be explained through the water pH. The laboratory simulation showed a slight alkalinization (pH increased by 0.41) of the pore water over the sampling period. Contrary to the expected acidification of alkaline soils in flooded conditions, others also observed an increase in pH in manured alkaline soils (Amarawansha et al., 2015). The laboratory simulation suggests that changes in the soil water pH over the course of snowmelt flooding can alter the chemistry of the subsurface environment and an increase in pH increases the concentration of 17β-estradiol released into the environment. Under a laboratory experiment measuring the solubility of 17β-estradiol in acidic, neutral, and alkaline solutions (pH was 4, 7, and 10, respectively), Shareef et al. (2006) showed that 17β-estradiol solubility increased as pH increased. The pK_a of 17β-estradiol is about 10 (Prater et al., 2016; Adeel et al., 2017; Dai et al., 2021), and as the pH approaches 10, the H found on the phenolic group of 17β-estradiol will gradually start to dissociate (Tong et al., 2019; Dai et al., 2021), explaining the solubility of 17β-estradiol in more alkaline conditions. However, the pH in this study ranged between 7 – 8, so the pH alone may not be enough to explain the mobilization of 17β-estradiol from the soil particles into the aqueous phase. 17β-estradiol has been observed to form complexes with dissolved organic matter in humic soils, which increases its susceptibility to being desorbed from soils (Durán-Álvarez et al., 2014). The desorption of 17β-estradiol may also be a result of the weaker hydrophobic interactions between 17β-estradiol and the soil dispersion with the introduction of water into the soils as the water forms hydrogen bonds with the soil (Van Emmerick et al., 2003).

The lab simulation was meant to be used as a predictor of the transport mechanisms occurring in the field, and the two components were fundamentally different in two ways. The first difference is that in the field study, the soil was frozen at the start of the snowmelt period and gradually thawed over time (whereas the soil in the lab simulation remained at a constant temperature). This resulted in less interaction between the snowmelt and soil particles in the field, which is reflected in the lower 17β-estradiol concentrations measured in the field snowmelt. The second difference is that pore water measurements were not easily accessible in the field study; thus, there are no measurements from the field study preventing the theorization of mechanisms possibly resulting in the release of 17β-estradiol into the snowmelt from the soil. Therefore, this study combined laboratory and field techniques, the laboratory simulation of snowmelt to closely study the 17β-estradiol in both pore water and flood water to potentially identify mechanisms that can contribute to the release of 17β-estradiol from the soil colloidal fraction. The field study allowed for the assessment of cumulative loads of 17β-estradiol in real-world conditions, which could not be performed in the laboratory simulation due to study conditions. The assessment of cumulative loads is critical, especially in the context of offloading from agricultural fields into surface waters.

Contrary to what was observed in the laboratory simulation, there was no observable trend in the 17β-estradiol concentration over time in the field study, perhaps due to the high degree of day-to-day variability of snowmelt volume among samples. Despite not seeing an increase in 17β-estradiol concentrations over time, there is an increase in cumulative load over time as the snowmelt progresses. Maximum volumes were observed on day 3 (up to 40 L), then decreasing to as low as 8.8 L by the final sampling day. Thus, there is a sharp increase in the cumulative load on the third day, followed by an increase in load over time, although at a slower rate. This suggests that snowmelt volume is what is driving the pattern rather than the 17β-estradiol concentration. While the soil was likely to be partially frozen on days 3 to 5, there was sufficient interaction between the soil and the snowmelt, mobilizing some of the 17β-estradiol. Since snowmelt is driving the load of 17β-estradiol introduced in surface water, management practices that retain water on the landscape could be more beneficial than changing the manure application technique (especially in long-term manured fields).

There was a difference in cumulative load (measured on day 17) among application methods, such that plots that had liquid swine manure applied on the sub-surface had a significantly larger cumulative load than in plots that had manure applied on the surface or no manure applied at all, although there were no significant differences in either cumulative snowmelt volume, unweighted 17β-estradiol concentration, or volume weighted 17β-estradiol concentration among treatments. While the differences in concentrations for the field study were not significant, the median weighted and unweighted concentrations were higher in snowmelt collected from plots with manure applied on the sub-surface, which is consistent with the 17β-estradiol concentrations measured in the flood water from the laboratory simulation. Estrogen degradation was reported to be slower under cold, anaerobic conditions (Colucci and Topp, 2001; Ying and Kookana, 2005; Zhang et al., 2020), possibly due to delayed metabolic functions of bacterial species that break down estrogenic compounds (Zhang et al., 2020). Thus, the higher load of 17β-estradiol in the snowmelt collected from plots with manure applied in the sub-surface could be due to the preservation of 17β-estradiol in the sub-surface from the manure application in the fall. The sub-surface application of manure might increase the persistence of 17β-estradiol from manured agricultural fields and therefore will need to be assessed in future studies to develop manure management strategies that will also reduce offsite transport potential of manure-borne organic contaminants like 17β-estradiol.

2.5.2 Implications

Many studies have shown that relatively low concentrations of 17β-estradiol can result in devastating side effects on the health of aquatic life and humans. For example, clearhead ice fish embryos (*Protosalanx hylocranius*) exposed to 17β-estradiol concentrations as low as 0.05 ng/L ~ 0.2 ng/L showed congenital deformations and a delay in hatching (Odinga et al., 2022). Other studies have shown the introduction of 17β-estradiol into aquatic ecosystems has resulted in the feminization of male fish (Arnold et al., 2014) and reduced reproductive capacity and hampered the development of secondary sexual characteristics of Japanese medaka (*Oryzias latipes*; Lee Pow et al., 2017). In humans, exposure to non-endogenous estrogens has been linked to the creation of cancerous cells (Liang and Shang, 2013). There is evidence that range of concentrations (0.1 – 45 500 ng/L) of 17β-estradiol observed in surface waters globally have impacts on humans and aquatic wildlife (Odinga et al., 2022). This study shows that there is a greater possibility for the transport of 17β-estradiol into surface waters as a result of snowmelt runoff ending up in surface waters. Thus, mitigating the daily release of 17β-estradiol during snowmelt and the loading to surface waters over time will help reduce the direct impact 17β-estradiol has on animals and humans, especially in regions that have large animal production and are heavily manured.

Typically, manure management practices, such as incorporating surface-applied manure shortly after application (Amarakoon et al., 2014), is the first step taken to reduce the introduction of organic contaminants into the environment. However, as observed here, there was no significant difference in the 17β-estradiol concentration dissolved in snowmelt among the different manure application methods. Sub-surface application or incorporation may also not be enough as a recommendation as this study showed sub-surface application increased the cumulative load of 17β-estradiol introduced into the environment. Thus, in tandem with manure application methods, other management practices should also be considered to mitigate losses of contaminants into water. For example, snowmelt volume is one of the biggest drivers of 17β-estradiol loads into the environment, but snowmelt volume is highly variable and is primarily controlled by climactic factors, rather than specific management practices (Meyer and Wania, 2008; Pomeroy et al., 2010). Recommendations should consider regulating the drainage of snowmelt to mitigate the losses of 17β-estradiol into surface water and groundwater. Some suggestions have been to increase the surface roughness (i.e., modifying the topsoil through tillage to reduce the movement of particles in the soil) to reduce the runoff volume of snowmelt (Dolliver and Gupta, 2008; Wilson et al., 2019). Additionally, given the trend towards higher 17β-estradiol concentrations in the sub-surface treatment, the implications of tile drainage (i.e., increasing the mobility of shallow groundwater which may have higher levels of contamination) should be carefully considered in manured fields. A better understanding of transport mechanisms occurring during snowmelt (a critical hydrological period) of organic contaminants like 17β-estradiol would be important in suggesting better recommendations on mitigating contaminant losses into surface and pore waters.

