# Sourcing of microplastics entering Lake Winnipeg, Manitoba, Canada

by

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## Abstract

The accumulation of plastic pollution is a pressing issue, especially the presence of microplastics in the environment. Microplastics have been found around the world, however a research gap exists in understanding the sources of microplastics in freshwater environments. Studying urban watersheds is important for learning about the sources of microplastics, as most plastic pollution originates there. The lack of standardized methodologies for the collection, quantification, and identification of microplastics hinders our understanding of the sources and amounts of microplastics in the environment.

This thesis evaluates the potential of carbon and hydrogen stable isotope ($\delta^{13}C$ and $\delta^{2}H$) and elemental (%C and %H) analysis as a method to identify the polymer type of microplastics. The polymer type of the microplastics provides important information on their source. Commercial plastic products (n = 53) from six different polymer types were used to create a database of characteristic $\delta^{13}C$, $\delta^{2}H$, %C, and %H values for each polymer type. Environmental microplastics (n = 18) of unknown polymer types were also analyzed and their values were compared to the characteristic values for each polymer type in the database. Fourier transform infrared spectroscopy was used to confirm the identification of the polymer type of the unknown microplastics. The combination of the $\delta^{2}H$ and %H values was best able to distinguish the different polymer types and was most useful for identifying the unknown microplastics. The identification of the unknown microplastics using their $\delta^{2}H$ and %H values correlated strongly with identifications using FTIR, confirming that this represents a novel method that can be used to identify the polymer type of microplastics. Because it is a quantitative analysis, it is more attractive than other qualitative methods of identifying the polymer type of microplastics.

Additionally, this thesis investigates spatial and temporal trends in microplastic concentrations in the Red River in Manitoba. Surface water samples were collected from nine sites in the spring, summer, and fall of 2022 to evaluate spatial and temporal trends in microplastic concentrations. Microplastics were found in all samples, with concentrations ranging from 70.0 to 268.7 particles $m^{-3}$. Increased flow rates and stormwater runoff in the spring likely influenced seasonal variations observed in microplastic concentrations. Stormwater runoff, combined sewage overflows, and agricultural runoff are potential sources of microplastics to the Red River.

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## Contributions of Authors

**Chapter 1:** The general research question of this thesis was proposed by my thesis supervisor, Dr. Mostafa Fayek. I conducted the literature review and wrote the first draft of the chapter. Dr. Fayek and committee members Dr. Kirstin Brink and Dr. Feiyue Wang provided edits and feedback. I revised the chapter, implementing their feedback.

**Chapter 2:** Dr. Fayek and I collaborated on the experiment design and acquisition of samples for the commercial plastic database. Dr. Mark Hanson and Sarah Warrack collected some of the microplastics used in the study. All other microplastics were collected by me, with help from field assistants. I prepared all of the samples for analysis. Misuk Yun and Adam Snyder provided assistance in the Stable Isotope Lab and ran the samples. I was responsible for data management and data analysis and received input and feedback from Dr. Fayek. I wrote the first draft of the chapter and created all the figures. Dr. Fayek, Dr. Brink, and Dr. Wang provided edits and feedback. I revised the chapter, implementing their feedback.

**Chapter 3:** Dr. Fayek and I collaborated on the experiment design and construction of the microplastic sampling system. I collected the microplastics with assistance from Dr. Fayek and four field assistants. I identified the microplastics and was responsible for data management and data analysis. I wrote the first draft of the chapter and created the figures. Dr. Fayek, Dr. Brink, and Dr. Wang provided edits and feedback. I revised the chapter, implementing their feedback.

**Chapter 4:** I wrote the first draft of the chapter. Dr. Fayek, Dr. Brink, and Dr. Wang provided edits and feedback. I revised the chapter, implementing their feedback.

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# 1. Introduction

## 1.1. Plastic Waste

The first synthetic plastics were produced in the early 20th century, but it was only after World War II that plastics were mass produced and widely used. Global production of plastics has exploded since then. Between 1950 and 2017, 7.8 billion metric tons of virgin plastics were manufactured, with half of that amount only being produced since 2004 (Geyer et al., 2017). Plastics are lightweight, durable, relatively low cost to produce, and can be molded and cast into a variety of shapes, which makes them very economic materials (Andrady and Neal, 2009).

Plastics are synthetic materials composed of hydrocarbon polymers, typically derived from petroleum feedstocks. There are hundreds of different polymer types, but the market is dominated by just six polymers, which together accounted for almost 75% of all plastics used in

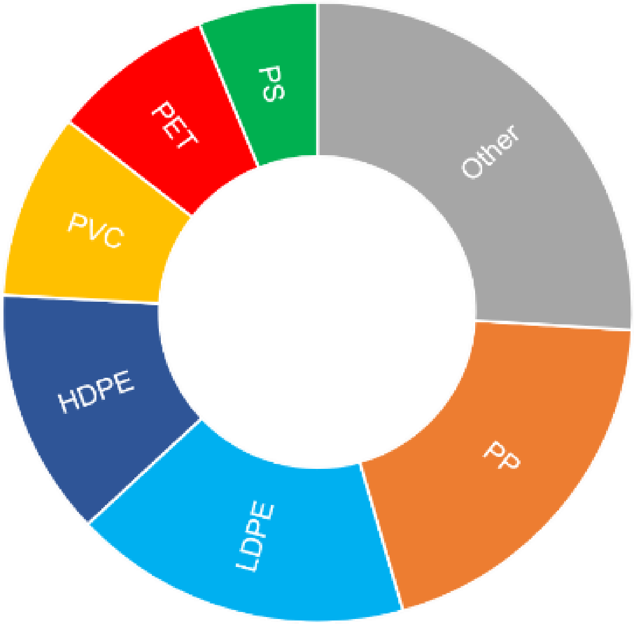

***Figure 1*** *Global plastic demand by polymer type in 2020, data from Plastics Europe, 2021*

2020 (Figure 1). These six polymers, which are known as the commodity plastics, are polyethylene terephthalate (PET), high density polyethylene (HDPE), polyvinyl chloride (PVC), low density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS).

As production and consumption of plastic continues to increase, the issue of waste management is also exacerbated. Geyer et al., (2017) estimate that 4.9 billion metric tons of plastic, which represents 60% of all plastics ever produced, have been discarded and are now residing in landfills or the environment (Geyer et al., 2017). None of the commodity plastics and other commonly used synthetic polymers are biodegradable. As a result, once discarded they accumulate rather than decompose. Exposure to UV radiation and physical abrasion in the natural environment weakens the plastics, causing them to fragment into smaller particles (Barnes et al., 2009).

## 1.2. Microplastics

Pieces of plastic smaller than 5 mm are known as microplastics. Microplastics can be classified as either primary or secondary microplastics. Primary microplastics are plastics that were manufactured to be of microscopic size. These include microbeads used in personal care products or virgin plastic nurdles (sizes typically ranging from 2-5 mm), which can be present in wastewater. Secondary microplastics result from the breakdown of larger plastic products. The plastics can fragment as a result of physical, chemical, or biological processes that reduce the structural integrity of the plastic. Exposure to ultraviolet radiation in sunlight can cause photo-degradation of plastics, making them more brittle, and fragmentation can occur as a result of wave action and physical abrasion in aquatic environments (Andrady, 2011; Barnes et al., 2009;

Browne et al., 2007). As well, some plastic can be biodegraded by insects, bacteria and fungi (Pham et al., 2023; Shimao, 2001).

Microplastics are ubiquitous in the environment. They have been found in every ocean (Borrelle et al., 2020), in freshwaters and drinking water (Koelmans et al., 2019), in sediments (Nizzetto et al., 2016), in Antarctic snow (Aves et al., 2022), in remote areas (Allen et al., 2019; Free et al., 2014), in the deep sea (Van Cauwenberghe et al., 2013; Woodall et al., 2015), and even in human blood (Leslie et al., 2022). The first observations of small plastic fragments in the oceans were reported in the 1970s (e.g., Buchanan, 1971; Carpenter and Smith, 1972). The term ‘microplastic’ was only formally adopted in 2004, with interest in the field increasing exponentially since then.

### 1.2.1. Microplastics in Freshwater Ecosystems

Until recently, the majority of microplastics research has been focused on marine environments, with only about 4% of microplastics publications relating to their occurrence in freshwaters (Wagner and Lambert, 2018). Microplastics are present in many freshwater ecosystems around the world (Dris et al., 2015; Wagner and Lambert, 2018). Rivers have been identified as a major pathway for microplastics to enter the oceans, with about 80% of marine microplastics originating from land-based sources (Andrady, 2011; Klein et al., 2015; Rodrigues et al., 2018; Wagner et al., 2019). Much remains to be understood about microplastics in freshwaters, including their sources, spatial and temporal distribution, the mechanisms controlling their transport, and their eventual fate.

Upstream land use and the amount of urbanization and industrialization surrounding rivers can be correlated with levels of microplastic pollution (Baldwin et al., 2016; Kunz et al., 2023). Microplastics can enter freshwater ecosystems through several pathways (Figure 1.2). Larger plastic items that were improperly disposed of or lost from landfills can enter freshwaters by wind action or runoff and can fragment into secondary microplastics. Direct inputs of microplastics to freshwaters include urban and agricultural runoff (Grbić et al., 2020; Huang et al., 2023; Werbowski et al., 2021), wastewater effluent (Blair et al., 2017; Estahbanati and Fahrenfeld, 2016), combined sewer overflows (Forrest et al., 2022), and atmospheric deposition (Dris et al., 2016).

Freshwaters represent a complex system for microplastics. Freshwaters receive microplastics from the terrestrial environment as well as atmospheric deposition. Freshwaters can act as sinks and retain microplastics in sediments. Features of the different polymers, such as their density, size, and shape, can influence their distribution in freshwaters and their eventual fate. Plastics that are denser than water, such as PVC or PET, are more likely to sink and be retained in sediments. Larger or irregularly shaped microplastic particles also tend to settle in sediments, while spherical microplastics and microfibres are more likely to float (Horton and Dixon, 2018). High flow events can resuspend settled microplastics, and wind, currents, and waves can also affect the distribution of microplastics in freshwaters (Hurley et al., 2018; Prata et al., 2019).

### 1.2.2. Impacts of Microplastics on Freshwater Ecosystems

The true extent of microplastic contamination in the environment and the impacts that they have on organisms is not well known, however it has been demonstrated that microplastics can be ingested by organisms and bioaccumulate through the food chain (Andrady, 2011). The density of some of the most common plastics allows them to reside in the parts of the water column where they are most available to organisms, and their small size means that microplastics can be confused for food and ingested by organisms (Andrady, 2017). Microplastic ingestion can affect the survival (Lee et al., 2013; Mazurais et al., 2015; Ogonowski et al., 2016; Ziajahromi et al., 2018), reproduction (Au et al., 2015; Jeong et al., 2016; Lee et al., 2013), embryonic development (Nobre et al., 2015), mobility (Gambardella et al., 2017; Rehse et al., 2016), feeding (Cole et al., 2013; Green et al., 2016; Murphy and Quinn, 2018), and growth of organisms (Blarer and Burkhardt-Holm, 2016; Jeong et al., 2016; Straub et al., 2017; Wright et al., 2013). These effects can be caused by physical damage from the microplastics (i.e., blocking food passages or accumulating on the organism), leaching of chemicals from the microplastics, and the presence of pollutants sorbed to the surface of the microplastics (Koelmans et al., 2022).

The severity of the effects is dependent on the plastic properties, their concentrations in the environment, and rates of exposure. Different chemical additives, such as plasticizers, flame retardants, and dyes, are incorporated into plastic polymers during production (Lithner et al., 2011). These additives can leach from the plastics as they degrade in the environment (Campanale et al., 2020; Hahladakis et al., 2018). Additionally, microplastics can accumulate persistent organic pollutants (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and pesticides), which can then be transferred to organisms upon ingestion and can bioaccumulate (Engler, 2012; Mato et al., 2001; Rochman et al., 2013). In humans, microplastics have been found in feces (Liu et al., 2023; Yan et al., 2022), lung tissue (Amato-Lourenço et al., 2021; Jenner et al., 2022), liver tissue (Horvatits et al., 2022), blood (Leslie et al., 2022), breast milk (Liu et al., 2023; Ragusa et al., 2022), and in placentas (Liu et al., 2023; Ragusa et al., 2021). Sources of human exposure to microplastics include inhalation of airborne microplastics and oral ingestion: drinking water, seafood, honey, alcohol, and salt have been found to be contaminated by microplastics (Cox et al., 2019; Revel et al., 2018).

## 1.3. Identification of Microplastics

Because microplastics research is still an emerging field, analytical methods for the identification of microplastics are still being developed and there is much methodological variation between studies, which makes data comparison difficult. Microplastics exhibit great diversity in their size, shape, and polymer type, complicating microplastics identification from environmental samples. A combination of analytical techniques is typically used for their identification. First, initial identification of microplastics is generally done through visual classification using a microscope, then a chemical characterization is performed to confirm that the identified particles are actually microplastics (Shim et al., 2017). Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy are the most common methods used for the chemical characterization of microplastics. However, environmental exposure causes weathering of the microplastics, and those effects can interfere with the identification of microplastic polymer type (Xu et al., 2019). Additionally, the presence of additives in the plastics, particularly pigments, can also confound identification (Shim et al., 2017; Van Cauwenberghe et al., 2013).

Because of these issues, there is a need to investigate new methods that can complement existing techniques for the identification of the polymer type of microplastics.