2.6 Conclusion

This study had detectable concentrations of 17β-estradiol in all manure application methods (i.e., surface, sub-surface, or no manure application) in the field and lab simulation. However, there were no differences in the 17β-estradiol concentrations in snowmelt collected from the different plots, suggesting that an agricultural field that is heavily manured has the potential for 17β-estradiol transport. This lab simulation and field study provides a preliminary look into possible mechanisms responsible for the transport of 17β-estradiol into surface waters. The lab simulation showed an increase in pH during the flooding which may be related to the desorption 17β-estradiol from the soil into the pore water, mobilizing 17β-estradiol into the aqueous phase. This study also shows that snowmelt volume drives the 17β-estradiol load in environmental conditions. In the plots with manure applied on the sub-surface, there was a significantly larger load of 17β-estradiol released, likely due to the delayed degradation of estrogenic compounds under anaerobic conditions. Therefore, management practices should focus on retaining snowmelt on the landscape and using appropriate manure application techniques, especially on long-term manured fields. The 17β-estradiol in surface waters from agricultural runoff has been shown to detrimentally affect human and aquatic species' physiology. The results from this study will help develop manure management practices by providing insights on 17β-estradiol concentrations in snowmelt in the Canadian Prairies, thus mitigating the losses of 17β-estradiol into surface and groundwaters. This study's data will help to develop/ improve predictive models of contaminant transport under prairie conditions.

2.7 References

Adeel, M., Song, X., Wang, Y., Francis, D. and Yang., Y. 2017. Environmental impact of estrogens on human, animal, and plant life: a critical review. *Environ. Int.* **99**: 107 – 119. doi: 10.1016/j.envint.2016.12.010

Amarakoon, I.D., Zvomuya, F., Cessna, A.J., Degenhardt, D., Larney, F.J. and McAllister, T.A. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosine from surface-applied and soil-incorporated beef cattle feedlot manure. *J. Environ. Qual.* **43**: 547 – 557.

Amarawansha, G., Kumaragamage, D., Flaten, D., Zvomuya, F. and Tenuta, M. 2015. Phosphorus mobilization from manure-amended and unamended alkaline soils to overlaying water during simulated flooding. *J. Environ. Qual.* **44**: 1252 – 1262. doi: 10.2134/jeq2014.10.0457

Arnold, K.E., Brown, A.R., Ankley, G.T. and Sumpter, J.P. 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. *Philos. Trans. R. Soc. B Biol. Sci.* **369**. doi: 10.1098/rstb.2013.0569

Bavumiragira, J.P., Ge, J. and Yin, H. 2022. Fate and transport of pharmaceuticals in water systems: a process review. *Sci. Total Environ.* **823.** doi: 10.1016/j.scitotenv.2022.153635

Christou, A., Agüera, A., Bayona, J.M., Cytryn, E., Fotopoulos, V., Lambropoulou, D., Manaia, C.M., Michael, C., Revitt, M., Schröeder, P. and Fatta-Kassinos, D. 2017. The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: the knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes – a review. *Water Res*. **123**: 448 – 467. doi:10.1016/j.watres.2017.07.004

Colucci, M.S., Bork, H. and Topp, E. 2001. Persistence of estrogenic hormones in agricultural soils: I.17β-estradiol and estrone. *J. Environ. Qual.* **30**: 2070 – 2076. doi: 10.2134/jeq2001.2070

Colucci, M.S. and Topp, E. 2001. Persistence of estrogenic hormones in agricultural soils: II. 17α-ethynylestradiol. *J. Environ. Qual*. **30**: 2077 – 2080. doi: 10.2134/jeq2001.2077

Combalbert, S. and Hernandez-Raquet, G. 2010. Occurrence, fate, and biodegradation of estrogens in sewage and manure. *Appl. Microbiol. Biotechnol.* **86**: 1671 – 1692. doi: 10.1007/s00253-010-2547-x

Costa, D., Pomeroy, J., and Wheater, H. 2018. A numerical model for the simulation of snowpack solute dynamics to capture runoff ionic pulses during snowmelt: the PULSE model. *Adv. Water Resour.* **122**: 37 – 48.

Dai, X., Yang, X., Xie, B., Jiao, J., Jiang, X., Chen, C., Chang, Z., He, Z., Lin, H., Chen, W. and Li, Y. 2021. Sorption and desorption of sex hormones in soil- and sediment-water systems: A review. *Soil Ecol. Lett.* doi: 10.1007/s42832-020-0074-y

Dolliver, H. and Gupta, S. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *J. Environ. Qual.* **37**: 1227 – 1237. doi: 10.2134/jeq2007.0392

Durán-Álvarez, J.C., Prado, B., Ferroud, A., Juayerk, N. and Jimenez-Cisneros, B. 2014. Sorption, desorption and displacement of ibuprofen, estrone, and 17β estradiol in wastewater irrigated and rainfed agricultural soils. **473 – 474:** 189 – 198. doi: 10.1016/j.scitotenv.2013.12.018

Government of Manitoba. 2007, April. *Farm Practices Guidelines for Pig Producers in Manitoba*. Gov.mb. https://www.gov.mb.ca/agriculture/livestock/production/pork/pubs/farm-practices-guidelines_complete.pdf

Government of Manitoba. 2022, April. *Sector Profile at a Glance: Hog Highlights*. Gov.mb. https://www.gov.mb.ca/agriculture/markets-and-statistics/livestock-statistics/pubs/hog-sector-profile.pdf

Havens, S.M., Hedman, C.J., Hemming, J.D.C., Mieritz, M.G. Shafer, M.M. and Schauer, J.J. 2020. Occurrence of estrogens, androgens and progestogens and estrogenic activity in surface water runoff from beef and dairy manure amended crop fields. *Sci. Total Environ.* **710**. doi: 10.1016/j.scitotenv.2019.136247

Hildebrand, C., Londry, K.L. and Farenhorst, A. 2006. Sorption and desorption of three endocrine disrupters in soil. *J. Environ. Sci. Health B.* **41**: 907 – 921. doi: 10.1080/03601230600806020

Hofmann, N.A. 2001. Geographical profile of manure production in Canada, Canadian 945 Electronic Library. Retrieved from: https://www150.statcan.gc.ca/n1/pub/16f0025x/manure-fumier/4058921-eng.htm on 14 Mar 2023

Johnston, A.M. and Bruulsema, T.W. 2014. 4R nutrient stewardship for improved nutrient use efficiency. *Procedia Eng.* **83**: 365 – 370. doi: 10.1016/j.proeng.2014.09.029

Kuchta, S.L. Cessna, A.J., Elliot, J.A., Peru, K.M. and Headly, J.V. 2009. Transport of lincomycin to surface and ground water from manure-amended cropland. *J. Environ. Qual.* **38**: 1719 – 1727. doi: 10.2134/jeq2008.0365

Lee Pow, C.S.D., Law, J.M., Kwak, T.J., Cope, W.G., Rice, J.A., Kullman, S.W. and Aday, D.D. 2017. Endocrine active contaminants in aquatic systems and intersex in common sport fishes. *Environ. Toxicol. Chem*. **36:** 959 – 968. doi: 10.1002/etc.3607

Liang, J. and Shang, Y. 2013. Estrogen and Cancer. *Annu. Rev. Physiol.* **75:** 225 – 240. doi: 10.1146/annurev-physiol-030212-183708

Liu, J., Baulch, H.M., Macrae, M.L., Wilson, H.F., Elliot, J.A., Bergstrom, L., Glenn, A.J. and Vadas, P.A. 2019. Agricultural water quality in cold climates: processes, drivers, management options, and research needs. *J. Environ. Qual.* doi: 10.2134/jeq2019.05.0220

MAFRI, 2010. Manitoba Agriculture and Rural Initiative. Soil Series Descriptions.