## 1.4. Stable Isotope Analysis

Stable isotope analysis is an analytical technique used in a variety of disciplines to track the chemical, biological, and geographic origins of materials (Muccio and Jackson, 2009). Several studies have observed that the carbon isotopic composition of plastic materials can be used to differentiate petroleum-based from plant-based polymers (Rogers et al., 2021; Suzuki et al., 2010; Telloli et al., 2019). As well, the technique showed promise for tracking changes to plastic polymers due to aging and weathering in the environment (Battulga et al., 2023; Berto et al., 2017; Birch et al., 2021; Kuznetsova et al., 2023). In forensic science, carbon stable isotope analysis has been used to characterize and discriminate plastic products used for committing crimes, including packing tapes, zip ties, and plastic bags (Dietz et al., 2012; Horacek et al., 2008; Howa et al., 2016; Meikle et al., 2021). The isotopic composition of petroleum can vary depending on the extraction source, resulting in differences between plastic feedstocks sourced from different areas. Additives to the plastics and different steps in the manufacturing process can also potentially affect the isotopic composition of plastic materials, meaning that it may be possible to distinguish some petroleum-derived plastics from each other (Birch et al., 2021). Preliminary studies have shown that the carbon isotopic composition of petroleum-derived plastics varied, but similar means were observed in most plastic types (Battulga et al., 2023; Berto et al., 2017; Birch et al., 2021; Kuznetsova et al., 2023). Wider ranges have been reported in the hydrogen isotopic composition of petroleum-derived plastics (Jones et al., 2018; Kuznetsova et al., 2023). However, only limited work has been done using the combination of carbon and hydrogen stable isotopes to characterize plastic polymers and the applicability of this technique to a wider range of plastic polymers warrants further investigation.

## 1.5. Elemental Analysis

The carbon concentration and hydrogen concentration are dependent on the chemical composition of each polymer. The carbon concentration of plastics has not yet been investigated as a method of distinguishing between different polymer types coming from the same feedstocks. Rogers et al., (2021) analyzed the carbon concentration in addition to the carbon and hydrogen stable isotope composition of petroleum and bio-based plastics to differentiate the plastics based on their source materials (Rogers et al., 2021). Very few studies have reported on the carbon concentration of plastics, however the research has shown that the carbon concentration characterized from different plastic polymers fits with the known composition of the material (Rogers et al., 2021; Zhang et al., 2020). No studies have characterized the hydrogen concentration of plastics, however it is expected that, like with the carbon concentration, the hydrogen concentration values will align with the known composition of the material. The carbon and hydrogen concentration of different plastic polymers should be characterized and evaluated for use as a tool to identify the polymer type of unknown microplastics.

## 1.6. Research Questions and Objectives

The research presented in this thesis is organized around two primary objectives. The first objective is to evaluate the utility of carbon and hydrogen stable isotope and elemental analysis as a method of identifying the polymer type of microplastics. More specifically, the research will characterize the carbon and hydrogen stable isotope and elemental compositions of various products from each of the six commodity plastics to establish a database of characteristic compositions for each polymer type. It will also investigate whether the isotopic and elemental compositions of each different polymer type are unique enough to be distinguished from one another. Finally, microplastics of unknown polymer types collected from environmental samples will have their isotopic and elemental composition characterized for comparison with the database and possible identification. I hypothesize that:

1. *The carbon stable isotope composition of the different polymer types will overlap, as observed in previous studies*
2. *The hydrogen stable isotope composition of the different polymer types will exhibit wider ranges than the carbon stable isotope composition*
3. *The different processes involved in manufacturing different polymers will result in distinct hydrogen isotopic compositions for each polymer type*
4. *The carbon and hydrogen elemental composition of HDPE, LDPE, and PP will be the same, but PET, PVC, and PS will be distinct*
5. *It will be possible to identify the polymer type of the unknown microplastics by their carbon and hydrogen isotopic and elemental composition*

The second objective of this thesis is to assess variations in the spatial and temporal distributions of microplastics in the Red River, Manitoba. The research will measure microplastic concentrations at several sampling sites along the Red River, including sites upstream, within, and downstream of the city of Winnipeg during the spring, summer, and fall of 2022 to evaluate seasonal changes in microplastic concentrations. Additionally, potential sources of microplastics to the Red River will be identified. I hypothesize that:

1. *Microplastic concentrations will be lower upstream of the city of Winnipeg compared to concentrations downstream of and within the city*
2. *Microplastic concentrations will be higher downstream of wastewater treatment plants, as wastewater effluent has been identified as a significant source of microplastics to rivers*
3. *In the spring, microplastic concentrations will be higher than during the summer or fall due to increased flow rates and surface runoff*

For regulatory bodies to be able to address the issue of microplastics in freshwaters, there needs to be information on the concentrations currently in the environment and their possible sources. Understanding the sources of microplastics to the environment is of critical importance as it informs mitigation efforts to reduce the amount of microplastics entering ecosystems. Specific information on the polymer type of microplastics is key to understanding their potential effects on organisms, as polymer type influences their toxicity. This research will contribute to our knowledge of the concentrations and sources of microplastics to the Red River and can be used to guide policy decisions relating to plastic pollution in Manitoba. Additionally, this research investigates a novel method for the characterization of plastic polymers and the identification of microplastics, contributing to the toolbox of techniques used to identify and understand the characteristics of microplastics in the environment.

## 1.7. Thesis Organization

This thesis is organized into four chapters: Chapter One is an introduction to the topics discussed in the thesis, Chapters Two and Three are comprised of standalone primary research papers compiled for the purpose of publication as peer-reviewed scientific articles, and Chapter Four is the thesis conclusion chapter. The overarching subject of this thesis is the identification and characterization of microplastics, with a focus on technique development for characterization of microplastic polymer type. Chapter One represents the introduction and provides a review of the literature and background information on the topics discussed in this thesis. Chapter Two investigates the use of carbon and hydrogen stable isotope and elemental analysis as a method of identifying the polymer type of microplastics. Chapter Three assesses variations in the spatial and temporal distributions of microplastics in the Red River, Manitoba and evaluates potential sources of microplastics to the river. Chapter Four summarizes the results of Chapters Two and Three and summarizes their key findings and implications. This concluding chapter also discusses directions for future work on microplastics characterization and management of freshwater microplastics.

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# 2. Characterization of Microplastic Polymers Using Carbon and Hydrogen Stable Isotope and Elemental Analysis

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## 2.1. Abstract

The prevalence of plastic waste in the environment is a pressing research concern. Microplastics have been found in marine and freshwaters worldwide, however the absence of standardized methods to characterize microplastics in the environment has hindered our understanding of their sources and impacts. The development of novel methodologies for the characterization and identification of microplastics is key for understanding the effects of their presence in the environment. In this study, we investigate using a combination of carbon and hydrogen stable isotope and elemental analysis as a complementary technique to distinguish between plastic polymer types. The carbon and hydrogen isotopic composition and elemental concentration of various common plastic products from six different polymer types were characterized to create a database of typical values for each polymer type. Environmental microplastics of unknown polymer types were also analyzed and their values were compared to the characteristic values for each polymer type in the database. The identification using the hydrogen isotopic composition and hydrogen concentration of microplastics correlated with identification using FTIR for all but one of the unknown microplastics. The isotopic and elemental composition is a quantitative method of identification, which reduces biases and uncertainty compared to other qualitative methods of identification, which makes it an attractive method for identifying the polymer type of microplastics. This study represents the first characterization of

the hydrogen concentration of plastics and introduces a complementary technique that can be used to identify unknown plastics, with applications for the study of plastic pollution as well as forensic and regulatory investigations of plastic materials.

## 2.2.Introduction

Our hyper-consumption of plastic materials has resulted in the accumulation of plastic debris in the environment, with 19-23 million metric tonnes of plastic entering aquatic ecosystems annually, an amount that could increase to 53 million metric tonnes per year by 2030 (Borrelle et al., 2020). Plastics do not completely decompose. Instead, once in the environment they degrade and fragment into smaller particles through exposure to UV radiation and physical abrasion (Andrady, 2003; Barnes et al., 2009). Microplastics, which are pieces of plastic <5 mm in size, have been found in aquatic environments worldwide (Andrady, 2011; Lusher et al., 2015; Van Cauwenberghe et al., 2013; Wu et al., 2019; Yang et al., 2022). Microplastics can be ingested by organisms and bioaccumulate through the food chain. Negative effects resulting from the consumption of microplastics have been reported in various organisms in laboratory situations (Eltemsah and Bøhn, 2019; Gambardella et al., 2017; Jeong et al., 2016), however the extent of the effects they may have on organisms in the wild remains unknown (Andrady, 2011; Rochman, 2015; Weis and Palmquist, 2021).

The severity of the effects of microplastic ingestion by organisms is influenced by the properties of the plastic, their concentration in the environment, and rates of exposure. Microplastics originate from a wide variety of products and plastic polymers, each with different properties influenced by their specific chemical structure and composition (Koelmans et al., 2022). Additives such as flame retardants, UV stabilizers, plasticizers, and dyes are often incorporated into plastics during production (Lithner et al., 2011). As plastics fragment and degrade in the environment, additives can leach from the plastics (Campanale et al., 2020; Hahladakis et al., 2018). As well, microplastics can accumulate persistent organic pollutants (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and pesticides), which can then be transferred to organisms upon ingestion and can bioaccumulate (Engler, 2012; Mato et al., 2001; Rochman et al., 2013). The polymer type of the microplastic influences where in the water column the particle resides, the toxicity of the polymer, the amount of additives incorporated, the sorption of persistent organic pollutants to the particle, and degradation rates (Andrady, 2017; Bakir et al., 2012; Koelmans et al., 2022; Kowalski et al., 2016; Lithner et al., 2011; Menéndez-Pedriza and Jaumot, 2020; Rochman, 2015). Knowledge on the polymer type of the microplastic is key to understanding the potential effects that they may have on organisms and the risks that their presence poses in the environment.

Currently, the polymer type of microplastics is identified using spectroscopic techniques such as Fourier-transform infrared (FTIR) spectroscopy and Raman spectroscopy. However, the presence of additives in the plastics, especially colourants, and the effects of weathering can confound the identification of polymer type using these methods (Shim et al., 2017). There is a need to develop new methods that can complement existing techniques used to identify the polymer type of microplastics. Stable isotope analysis is a technique used in a variety of disciplines to track the chemical, biological, and geographic origins of materials (Förstel, 2007; Matos and Jackson, 2019; Rubenstein and Hobson, 2004; Tsutaya and Yoneda, 2015; Wiederhold, 2015). Petroleum has been shown to be isotopically variable, and previous studies have demonstrated that the stable carbon (C) isotope composition ($\delta^{13}C$) of plastics are consistent with their pre-cursor materials, suggesting that the technique can be used to distinguish between petroleum-based and bio-based plastics (Suzuki et al., 2010; Wang et al., 2015; Yeh and Epstein, 1981).

Two preliminary studies (Berto et al., 2017; Birch et al., 2021) analyzed the $\delta^{13}C$ values in a variety of plastics to distinguish between different polymer types derived from petroleum and bio-based sources. Results showed that there were significant differences in the $\delta^{13}C$ values from the petroleum-based and bio-based plastics and that the presence of colourants did not affect the isotopic composition of the plastics (Berto et al., 2017; Birch et al., 2021). However, the $\delta^{13}C$ values of the different petroleum-based plastics overlapped, making it impossible to distinguish between different polymer types when solely using the C stable isotope composition. To improve the distinguishing capabilities and overcome the limitations of C stable isotope analysis, C and hydrogen (H) isotope analysis and carbon concentration (%C) were used to distinguish petroleum-based, bio-based, and mixed bio-petroleum plastics (Rogers et al., 2021). The H isotope compositions ($\delta^{2}H$) for plastics exhibit wider ranges than the $\delta^{13}C$ values, possibly because the different manufacturing processes involved in the production of plastics may influence the final $\delta^{2}H$ values of different polymer types (Jones et al., 2018; Kuznetsova et al., 2023; Rogers et al., 2021; Taylor et al., 2008). Very few %C values have been reported for plastics, aside from Rogers et al., (2021), which reported a range of 56.4% to 85.3%, and Zhang et al., (2023), which reported a range of 69.8% to 92.1% (Rogers et al., 2021; Zhang et al., 2023).

Previous studies using the isotopic composition of plastics have focused more on the ability of the technique to distinguish between petroleum-based and bio-based plastics. This study builds on these previous works to focus on distinguishing different polymer types of petroleum-based plastics by using a combination of C and H stable isotope analysis with %C and H concentration (%H). Here, we report the $\delta^{13}$C and $\delta^{2}$H values and %C and %H of 53 commercial petroleum-based plastic products from six polymer types for use as a reference database. The $\delta^{13}$C and $\delta^{2}$H values and %C and %H of 18 environmental microplastics of unknown polymer types are also reported and compared to the database. We use FTIR spectroscopy to confirm the utility of using C and H stable isotopes, and %C and %H to distinguish between microplastic polymer types from the natural environmental. This work represents the first characterization of the %H of plastics and provides a complementary analytical method that can be used to identify the polymer type of microplastics and other unknown plastics, which can be applied to the environmental tracking of microplastics as well as forensic studies and regulatory investigations of plastic materials.

## 2.3. Methodology

### 2.3.1. Polymer Samples

Samples were selected for analysis based on their commercial prevalence and evidence for their accumulation in the environment (Bond et al., 2018; Plastics Europe, 2019). Polymer types included in this study are polyethylene terephthalate (PET), high density polyethylene (HDPE), polyvinyl chloride (PVC), low density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS). Commercial plastic products (n = 53, i.e., packaging, food containers, household items, etc.) composed of each polymer type were chosen for analysis to assess homogeneity in the isotopic and elemental composition of each polymer type. Commercial plastic samples were cut into small pieces and cleaned with deionized (DI) water prior to analysis.