Meyer, T. and Wania, F. 2008. Organic contaminant amplification during snowmelt. *Water Res.* **42**: 1847 – 1865. doi: 10.1016/j.watres.2007.12.016

Moore, S.C., Matthews, C.E., Shu, X.O., Yu, K., Gail, M.H., Xu, X., Ji, B., Chow, W., Cai, Q., Li, H., Yang, G., Ruggieri, D., Boyd-Morin, J., Rothman, N., Hoover, R.N., Gao, Y., Zheng, W. and Ziegler, R.G. 2016. Endogenous estrogens, estrogen metabolites, and breast cancer risk in postmenopausal Chinese women. *J Natl Cancer Inst*. **108**. doi: 10.1093/jnci/djw103

Odinga, E.S., Zhou, X., Mbao, E.O., Ali, Q., Waigi, M.G., Shiraku, M.L. and Ling, W. 2022. Distribution, ecological fate, and risks of steroid estrogens in environmental matrices. *Chemosphere*. **308**. doi: 10.1016/j.chemosphere.2022.136370

Pomeroy, J., Fang, X., Westbrooke, C., Minke, A., Guo, X. and Brown, T. 2010. Prairie hydrological model study final report. Univ. Saskatchewan, Saskatoon, SK, Canada.

Reid, K., Schneider, K. and Joosse, P. 2019. Addressing imbalances in phosphorus accumulation in Canadian agricultural soils. *J. Environ. Qual.* **48**: 1156 – 1166. doi: 10.2134/jeq2019.05.0205

Prater, J.R., Horton, R. and Thompson, M.L. 2016. Impacts of environmental colloids on the transport of 17β-estradiol in intact soil cores. *J. Soil Sediment Contam.* **25**: 164 – 180. doi: 10.1080/15320383.2016.1112360

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raman, D.R., Williams, E.L., Layton, A.C., Burns, R.T., Easter, J.P., Daugherty, A.S., Mullen, M.D. and Sayler, G.S. 2004. Estrogen content of dairy and swine wastes. *Environ. Sci. Technol.* **38:** 3567- 3573. doi: 10.1021/es0353208

Shareef, A., Angrove, M.J., Wells, J.D. and Johnson, B.B. 2006. Aqueous solubilities of estrone, 17β-estradiol, 17α-ethynylestradiol, and bisphenol a. *J. Chem. Eng. Data.* **51**: 879 – 881. doi: 10.1021/je050318c

Sun, W.L., Ni, J.R. and Liu, T.T. 2006. Effect of sediment humic substances on sorption of selected endocrine disruptors. *Water Air Soil Pollut.* **6**: 583 – 591. doi: 10.1007/s11267-006-9043-4

Tong, X., Li, Y., Zhang, F., Chen, X., Zhao, Y., Hu, B. and Zhang, X. 2019. Adsorption of 17β-estradiol onto humic-mineral complexes and effects of temperature, pH, and bisphenol a on the adsorption process. *Environ. Pollut*. **254**. doi: 10.1016/j.envpol.2019.07.092

Van Emmerik, T., Angrove, M.J., Johnson, B.B., Wells, J.D. and Fernandes, M.B. 2003. Sorption of 17β-estradiol onto selected soil minerals. *J. Colloid Interface Sci.* **266**: 33 – 39. doi: 10.1016/S0021-9797(03)00597-6

Wilson, H., Elliot, J., Macrae, M. and Glenn, A. 2019. Near-surface soils as a source of phosphorous in snowmelt runoff from cropland. *J. Environ. Qual.* **48:** 921 – 930. doi: 10.2134/jeq2019.04.0155

Yamamoto, H., Liljestrand, H.M., Shimizu, Y. and Morita, M. Effects of physical – chemical characteristics on the sorption of selected endocrine disruptors by dissolved organic matter surrogates. *Environ. Sci. Technol.* **37**: 2646 – 2657. doi: 10.1021/es026405w

Ying, G.G. and Kookana, R.S. 2005. Sorption and degradation of estrange-like-endocrine disrupting chemicals in soil. *Environ. Toxicol. Chem.* **24**: 2640 – 2645.

Zhang, K., Zhang, Z., Hu, Z., Zeng, F. Chen, C., Yang, X. and Li, Y. 2020. Bacterial community composition and function succession under anaerobic conditions impacts the biodegradation of 17β-estradiol and its environmental risk. *Environ Poll*. doi: 10.1016/j.envpol.2020.115155

Chapter 3: The fate of dissolved sulfamethoxazole during spring-thaw snowmelt in a field with a history of manure application.

3.1 Abstract

The fate of sulfamethoxazole (SMX) in Prairie agroecosystems during snowmelt is not well understood. This study aims to provide the first estimates of concentrations and loads of SMX in snowmelt in a field with a history of manure application. The mean concentration of SMX throughout the snowmelt period was 0.0345 ± 0.066 µg/L. The SMX cumulative load was 3.81 ± 3.4 µg/L with a range of 1.03 – 12.8 µg/L. Both the concentration and load were not influenced by the method of manure application (i.e., surface applied versus sub-surface applied).

3.2 Introduction

Sulfamethoxazole (SMX) is a broad-spectrum sulfonamide antibiotic used to treat bacterial infections in livestock operations (Kim et al., 2011). SMX is not completely metabolized by livestock, and up to 90% of SMX is excreted in manure as its original form (Kim et al., 2011). Application of liquid swine manure (LSM) has been found to lead to higher concentrations of both SMX and antibiotic-resistant genes (ARG) in the soil compared with manure from other animals (Yue et al., 2021). This is especially concerning when the dissipation of sulfonamides has been shown to be slower in prairie wetlands (Cessna et al., 2020). Given the high rates of LSM use on agricultural fields in Manitoba, there is a pressing concern to understand SMX fate in the prairie environment and to develop management practices specific to this cold agricultural region. The hydrological regime on Canadian Prairies is dominated by the spring snowmelt, which contributes up to 80% of the annual water runoff (Liu et al., 2018). Snowmelt can result in an extended period of flooding on agricultural fields and can potentially mobilize contaminants from soils to flood waters. While considerable attention has been given to the fate of nutrients during snowmelt (e.g., Costa et al., 2018), antibiotics have received less research. Furthermore, manure management efforts have primarily focused on preventing nutrient pollution in surface water (Liu et al., 2018). Research conducted during the growing season suggested incorporating surface-applied manure shortly after application can minimize the introduction of antibiotics into aquatic environments during rainstorms (Amarakoon et al., 2014). Comparable work has not been done during snowmelt when prolonged flooding and runoff present an increased risk of antibiotic transport.