### 2.3.2. Microplastic Samples

Microplastics that had previously been collected from the Red River in Manitoba, Canada, were selected for analysis. Microplastics were collected as part of work done for other projects through surface water sampling using either a custom-made mobile pump-based sampling device following the design of Donovan et al., (2019) or by manta trawl sampling. After collection, samples were processed using a wet peroxide oxidation treatment following the protocol outlined by Masura et al., (2015). Samples were processed using a wet peroxide oxidation treatment following the protocol outlined by Masura et al., (2015) to remove organic materials from the samples. The collected filters were rinsed into glass beakers with deionized (DI) water to reconstitute the sample to 100 ml. To facilitate the digestion of organic material, 20 mL of a 0.05 M Fe (II) solution and 20 mL of 30% hydrogen peroxide ($H_2O_2$) was added to the sample. The 0.05 M Fe (II) solution was prepared by adding 7.5 g of $FeSO_4$ to 500 mL of water and 3 mL of concentrated sulfuric acid. The mixture was left to stand on the lab bench at room temperature for five minutes. Then, a stir bar was added to the beaker, and it was covered with a watch glass. The beaker was then placed on a hot plate and heated to 75$^{o}$C. The sample was left to digest for 30 minutes on the hot plate. At 30-minute intervals, samples were re-examined and an additional 20 mL of $H_2O_2$ was added if organic material was still present. This process was repeated until all visible organic material was digested. Samples were then covered and left for 24 hours to allow for complete digestion. Samples were then rinsed with DI water to remove the $H_2O_2$ and were stored in glass containers before counting. The filters were visually examined using a stereoscopic microscope and the number, type, and colour of the microplastics were recorded. Microplastics were categorized into five types based on their shape: fragments, fibres, foams, films, and pellets.

Fragments are irregularly shaped microplastics, fibres are thin and elongated microplastics, foams are soft and porous, films are thin and transparent microplastics, and pellets are globular in shape and hard microplastics. Visible microplastic samples (n = 18) were selected for isotopic and elemental analysis.

### 2.3.3. Stable Isotope and Elemental Analysis

The $\delta^{13}C$ values and the %C of the plastic polymers were determined by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS) in continuous flow mode. Samples and standards (100-250 µg) were loaded into tin capsules and dropped into the reactor via an autosampler.

The $\delta^{2}H$ and the %H of the plastic polymers were determined by thermal combustion elemental analyzer-isotope ratio mass spectrometry (TCEA-IRMS) in continuous flow mode. Samples and standards were placed in a vacuum desiccator for 3 days prior to analysis to remove any surface hydrogen. Samples and standards (100-250µg) were loaded into silver capsules and dropped into the reactor via autosampler.

Delta notation (δ) is used to report the isotopic composition of a sample, in per mil (‰), calculated using equation [1]:

$$\delta^{h}E = \left( \frac{\left(\frac{^{h}E}{^{l}E}\right) sample}{\left(\frac{^{h}E}{^{l}E}\right) standard} - 1 \right) x\ 1000 \qquad [1]$$

where "*h*" refers to the heavy isotope, "*l*" refers to the light isotope, and *"E"* refers to the element being measured. The standard reference material for carbon isotope analysis is Vienna Pee Dee Belemnite (VPDB) and for hydrogen it is Vienna Standard Mean Ocean Water (VSMOW). Two Lglutamic acid standards (USGS40, $\delta^{13}C$ = -26.39 ± 0.04‰; USGS41a, $\delta^{13}C$ = 36.55 ± 0.08‰) were used as certified reference materials for carbon and two polyethylene standards (IAEA-CH7, $\delta^{2}H$ = -100.3 ± 2‰; USGS77, $\delta^{2}H$ = -75.9 ± 0.6‰) were used as certified reference materials for hydrogen. Calibration was performed by analyzing the certified reference materials at the beginning, middle, and end of each run and then calculating a calibration line using the known and measured isotope values of the reference materials. To monitor the quality of analysis performance, internal standards were analyzed. A keratin standard was used for carbon and a polyethylene standard was used for hydrogen.

### 2.3.4. FTIR Analysis

In order to confirm the classification of the unknown microplastics based on their isotopic and elemental composition, FTIR spectroscopy was performed using a Spectrum 2 FTIR spectrometer (Perkin-Elmer, USA) with a universal attenuated total reflectance accessory (Perkin-Elmer, USA). Spectra were generated from 16 scans, covering the spectral range of 400-4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. A spectral library search (Perkin-Elmer ATR of Polymers Library, Perkin-Elmer) was used for polymer identification in the Perkin-Elmer Spectrum software.

### 2.3.5. Data Analysis

Statistical tests were performed using XLSTAT (Addinsoft, New York, NY, USA). The averages, standard deviation, and the minimum and maximum values were calculated for each polymer type. A Shapiro-Wilk’s normality test was used to test for normality. If groups were normally distributed, an ANOVA test was used to test for statistically significant differences between the groups. If groups were not normally distributed, the Kruskal-Wallis test was used to test for. Dunn's post hoc test was used to determine which groups are different between the polymer types. Graphics were prepared using Microsoft EXCEL (version 2311; Microsoft, Redmond, WA, USA).

## 2.4. Results and Discussion

### 2.4.1. Carbon and Hydrogen Isotopic Composition and Concentration of Commercial Plastics

The carbon and hydrogen stable isotope ratios were obtained for commercial plastic products from six polymer types and mean values and ranges were reported for each polymer type (Table 1). The $\delta^{13}C$ values of the commercial plastics ranged from -25.0‰ to -38.0‰. The lowest mean $\delta^{13}C$ values were seen in PVC (-35.3 ± 3.8‰) and the highest mean $\delta^{13}C$ values were observed in PET (-28.0 ± 0.6‰). Significant differences (Kruskal-Wallis test, $P < 0.05$) were observed in the $\delta^{13}C$ values between the different plastic polymer types. Polyethylene terephthalate (PET) was significantly different from all other polymer types ($P < 0.05$), PP was significantly different from all other polymer types ($P < 0.05$) except for PS ($P = 0.240$), and PS was significantly different from PET, HDPE, and PVC ($P < 0.05$), but not LDPE ($P = 0.240$) and PP. High density polyethylene (HDPE) was significantly different from PET, PP, and PS ($P < 0.05$), but not LDPE ($P = 0.248$) or PVC ($P = 0.485$), and PVC was significantly different from PET, PP, and PS ($P < 0.05$) but not HDPE or PVC ($P = 0.073$). All plastic products included in this study came from petroleum feedstocks, and the $\delta^{13}C$ values of all polymer types were within the range of $\delta^{13}C$ values reported for petroleum, suggesting that the different reactions that occur during plastic synthesis do not result in significant carbon isotope fractionation (Yeh and Epstein, 1981). Therefore, the $\delta^{13}C$ values of plastic polymers likely represent the C isotopic composition of their feedstocks (Rogers et al., 2021). The $\delta^{13}C$ values reported here are consistent with those reported in other studies, with similar ranges observed for PE (-29.2 to -23.5‰, Battulga et al., 2023; -35.5 to -27.3‰, Berto et al., 2017; and -32.1 to -25.4‰, Suzuki et al., 2010), PP (-31.4 to -24.9‰, Battulga et al., 2023; and -30.1 to -26.2‰, Santos and Rodrigues, 2021), and PVC (-41.3 to 23.5‰, Dietz et al., 2012). Previous studies have noted that the carbon isotopic composition of plastics can vary depending on the geographical source of their feedstock material and the country the plastic was produced in (Birch et al., 2021). The means and standard deviations for all the polymer types were similar and ranges for some overlapped, so care should be taken if solely relying on the $\delta^{13}C$ values to discriminate between polymer types.

***Table 1*** *Summary table of $\delta^{13}C$ and $\delta^{2}H$ values of plastic polymers in this study*

| **Polymer type** | **Mean $\delta^{13}C$ ± SD (‰)** | **Range $\delta^{13}C$ ‰** | **Mean $\delta^{2}H$ ± SD (‰)** | **Range $\delta^{2}H$ ‰** |
|---|---|---|---|---|
| **PET (n = 10)** | -28.0 ± 0.6 | -28.9 to -26.8 | -59.2 ± 9.3 | -75.7 to -44.0 |
| **HDPE (n = 9)** | -32.0 ± 1.9 | -34.6 to -28.2 | -77.2 ± 8.9 | -94.1 to -62.1 |
| **PVC (n = 7)** | -35.3 ± 3.8 | -38.0 to -29.6 | -14.8 ± 49.3 | -112.9 to 25.3 |
| **LDPE (n = 10)** | -31.0 ± 1.5 | -34.3 to -28.0 | -66.5 ± 14.9 | -90.5 to -40.6 |
| **PP (n = 9)** | -29.0 ± 2.3 | -31.9 to -25.0 | -107.0 ± 46.6 | -207.0 to -45.9 |
| **PS (n = 8)** | -30.2 ± 1.0 | -31.3 to -27.8 | -20.5 ± 54.9 | -81.1 to 77.8 |

The $\delta^{2}H$ values for all samples ranged from -207.0 to 77.8‰. The lowest mean $\delta^{2}H$ values were observed in PP (-107.0 ± 46.6‰) and the highest mean $\delta^{2}H$ values were observed in PVC (-14.8 ± 49.3‰). The $\delta^{2}H$ values of polymers characterized in this study have a wide range of $\delta^{2}H$ values relative to previously reported values for petroleum (-85 to -181‰, Yeh & Epstein, 1981). Results in this study are consistent with previous studies reporting $\delta^{2}H$ values for plastics.

Previous studies have observed ranges of -48 to -105‰ (Taylor et al., 2008), -31.3 to -60.8‰ (Rogers et al., 2021), -46.91 to -121.58‰ (Kuznetsova et al., 2023), and -6.03 to -109.61‰ (Jones et al., 2018) for PE. Rogers et al., (2021) reported one $\delta^2$H value for PP, -102.8‰, and a range of -28.8 to 97.8‰ for PS (Rogers et al., 2021). Statistically significant differences (Kruskal-Wallis test, $P < 0.05$) were observed in the $\delta^2$H values between the different plastic polymer types. All polymer types were significantly different from each other ($P < 0.05$) except for two pairings: HDPE and PP ($P = 0.140$), and PVC and PS ($P = 0.892$).

The $\delta^2$H values in PVC, PP, and PS revealed two distinct groupings of different samples within each of those polymer types (Table 2). In PVC, one grouping (PVC 1) had positive $\delta^2$H values (mean 9.9 ± 9.6‰) and one (PVC 2) had more negative values (mean -107.6 ± 4.4‰). In PP, one group (PP 1) had more positive $\delta^2$H values (mean -87.4 ± 24.9‰) while the other group (PP 2) had the most negative $\delta^2$H values of all the samples (mean -188.1 ± 15.4‰). In PS, one group (PS 1) also exhibited positive $\delta^2$H values (mean 45.1 ± 26.6‰) and the other group (PS 2) had more negative $\delta^2$H values (mean -59.9 ± 13.2‰). Rogers et al., (2021) reported positive $\delta^2$H values for two samples of recycled petroleum-based plastic. They suggested that the heating and reforming of the plastics with steam resulted in the more positive $\delta^2$H values (Rogers et al., 2021). Plastic synthesis occurs using either condensation or addition reactions, both of which could produce hydrogen isotope fractionation (Rogers et al., 2021). As well, the temperature of polymer synthesis may also influence isotopic fractionation, resulting in measurable differences in $\delta^2$H values between polymer types, or even within the same polymer type, depending on the specific method used for each individual plastic sample.

Differences in carbon isotopic composition have been observed between more flexible bags and other, more rigid products in some polymers, although other polymers do not exhibit similar trends (Berto et al., 2017). The samples in the PVC 1 group were much more rigid than those in PVC 2, and those in PS 1 were more flexible than the PS 2 group. Some polymers may be more likely than others to have their isotopic composition affected by the presence of additives, and certain polymer types may have more additives incorporated than others. For example, PVC has low thermal stability and requires the addition of heat stabilizers (Hahladakis et al., 2018). PVC also is quite rigid unless plasticizers are added. Plasticized PVC is the polymer that requires the highest volumes of additives; flexible PVC can be 60-70% plasticizer by mass (Andrady and Rajapakse, 2019). Some of the most common additives used in plastic production include flame retardants, UV stabilizers, plasticizers, fillers, and pigments (Wiesinger et al., 2021). The effect of pigments on the hydrogen isotopic composition of plastics has not yet been investigated, however some studies have reported changes to $\delta^{13}C$ values of differently coloured plastics (Battulga et al., 2023; Birch et al., 2021).

***Table 2*** *Summary table of isotope ratios and %C and %H of PVC, PP, and PS groups in this study*

| Polymer type | Mean $\delta^2H$ ± SD (‰) | Mean $\delta^{13}C$ ± SD (‰) | Mean %H ± SD | Mean %C ± SD |
|---|---|---|---|---|
| **PVC 1 (n = 5)** | 9.9 ± 9.6 | -29.6 ± 0.1 | 4.3 ± 0.3 | 41.8 ± 0.4 |
| **PVC 2 (n = 2)** | -107.6 ± 4.4 | -37.6 ± 0.2 | 4.6 ± 0.1 | 40.1 ± 0.7 |
| **PP 1 (n = 7)** | -87.4 ± 24.9 | -28.6 ± 2.5 | 13.5 ± 1.0 | 83.8 ± 5.8 |
| **PP 2 (n = 2)** | -188.1 ± 15.4 | -30.3 ± 0.1 | 13.0 ± 0.9 | 79.1 ± 7.2 |
| **PS 1 (n = 3)** | 45.1 ± 26.6 | -30.6 ± 0.4 | 7.9 ± 0.2 | 92.2 ± 1.3 |
| **PS 2 (n = 5)** | -59.9 ± 13.2 | -29.9 ± 1.1 | 7.8 ± 0.5 | 94.1 ± 2.9 |