This study aimed to quantify the concentrations and loads of SMX in a field with a history of manure application during snowmelt under different LSM management practices (no LSM applied, LSM applied on the surface, and LSM applied to the subsurface of soil). We hypothesized: 1) SMX concentrations and loads will depend on the LSM application methods, and 2) the concentrations and loads of SMX will increase as snowmelt progresses when there is increased contact between snowmelt water and soil.

3.3 Materials and Methods

3.3.1 Experimental design and field sampling

In the fall of 2021, 12 plots were established in an agricultural field that routinely receives manure applications based on the nutrient requirements and the soil test phosphorus levels in southeastern Manitoba, Canada (49° 32′ 5″ N, 96° 51′5″ W). The soil at this site belongs to the Osborne series and is classified as Rego Humic Gleysol. The soil texture is Clay, with a pH of 7.9 and organic matter content of 7.1%. We used a randomized complete block design with four replicate plots, where three treatments were applied to each block: LSM spread on the surface of the plot using small pails to evenly distribute the manure to ensure the accuracy of the manure rate (i.e., surface applied), LSM applied 15 cm into the soil, using a hoe to dig narrow trenches 15 cm deep which were then re-covered with soil (i.e., sub-surface applied), or no LSM applied (i.e., control). The LSM (~2% solid content) was applied with an even distribution at an application rate of 170 000 L/ha, the manure application rate practiced by the producer in that year. The plots were 3 m x 1 m in size, with a 0.6 m alley between plots. Runoff boxes made of high-density polyethylene puckboard supported by wooden frames (1.2 m x 0.9 m x 0.6 m) were placed 15 cm into the soil surface to collect snow over the winter for each plot.

From March 19 to April 5, 2022, snowmelt samples were collected on days when the temperature was above 0 °C (between 12 pm and 2 pm), and liquid water was present on the surface of the soil. The average maximum temperature on sampling days was 4.2 °C, and the total precipitation over this period was 10.3 mm, including rain on days 3 and 7. Over the snowmelt period, there were 10 days where there was meltwater to collect. Floodwater in each runoff box was pumped out with a handheld water pump, and the total snowmelt volume per runoff box (i.e., replicate) was recorded, and a 250 mL subsample was taken from each plot. Samples were composited over two sampling days to ensure a sufficient mass of SMX for analysis from each subsample.

3.3.2 Sample preparation

The samples were pre-concentrated using solid-phase extraction (SPE). SPE Restek Hydrophilic Lipophilic Balance (HLB) cartridges (6 mg of sorbent, 3 mL; Oasis Waters) were conditioned, and the samples were passed through the HLB cartridges with a vacuum at a flow rate of 1 mL/min. Once all samples were pulled through the HLB cartridges, the cartridges were rinsed with 3 mL ultrapure water (Milli-Q; 18 MΩ cm) to remove excess salts and allowed to dry for 1 min under vacuum. The samples were stored at -20°C until they were transferred, 24 hr before elution, to a refrigerator at 4°C.

The samples were eluted with 3 mL of MeOH, allowing gravity to pull the samples through the HLB cartridges, followed by drying the samples to completeness using a 45°C water bath and a nitrogen evaporator (OA-SYS Heating system and N-Evap 111, Organomation Associates, Inc.) with a gentle stream of Ultrapure N2 (Praxiar Canada Inc.). The samples were reconstituted with 50/50 (v/v) MeOH: Milli-Q to 0.5 mL. Finally, the samples were filtered using a 0.22 µm syringe filter (Resteck) into 2 mL amber glass vials (Chromatographic Specialties Inc.). The samples were stored at -20 °C until analysis.

3.3.3 Liquid chromatography tandem mass spectrometry (LC-MS/MS)

Chromatography was performed with an Agilent 1260 Infinity II UHPLC (Agilent Technologies), with separation using an Agilent Eclipse Plus C18 column (2.1 mm x 50 mm, 1.8 µm dp) coupled to an Agilent Eclipse Plus C18 guard column (2.1 mm x 5 mm) at 42 ºC at 0.3 mL/min. The injection volumes were 2 µL during optimization and 10 µL during analysis. Mobile phase A was Milli-Q water, and mobile phase B was MeOH.

Gradient elution was performed as follows: 0 - 2.00 min linear ramp from 5% to 95% B, 2.01 - 4.00 min hold at 95% B, 4.01-5.00 linear ramp from 95% to 5% B, followed by reequilibration from 5.01- 8.00 min at 5% B. Qualitative assessment and quantification were performed through multiple reaction monitoring (MRM) on an Agilent 6470 triple quadrupole mass spectrometer in positive electrospray ionization mode (ESI+), a capillary voltage of 4000 V, and a source temperature of 300°C. Nitrogen was used for desolvation and drying gas at 11 L/min, and for nebulization at 15 psi. Ultrapure nitrogen was used as collision gas at a flow of rate 16.8 L/min. The MS1 and MS2 heaters were set at 100°C. The samples were spiked with deuterated SMX (Sigma-Aldrich) as the internal standard. The method limit of detection 0.0094 µg/L, and the method limit of quantification was 0.015 µg/L. The percent recovery of the method for SMX was 73.0 ± 38%. The linearity was $R^2 = 0.9999$.

3.3.4 Statistical analysis

A repeated measures analysis was used to test the effects of manure application method and sampling day on SMX concentration. The treatment and sampling day were fixed effects, with block as a random effect, and the sampling day was the repeated factor.

The cumulative load of SMX was calculated by multiplying the SMX concentration by the mean daily snowmelt volume to account for high variance in snowmelt volume among replicates. A one-way ANOVA was used to test the effects of manure application method on the cumulative load of SMX. The data were log-transformed when residuals were not normally distributed. All statistical analyses were performed in R (version 4.2.2, R Development Core Team, 2022).

3.4 Results

The mean SMX concentration over the entire sampling period of all treatments was 0.0345 ± 0.066 µg/L and did not vary significantly among treatments (i.e., LSM application method; F-calculated =0.223, p = 0.805; Table 3.1) or sampling days (F=1.023, p = 0.410; Table 3.1). The maximum concentration introduced into the environment was 0.36 µg/L. Snowmelt volumes collected were highly variable among plots (i.e., replicates), as is expected on the prairies where wind redistribution of snow is widespread (Costa et al., 2018). The volumes also varied throughout the sampling period; the maximum volumes generally occurred on day 3 (37.1 ± 6.5 L) and went as low as 8.8 L by the final sampling day. The mean cumulative load of SMX was 4.12 ± 3.6 ng/m2 for the 17-day snowmelt period, with a range of 1.03 – 12.8 ng/m2 , and there was no significant difference among the manure application method (F=0.241, p = 0.787; Table 3.1).

Table 3.1: The least square mean concentration (µg/L) and cumulative load (ng/m^2) with the standard error (in brackets) of sulfamethoxazole in snowmelt for the treatments (control, sub-surface applied, and surface applied liquid swine manure (LSM)) and the sampling day (1, 3, 5, 13, 17).