Carbon and hydrogen concentrations were measured in commercial plastic products from six polymer types and mean values and ranges were reported for each polymer type (Table 3). The %C values ranged from 39.1% to 98.4%. The lowest mean %C values were observed in PVC (40.8 ± 1.1%) and the highest mean %C values were observed in PS (93.2 ± 2.4%). Significant differences (Kruskal-Wallis test, $P < 0.05$) were observed between mean %C values of the different polymer types. High density polyethylene (HDPE) was not significantly different from LDPE ($P = 0.999$) and PP ($P = 0.558$) but was significantly different from PET, PVC, and PS ($P < 0.05$). Polyethylene terephthalate (PET) and PVC were not significantly different from each other ($P = 0.197$), however they both were significantly different from HDPE, LDPE, PP, and PS ($P < 0.05$). Polystyrene (PS) was significantly different from all other polymer types ($P < 0.05$). The %H values ranged from 4.0% to 15.1%. The lowest mean %H values were observed in PET (4.3 ± 0.1%) and the highest mean %H values were observed in LDPE (13.3 ± 0.3%). Significant differences ($P < 0.05$) were observed between mean %H values for the different polymer types. High density polyethylene (HDPE) was not significantly different from LDPE ($P = 0.772$) and PP ($P = 0.313$), and LDPE was also not significantly different from PP ($P = 0.180$); however, HDPE and LDPE both were significantly different from all the other polymer types included in this study ($P < 0.05$). Polyethylene terephthalate (PET) and PVC were not significantly different from each other ($P = 0.784$), although they were significantly different from all other polymer types included in this study ($P < 0.05$). As well, PS was significantly different from all other polymer types in this study ($P < 0.05$) except for PP ($P = 0.089$).

*Table 3 Summary table of %C and %H of plastic polymers in this study*

| Polymer type | Mean %C ± SD | Range %C | Mean %H ± SD | Range %H |
|---|---|---|---|---|
| **PET (n = 10)** | 63.2 ± 0.9 | 61.6 to 65.3 | 4.3 ± 0.1 | 4.1 to 4.5 |
| **HDPE (n = 9)** | 85.9 ± 2.9 | 79.8 to 88.6 | 14.0 ± 0.6 | 12.9 to 14.7 |
| **PVC (n = 7)** | 40.8 ± 1.1 | 39.1 to 42.1 | 4.4 ± 0.3 | 4.0 to 5.0 |
| **LDPE (n = 10)** | 86.3 ± 2.2 | 82.7 to 89.7 | 14.3 ± 0.3 | 13.6 to 15.1 |
| **PP (n = 9)** | 82.3 ± 6.3 | 71.6 to 89.3 | 13.3 ± 0.9 | 11.8 to 14.5 |
| **PS (n = 8)** | 93.2 ± 2.4 | 90.5 to 98.4 | 7.9 ± 0.4 | 7.2 to 8.6 |

Most of the mean %C and %H values reported in this study fit with the expected %C and %H values for each polymer according to their chemical composition, except for PP, although all polymer types exhibited a range of up to 18% in their %C and 3% in their %H. Some variation in %C and %H values within each polymer type may be attributed to the presence of additives in different samples, as additives are often included in plastic products (Andrady and Rajapakse, 2019; Hahladakis et al., 2018). Other studies have also observed variations from expected %C and %H compositions of different polymer types, with Zhang et al., (2023) observing lower than expected mean %C values for PET (60.4% ±1.4), HDPE (80.5% ±3.4), LDPE (83.8% ± 1.0), and PP (84.1% ± 0.7) samples (Zhang et al., 2023). Rogers et al., (2021) also reported wide ranges of %C values for some polymers, reporting a range of 78.3% to 84.4% for PE and 70.1% to 92.1% for PS (Rogers et al., 2021). There is a lack of transparency regarding reporting on the additive substances present in plastics, making it difficult to understand the effects that additives may have on the chemical composition of the plastics (Wiesinger et al., 2021). The variation in %C and %H observed here and in other studies is possibly explained by differences in the amount and type of additives used in different plastic products.

### 2.4.2. Polymer Type Identification

Biplots were used to assess the ability of carbon and hydrogen stable isotope composition and concentration to discriminate between different petroleum-based plastic polymers (Figures 1A-1D). The $\delta^{13}$C vs. %C biplot (Figure 1A) and %C vs. %H biplot (Figure 1D) showed separation between PET and PVC based on the %C. Polystyrene (PS) was slightly separated from the cluster of HDPE, LDPE, and PP, because of its higher %C values, although some variation was observed and some PS samples had a lower %C, causing some overlap with HDPE, LDPE, and PP. Unlike some other polymers, PET and PVC are not just composed of carbon and hydrogen, there is also oxygen in the structure of PET and chlorine in the structure of PVC. As a result, PET and PVC will have lower %C and %H than all the other polymer types. High density polyethylene (HDPE) and LDPE have the same chemical composition and PP has the same ratio of carbon to hydrogen atoms. Therefore, it was expected that they would be indistinguishable based on their %C and %H values. Polystyrene (PS) has a different ratio of carbon to hydrogen atoms in its structure, so it was expected to have higher %C and lower %H than HDPE, LDPE, and PP, which was observed in our results. As previously reported (Berto et al., 2017; Birch et al., 2021), the $\delta^{13}$C values of all polymer types overlap (Figures 1A and 1C), making it impossible to distinguish between the different polymer types using only their carbon isotopic composition. Visually, there was a large amount of overlap between the $\delta^{13}$C values, except for the PVC 2 group, which had much lower $\delta^{13}$C values than all other samples. The addition of the $\delta^{2}$H values to the $\delta^{13}$C values (Figure 1C) does not do much to differentiate between the polymer types included in this study.