Treatment	Concentration	Cumulative load
	µg/L	ng/m^2
Application method		
Control	0.028 (0.013)	1.06 (0.32)
Sub-Surface Applied LSM	0.040 (0.014)	1.40 (0.43)
Surface Applied LSM	0.030 (0.013)	1.36 (0.42)
Sampling day		
1	0.011 (0.017)	-
3	0.054 (0.018)	-
5	0.038 (0.018)	-
13	0.053 (0.018)	-
17	0.0077 (0.017)	-
	p - value	
Application method	0.805	0.787
Sampling day	0.410	-
Application method: sampling day	0.719	-

3.5 Discussion

Sulfonamides pose a risk to the ecosystem health of freshwaters and are also linked to the rise in antibiotic resistance in environmental bacteria (Yue et al., 2021), but research on SMX transport dynamics from agricultural soils is limited. In the Canadian Prairies, where most runoff comes during snowmelt, this study provides baseline data on the behaviour of this contaminant during snowmelt.

There were no significant differences in SMX concentration among the two manure application methods or the control treatments where LSM was not applied in Fall 2021 (Table 3.1). The presence of SMX in the snowmelt from control plots could be due to the long history of manure application at this site with elevated residual SMX levels. This also meant that there were no significant differences in the cumulative loads among treatments, although there was a peak in cumulative load during days 3 – 5 of the sampling period, coincident with the largest volume of snow (Figure 3.1). While it was likely that the soil was still partially frozen on days 3 to 5, there was sufficient contact between the snowmelt water and the shallow soil to mobilize some SMX (Costa et al., 2018). This suggests that a high risk of transport of SMX into surface waters occurs when the snowmelt volume is highest.

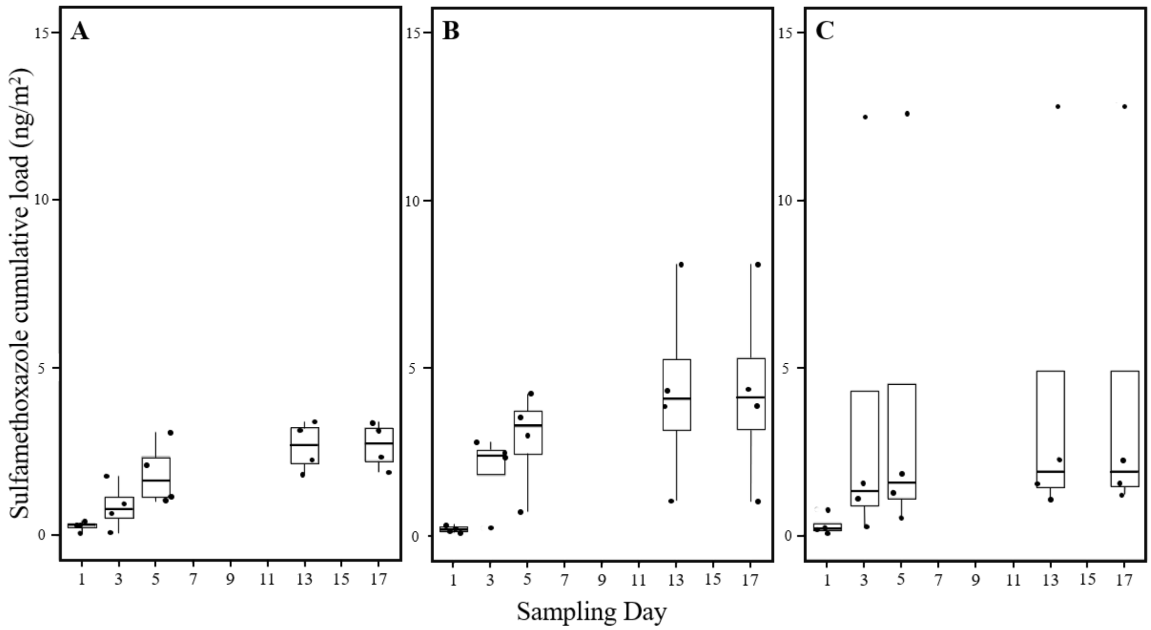


Figure 3.1: Boxplot of the cumulative load (ng/m^2) of sulfamethoxazole in snowmelt during the snowmelt period in three different treatments: A) control, B) sub-surface applied liquid swine manure, and C) surface applied liquid swine manure applied in the fall of 2021. The samples were collected over 17 days in the spring of 2022.

The concentration of SMX required for chronic ecotoxicity in aquatic species ranges from 5.3 – 253 000 µg/L (Straub et al., 2016). The concentrations of SMX in the snowmelt were below those levels, but the mean cumulative load of 4.12 ng/m2 still poses a risk to aquatic systems, considering the widespread land application of manure (Liu et al. 2018). Another risk of SMX pollution in aquatic systems is the possibility of increasing antibiotic-resistant bacteria. The prolonged application of swine manure has been shown to result in higher levels of ARGs relative to other types of livestock manure (Wu et al., 2022). Thus, even if SMX concentrations in snowmelt are below ecotoxicological levels, there are environmental risks associated with snowmelt-induced loading in prairie environments.

Snowmelt volume primarily drives the cumulative load of SMX over the snowmelt period since there were no significant differences in SMX concentrations among treatments or days. The cumulative load continued to increase until the end of the snowmelt, albeit at a slower rate in the last four days (Figure 3.1). Management practices that hold snowmelt runoff on the land (Liu et al., 2018) may help to mitigate the risk of SMX pollution in streams and lakes. Future research should investigate the efficacy of such management options, which may also mitigate nutrient pollution (Liu et al., 2018).

3.6 Conclusion

Mitigating the transport of antibiotics into soils and surface waters is important to reduce adverse impacts on the ecosystem and human health. This study is among the first to quantify SMX in snowmelt water from manure-amended agricultural land under field conditions, and as such, provides data on possible concentrations (up to 0.36 µg/L) and load (1.03 – 12.8 ng/m2). This research will guide future research in this understudied area of antimicrobial transport in the Canadian Prairies to assist multifaceted approaches to manure management for sustainable livestock cropping systems.

3.7 References

Amarakoon, I.D., Zvomuya, F., Cessna, A.J., Degenhardt, D., Larney, F.J. and McAllister, T.A. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosine from surface-applied and soil-incorporated beef cattle feedlot manure. *J. Environ. Qual*. **43**: 547 – 557.

Cessna, A.J., Kuchta, S.L., Waiser, M., Brua. R.B. and Bailey, J. 2020. Persistence of the antimicrobials lincomycin, chlortetracycline, and sulfamethazine in prairie wetlands. *J. Environ. Qual*. **49**: 236 – 245.

Costa, D., Pomeroy, J., and Wheater, H. 2018. A numerical model for the simulation of snowpack solute dynamics to capture runoff ionic pulses during snowmelt: the PULSE model. *Adv. Water Resour.* **122**: 37 – 48.

Kim, K.R., Owens, G., Kwon, S.I., So, K.H., Lee, D.B. and Ok, Y.S. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water Air Soil Pollut*. **214**: 163 – 174.

Liu, J., Kleinman, P.J.A., Aronsson, H., Flaten, D., McDowell, R.W., Bechmann, M., Beegle, D.B., Robinson, T.P., Bryant, R.B., Liu, H., Sharpley, A.N. and Veith, T.L. 2018. A review of regulations and guidelines related to winter manure application. *Ambio*. **47:** 657 – 670. doi: 10.1007/s13280-018-1012-4

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Straub, J. O. 2016. Aquatic environmental risk assessment for human use of the old antibiotic sulfamethoxazole in Europe. *Environ. Toxicol. Chem*. **35**: 767 – 779.

Wu, J., Wang, J., Li, W., Guo, S., Li, K., Xu, P., Ok, Y.S., Jones, D.L. and Zou, J. 2022. Antibiotics and antibiotic resistance genes in agricultural soils: a systematic analysis. *Crit. Rev. Environ. Sci. Technol.* doi: 10.1080/10643389.2022.2094693

Yue, Z., Zhang, J., Zhou, S., Ding, C., Wan, L., Liu, J., Chen, L. and Wang, X. 2021. Pollution characteristics of livestock faeces and the key driver of the spread of antibiotic resistance genes. *J. Hazard. Mater.* **409**. doi: 10.1016/j.jhazmat.2020.124957

Chapter 4: General Discussion

Veterinary pharmaceuticals such as antibiotics and steroids are frequently administered to livestock to improve animal health by preventing the spread of infectious diseases (Sarmah et al., 2006; Kim et al., 2011) or to improve growth and feeding rates (Adeel et al., 2017). These pharmaceuticals are ultimately introduced into the environment as it is excreted in manure and applied to agricultural fields (Adeel et al., 2017). There is elevated use of liquid swine manure in the province because Manitoba is the largest pork producers in the country (Government of Manitoba, 2022). When manure is applied to agricultural fields, there is an increased risk of contaminants being introduced into the environment, especially during the snowmelt period. The snowmelt period is a critical time in the Canadian Prairies because there is inflated nutrient release into the environment due to prolonged flooding and runoff over frozen soils, which reduce contaminant retention (Costa et al, 2018; Liu et al., 2019). There is also evidence of increased concentration of organic contaminants being released into the environment during/ shortly after the snowmelt period. There were elevated concentrations of chlortetracycline and tylosin in snowmelt collected from plots that had manure applied on the surface in Wisconsin, USA (Dolliver and Gupta, 2008). Similarly, Kuchta et al. (2009) showed elevated concentrations of lincomycin in snowmelt collected from a basin that originated from plots with liquid swine manure injected into the soil near Riverhusrt, Saskatchewan. Another study showed that elevated concentrations of estrogens were detected in surface waters from field plots that were amended with composted manure from dairy and beef cattle, especially after snowmelt events occurred in Wisconsin, USA (Havens et al., 2020). Despite the elevated concentrations of organic contaminants measured in surface waters, the temporal changes in organic contaminant release during snowmelt and loading to surface waters during the snowmelt period is not well understood.

4.1 Relevant Findings

In Chapter 2, there were detectable concentrations of 17β-estradiol present in the laboratory simulation. The concentrations measured changed over time and the largest concentration of 17β-estradiol in pore water was measured on the final day (day 21) of sampling. There is evidence that hydrophobic contaminants like 17β-estradiol, in neutral pH, resulting in a bulk release at the end of the snowmelt period due to the filtering capacity of snow (i.e., how many particles of contaminants can pass through the snowpack) improving over time, which is what initially slows the release of hydrophobic contaminants until the end of the snowmelt period (Meyer and Wania, 2008). There was also an increase in 17β-estradiol concentration as the laboratory pore water pH became more alkaline, suggesting that a change in pH influences the desorption of 17β-estradiol from the soil into the aqueous phase. In our study region, the pH was between 7 – 8, suggesting that the deprotonation of 17β-estradiol (and thus the mobilization into the aqueous phase as more 17β-estradiol becomes negatively charged repelled by the negative charges in the soil) in more alkaline environments is only a partial driver of the desorption occurring since a majority of deprotonation for 17β-estradiol occurs at pH greater than 10.6. The weaker hydrogen bonds between 17β-estradiol and the soil may be broken as the water is introduced into the system, resulting in the water binding with the soil more effectively than the 17β-estradiol (Van Emmerick et al., 2003). Another mechanism affecting the release of 17β-estradiol into the aqueous phase may be the complexation of 17β-estradiol with dissolved organic matter, which increases its susceptibility to being desorbed from soils (Durán-Álvarez et al., 2014).

In Chapters 2 and 3, there were no significant differences in the concentrations measured in snowmelt based on the different manure application methods. Rather, 17β-estradiol and sulfamethoxazole concentrations were detected in all the plots despite the manure application method (i.e., unamended, on the surface, and below the surface). This is consistent with our laboratory simulation and other studies reported that were able to detect the presence of organic contaminants in surface waters from agricultural fields introduced through snowmelt, regardless of manure management (Dolliver and Gupta, 2008; Kuchta et al., 2009; Havens et al., 2020). This suggests that our most recent manure application in the fall of 2021 did not significantly affect the contaminant concentrations present in the snowmelt water regardless of the manure application method. This also suggests that fields with a long history of manure application may have contaminants present at detectable concentrations (i.e., there are contaminant concentrations measured in snowmelt from unamended plots). The degradation of organic contaminants typically slows down in cold, anaerobic conditions, compared to aerobic conditions, especially for estrogenic compounds (Colucci and Topp, 2001; Ying and Kookana, 2005; Zhang et al., 2020) and sulfonamides (Awad et al., 2013; Conde-Cid et al., 2020; Adesanya et al., 2021). The cold, anaerobic conditions likely explain the persistence of these organic compounds in the environment after manure application.

When considering the effect of organic contaminants on surface waters, it is important to quantify the cumulative load over the whole snowmelt period, as this represents what will be introduced into the aquatic environments. For both 17β-estradiol and sulfamethoxazole, the snowmelt volume was the most important factor in determining the cumulative load; thus, managing snowmelt runoff from agricultural fields may be important in reducing loads of 17β-estradiol and sulfamethoxazole into surface waters. The cumulative load of 17β-estradiol collected from plots with manure applied on the sub-surface was significantly higher than from plots with manure applied on the surface or no manure applied at all. In contrast, there was no significant difference in the cumulative load of sulfamethoxazole among different manure application methods. Although there is no significant relationship between snowmelt volume and the manure amendments or a significant difference in 17β-estradiol concentration among manure amendments, there are slightly elevated concentrations of 17β-estradiol in plots with manure applied in the sub-surface in both the field study snowmelt and the flood water of the laboratory simulation, suggesting that the elevated cumulative load of 17β-estradiol found in plots with manure applied in the subsurface might be due to its slowed degradation in cold, anaerobic conditions (Colucci and Topp, 2001; Ying and Kookana, 2005; Zhang et al., 2020).

4.2 Implications and Recommendations

The agricultural industry is one of the most important industries in Canada, making up 1.6 % of the gross domestic product (approximately $ 31.9 billion) in 2021 and employing nearly 241 500 Canadians (Government of Canada, 2022). However, agricultural practices, especially livestock farming, come with risks to the environment. Many studies show that although many contaminants introduced into surface waters can come from multiple sources, runoff from agricultural fields is one of the main sources of contamination (Hanselman et al., 2003; Raman et al., 2004; Kumar et al., 2005;

Burkholder et al., 2007; Combalbert and Hernandez-Raquet, 2010; Adeel et al., 2017). The prolonged use of manure introduces antibiotics and steroids into the environment, resulting in the higher presence of antibiotic-resistant genes (ARGs) in bacteria found in soil (Yue et al., 2021; Wu et al., 2022) and estrogen-related toxicity in some aquatic species and humans (Liang and Shiang, 2013; Odinga et al., 2022), respectively. There is particular interest in mitigating antibiotic release into the environment as ARGs result in antibiotic resistance in animals and humans. Antibiotic resistance is becoming increasingly problematic, especially when approximately 26% of bacterial infections reported in Canada in 2018 were resistant to at least one first-line antibiotic (CCA, 2019).

In current agricultural practices, there is an emphasis on the 4R of nutrient management to improve the practicality and efficiency of agricultural practices while improving environmental protection (Johnston and Bruulsema, 2014). The 4R of nutrient management is described as the right source, right rate, right time, and right place and are specific to nutrient management, but based on the results from this thesis and future research quantifying organic contaminants in the prairie agroecosystem, the fundamentals of 4R can be updated in the context of organic contaminants. Endocrine-disrupting compounds and antibiotics are consistently detected in runoff from heavily manured fields (Dolliver and Gupta, 2008; Kuchta et al., 2009; Havens et al., 2020), thus perhaps applying manure with reduced concentrations of these contaminants will overall reduce the loading of these contaminants into the environment. In North America, antibiotics can be used to improve growth rates of animals (Cromwell, 2002; Holman and Chénier 2015), so an overall improvement of antibiotic stewardship (i.e., better-targeted use of antibiotics) in North America would mitigate antibiotic resistance in livestock and ARGs in soil bacteria. Composting of manure in optimal conditions have also been shown to boost the degradation of estrogens (Abdellah, et al., 2020; Sun et al., 2022) and antibiotics (Amarakoon et al., 2016). In Chapters 2 and 3, there is evidence that snowmelt volume is the driver for cumulative load; thus, a recommendation that can be applied on the field would be to increase surface roughness through tillage to improve the water-holding capacity of fields (especially in fields that have an incline and are more likely to release snowmelt runoff into surface waters; Hansen et al., 2000). However, Manitoba implements conservation or no-tillage practices to reduce soil erosion, so using crop stubble is potentially another effective way of increasing surface roughness. Maintaining higher/ more crop residue is more effective with retaining snow on fields compared to lower/ no crop residue, especially when used with conservation and no-tillage practices (Liu and Lobb, 2021). Due to the large variation in soil types, regional weather, and compounds of concern, recommendations that are suggested must be specific to the region's conditions.

These results should be interpreted cautiously and in the context of limitations of study design. In Chapter 2, the field samples were analyzed as composites, which potentially masked some temporal effects in 17β-estradiol concentrations. Performing a field study without compositing the samples could provide more insight into which stage of snowmelt is 17β-estradiol most likely to be released. It would also be beneficial to measure the concentrations of 17β-estradiol in pore water in the field study and the laboratory simulation provided an estimate of the release of 17β-estradiol into the soil solution.

An important conclusion of Chapter 3 is that the mechanisms for the release of sulfamethoxazole are not well understood: thus, a study examining the sorption of sulfamethoxazole in clay soils would be important in determining what is causing its release into snowmelt. Similar to Chapter 2, measuring the concentration of sulfamethoxazole dissolved in pore water in a field study would be important in determining its release into the pore water, which will give insight into the diffusion of sulfamethoxazole into flood water from pore water that causes an increase the risk for off-site transport.

Furthermore, measuring the concentration of 17β-estradiol and sulfamethoxazole in the liquid swine manure will provide insight into the starting concentrations of the contaminants before manure application, putting the concentrations measured after snowmelt into the context of what was originally present in the manure. Measuring the concentrations of these contaminants in the soils amended with different manure application methods, before and after the manure application, will provide more information on the persistence of these contaminants in soils with high clay and humic content.

This thesis helps to understand the transport potential of 17β-estradiol and sulfamethoxazole in snowmelt from long-term manured fields into surface waters. This thesis provides important data (i.e., initial values of 17β-estradiol and sulfamethoxazole concentrations in snowmelt in the Canadian Prairies) and can be used to help improve the predictability of contaminant transport models under prairie conditions. The data from this thesis can also guide future research in this understudied field, with the aim of assisting policy makers in improving multi-faceted approaches to manure management.

4.3 Conclusions

[1] 17β-estradiol and sulfamethoxazole were detected in snowmelt regardless of manure application method, suggesting agricultural fields that have a long-term manure history have the potential for transport of 17β-estradiol and sulfamethoxazole.

[2] Laboratory pore water 17β-estradiol concentrations changed over time in relation to changes to pH, and the prolonged flooding of soils caused the mobilization of 17β-estradiol from soil into the aqueous phase.

[3] Snowmelt volumes drive the cumulative load of 17β-estradiol and sulfamethoxazole; thus, retaining snowmelt volumes on the field is important in mitigating losses into surface waters.

[4] Field plots with manure applied on the sub-surface showed higher cumulative loads for 17β-estradiol, possibly due to the delayed degradation of estrogenic compounds under cold, anaerobic conditions.

4.4 References

Adeel, M., Song, X., Wang, Y., Francis, D. and Yang., Y. 2017. Environmental impact of estrogens on human, animal, and plant life: a critical review. *Environ. Int.* **99**: 107 – 119. doi: 10.1016/j.envint.2016.12.010

Abdellah, Y.A.Y., Zhang, H. and Li, C. 2020. Steroidal estrogens during composting of animal manure: persistence, degradation, and fate, a review. *Wat. Air and Soil Poll.* **231**. doi: 10.1007/s11270-020-04904-4

Adesanya, T., Zvomuya, F., Sultana, T., Metcalfe, C. and Farenhorst, A. 2021. Dissipation of sulfamethoxazole and trimethoprim during temporary storage of biosolids: a microcosm study. *Chemosphere*. **269.** doi: 10.1016/j.chemosphere.2020.128729

Amarakoon, I.D., Zvomuya, F., Sura, S., Larney, F.J., Cessna, A.J., Xu, S. and McAllister, T.A. 2016. Dissipation of antimicrobials in feedlot manure compost after oral administration versus fortification after excretion. *J. Environ. Qual.* **45**: 503 – 510. doi: 10.2134/jeq2015.07.0408

Awad, Y.M., Kim, S.C., Abd El-Azeem, S.A.M., Kim, K.H., Kim, K.R., Kim, K.J., Jeon, C., Lee, S.S. and Ok, Y.S. 2013. Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. *Environ. Earth Sci.* **71**: 1433 – 1440. doi: 10.1007/s12665-013-2548-z

Burkholder, J., Libra, B., Weyer, P., Heathcote, S., Koplin, D., Thorne, P.S. and Wichman, M. 2007. Impacts of waste from concentrated animal feeding operations on water quality. **115**. doi: 10.1289/ehp.8839

Colucci, M.S. and Topp, E. 2001. Persistence of estrogenic hormones in agricultural soils: II. 17α-ethynylestradiol. *J. Environ. Qual*. **30**: 2077 – 2080. doi: 10.2134/jeq2001.2077

Combalbert, S. and Hernandez-Raquet, G. 2010. Occurrence, fate, and biodegradation of estrogens in sewage and manure. *Appl. Microbiol. Biotechnol.* **86**: 1671 – 1692. doi: 10.1007/s00253-010-2547-x

Conde-Cid, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Fernández-Calviño, D. and Arias-Estévez, M. 2020. Tetracycline and sulfonamide antibiotics in soils: presence, fate and environmental risks. *Processes.* **8**. doi: doi:10.3390/pr8111479

Costa, D., Pomeroy, J., and Wheater, H. 2018. A numerical model for the simulation of snowpack solute dynamics to capture runoff ionic pulses during snowmelt: the PULSE model. *Adv. Water Resour.* **122**: 37 – 48.

Council of Canadian Academies (CCA), 2019. Forecasting the future of antimicrobial resistance (AMR) in Canada. Retrieved from: https://cca-reports.ca/forecasting-the-future-of-amr/ (accessed 01 April 2023).

Cromwell, G.L. 2002. Why and how antibiotics are used in swine production. *Anim. Biotechnol*. **13**: 7 – 27. doi: 10.1081/ABIO-120005767

Dolliver, H. and Gupta, S. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *J. Environ. Qual.* **37**: 1227 – 1237. doi: 10.2134/jeq2007.0392

Durán-Álvarez, J.C., Prado, B., Ferroud, A., Juayerk, N. and Jimenez-Cisneros, B. 2014. Sorption, desorption and displacement of ibuprofen, estrone, and 17β estradiol in wastewater irrigated and rainfed agricultural soils. **473 – 474**: 189 – 198. doi: 10.1016/j.scitotenv.2013.12.018

Government of Canada. 2022, August. Overview of Canada's agriculture and agri-food sector. Retrieved from: https://agriculture.canada.ca/en/sector/overview (accessed 30 March 2023)

Government of Manitoba. 2022, April. *Sector Profile at a Glance: Hog Highlights*. Gov.mb. https://www.gov.mb.ca/agriculture/markets-and-statistics/livestock-statistics/pubs/hog-sector-profile.pdf

Hanselman, T.A., Graetz, D.A. and Wilkie, A.C. 2003. Manure-borne estrogens as potential environmental contaminants: a review. *Environ. Sci. Technol.* **37**: 5471 – 5478. doi: 10.1021/es034410+

Hansen, N.C., Gupta, S.C. and Moncrief, J.F. 2000. Snowmelt runoff, sediment, and phosphorus losses under three different tillage systems. *Soil Tillage Res.* **57**: 93 – 100. doi: 10.1016/S0167-1987(00)00152-5

Havens, S.M., Hedman, C.J., Hemming, J.D.C., Mieritz, M.G. Shafer, M.M. and Schauer, J.J. 2020. Occurrence of estrogens, androgens and progestogens and estrogenic activity in surface water runoff from beef and dairy manure amended crop fields. *Sci. Total Environ.* **710**. doi: 10.1016/j.scitotenv.2019.136247

Holman, D.B. and Chénier, M.R. 2015. Antimicrobial use in swine production and its effect on the swine gut microbiota and antimicrobial resistance. *Can. J. Microbio.* **61:** 785 – 798.

Johnston, A.M. and Bruulsema, T.W. 2014. 4R nutrient stewardship for improved nutrient use efficiency. *Procedia Eng.* **83**: 365 – 370. doi: 10.1016/j.proeng.2014.09.029

Kim, K.R., Owens, G., Kwon, S.I., So, K.H., Lee, D.B. and Ok, Y.S. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water Air Soil Pollut*. **214**: 163 – 174.

Kuchta, S.L. Cessna, A.J., Elliot, J.A., Peru, K.M. and Headly, J.V. 2009. Transport of lincomycin to surface and ground water from manure-amended cropland. *J. Environ. Qual.* **38**: 1719 – 1727. doi: 10.2134/jeq2008.0365

Kumar, K., Gupta, S.C., Chander, Y. and Singh, A.K. 2005. Antibiotic use in agriculture and its impact on the terrestrial environment. *Adv. Agron*. doi: 10.1016/S0065-2113(05)87001-4

Liang, J. and Shang, Y. 2013. Estrogen and cancer. *Annu. Rev. Physiol.* **75**: 225 – 240. doi: 10.1146/annurev-physiol-030212-183708

Liu, J., Baulch, H.M., Macrae, M.L., Wilson, H.F., Elliot, J.A., Bergstrom, L., Glenn, A.J. and Vadas, P.A. 2019. Agricultural water quality in cold climates: processes, drivers, management options, and research needs. *J. Environ. Qual.* doi: 10.2134/jeq2019.05.0220

Liu, J. and Lobb, D.A. 2021. An overview of crop and crop residue management impacts on crop water use and runoff in the Canadian Prairies. *Water*. **13**. doi: **10.3390/w13202929**

Meyer, T. and Wania, F. 2008. Organic contaminant amplification during snowmelt. *Water Res.* **42**: 1847 – 1865. doi: 10.1016/j.watres.2007.12.016

Odinga, E.S., Zhou, X., Mbao, E.O., Ali, Q., Waigi, M.G., Shiraku, M.L. and Ling, W. 2022. Distribution, ecological fate, and risks of steroid estrogens in environmental matrices. *Chemosphere*. **308**. doi: 10.1016/j.chemosphere.2022.136370

Raman, D.R., Williams, E.L., Layton, A.C., Burns, R.T., Easter, J.P., Daugherty, A.S., Mullen, M.D. and Sayler, G.S. 2004. Estrogen content of dairy and swine wastes. *Environ. Sci. Technol.* **38:** 3567- 3573. doi: 10.1021/es0353208

Sarmah, A.K., Meyer, M.T. and Boxall, A.B.A. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*. **65**:725 – 759.

Sun, S., Abdellah, Y.A.Y., Miao, L., Wu, B., Ma, T., Wang, Y., Zhang, H., Zhao, X. and Li, C. 2022. Impact of microbial inoculants combined with humic acid on the fate of estrogens during pig manure composting under low-temperature conditions. *J. Hazard. Mater*. **424**. doi: 10.1016/j.jhazmat.2021.127713

Van Emmerik, T., Angrove, M.J., Johnson, B.B., Wells, J.D. and Fernandes, M.B. 2003. Sorption of 17β-estradiol onto selected soil minerals. *J. Colloid Interface Sci.* **266**: 33 – 39. doi: 10.1016/S0021-9797(03)00597-6

Wu, J., Wang, J., Li, W., Guo, S., Li, K., Xu, P., Ok, Y.S., Jones, D.L. and Zou, J. 2022. Antibiotics and antibiotic resistance genes in agricultural soils: A systematic analysis. *Crit. Rev. Environ. Sci. Technol.* doi: 10.1080/10643389.2022.2094693

Ying, G.G. and Kookana, R.S. 2005. Sorption and degradation of estrange-like-endocrine disrupting chemicals in soil. *Environ. Toxicol. Chem.* **24**: 2640 – 2645.

Yue, Z., Zhang, J., Zhou, S., Ding, C., Wan, L., Liu, J., Chen, L. and Wang, X. 2021. Pollution characteristics of livestock faeces and the key driver of the spread of antibiotic resistance genes. *J. Hazard. Mater.* **409**. doi: 10.1016/j.jhazmat.2020.124957

Zhang, K., Zhang, Z., Hu, Z., Zeng, F. Chen, C., Yang, X. and Li, Y. 2020. Bacterial community composition and function succession under anaerobic conditions impacts the biodegradation of 17β-estradiol and its environmental risk. *Environ Poll*. doi: 10.1016/j.envpol.2020.115155