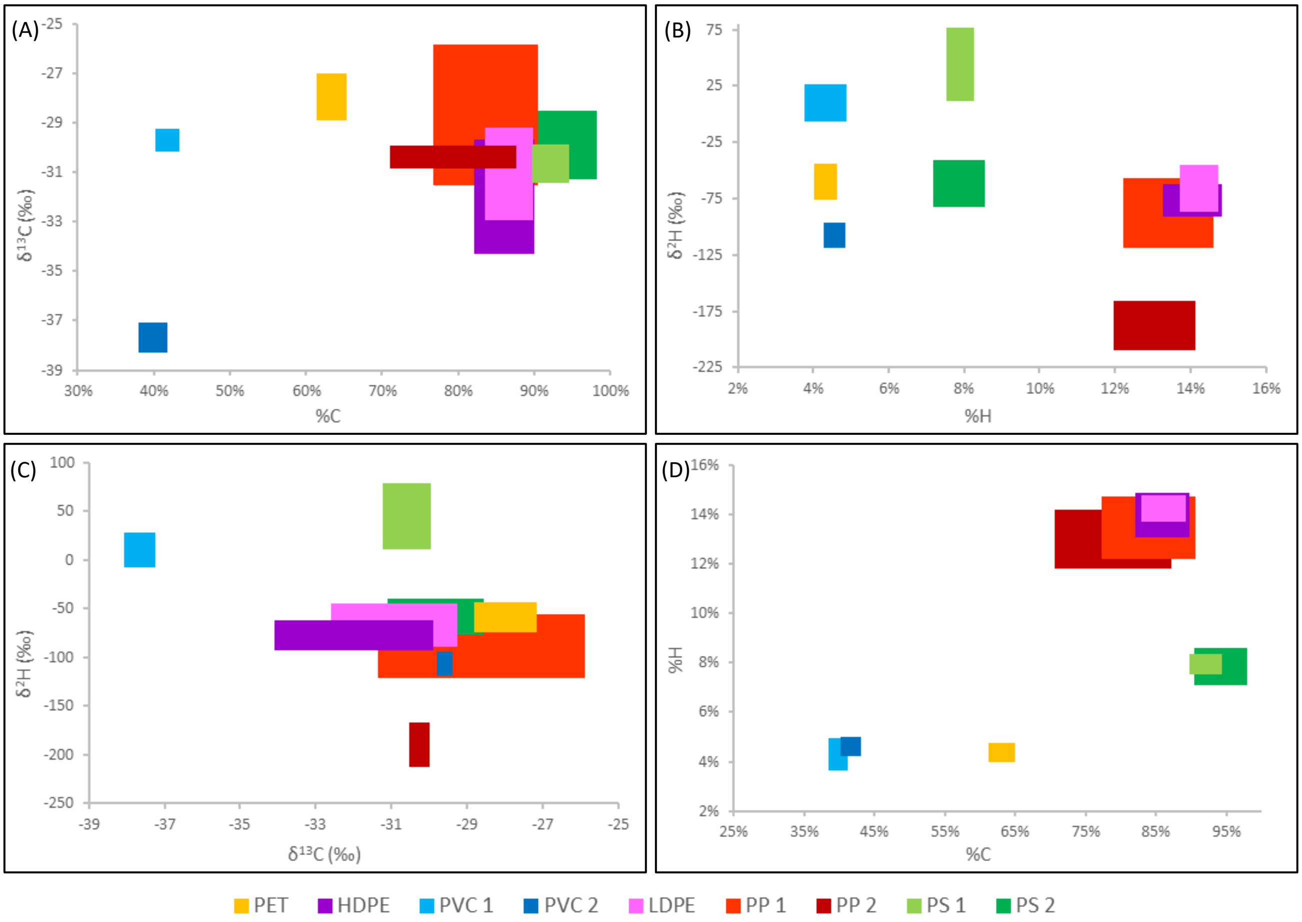

***Figure 1.*** *Biplots showing the separation of different polymer types based on the means ± 1 SD of the $\delta^{13}C$ vs. % C (A); $\delta^{2}H$ vs. % H (B); $\delta^{13}C$ vs. $\delta^{2}H$ (C); and % C vs. % H (D)*

Using $\delta^{2}H$ vs. %H (Figure 1B), it was possible to distinguish between several different polymer types. While PET and the two PVC groups have similar %H values, their $\delta^{2}H$ values are significantly different. PS is easily distinguishable from all other polymer types based on its %H. As observed in the %C vs. %H biplot (Figure 1D), HDPE, LDPE, and PP overlap, however there is more variation observed in the $\delta^{2}H$ values of PP, with the PP1 and PP 2 group having slightly lower and much lower $\delta^{2}H$ values, respectively, compared to HDPE and LDPE (Figure 1B). The combination of $\delta^{2}H$ and %H was able to distinguish between PET, PVC, and PS and between HDPE, LDPE, and PP.

### 2.4.3. Microplastics Identification

The carbon and hydrogen concentration and isotopic compositions of 18 microplastics collected from the Red River in Manitoba, Canada are reported in Table 4. The $\delta^{13}C$ values of the microplastics ranged from -28.1 to -31.3‰ and the $\delta^{2}H$ values ranged from -1.5 to -380.5‰. The %C values ranged from 65.0 to 86.4% and the %H values ranged from 3.4 to 14.3%.

The $\delta^{13}C$, $\delta^{2}H$, %C, and %H values of the samples MP-1, MP-2, MP-3, MP-4, MP-5, MP-6, MP-11, MP-15, and MP-16 all fit with the characterized $\delta^{13}C$, $\delta^{2}H$, %C, and %H composition of the HDPE, LDPE, and PP 1 polymer types. As mentioned previously, these polymer types were expected to be difficult to distinguish between because of their similar molecular structures. The $\delta^{13}C$, $\delta^{2}H$, %C, and %H values of the samples MP-7, MP-8, and MP-17 all fit with the characterized $\delta^{13}C$, $\delta^{2}H$, %C, and %H composition of the PP 1 polymer type. While being similar to HDPE and LDPE, MP-7 had a lower %H, which was only within the range of PP 1, and MP-8 and MP-17 had more negative $\delta^{2}H$ values, which were not observed to be consistent with HDPE and LDPE. The $\delta^{13}C$, $\delta^{2}H$, %C, and %H values of the sample MP-13 fit with the characterized $\delta^{13}C$, $\delta^{2}H$, %C, and %H composition of the PVC 1 polymer type. The $\delta^{13}C$, $\delta^{2}H$, %C, and %H values of the samples MP-14 and MP-18 fit with the characterized $\delta^{13}C$, $\delta^{2}H$, %C, and %H composition of the PS 2 polymer type. The sample MP-14 had a more negative $\delta^{2}H$ value than we observed in the PS commercial plastics, however the %H value of the sample was consistent with PS, so it was identified accordingly.

***Table 4*** *Summary table of isotope rations and %C and %H of microplastics in this study*

| Label | Description | Polymer type | $\delta^{13}$C (‰) | %C | $\delta^{2}$H (‰) | %H |
|---|---|---|---|---|---|---|
| **MP-1** | Pink fragment | HDPE | -31.0 | 83.6 | -61.7 | 14.1 |
| **MP-2** | Black fibre | PP | -29.4 | 84.4 | -86.1 | 14.0 |
| **MP-3** | White fragment | HDPE | -28.4 | 85.2 | -59.0 | 14.3 |
| **MP-4** | Pink fragment | HDPE | -31.3 | 80.8 | -66.3 | 14.2 |
| **MP-5** | White fragment | PP | -30.4 | 83.3 | -70.9 | 14.2 |
| **MP-6** | White pellet | PP | -28.1 | 86.4 | -70.7 | 14.1 |
| **MP-7** | White foam | PS | -29.1 | 78.4 | -78.0 | 12.6 |
| **MP-8** | White fragment | PP | - | - | -119.5 | 14.3 |
| **MP-9** | Black foam | PE (Cl) | -29.6 | 65.0 | -376.7 | 3.6 |
| **MP-10** | Black foam | PE (Cl) | - | - | -380.5 | 3.4 |
| **MP-11** | Black fragment | PP | - | - | -76.4 | 14.0 |
| **MP-12** | White film | PE (Cl) | - | - | -208.2 | 8.2 |
| **MP-13** | White fragment | PVC | - | - | -1.5 | 4.1 |
| **MP-14** | White foam | PS | - | - | -158.4 | 6.8 |
| **MP-15** | White fragment | PP | - | - | -52.3 | 14.2 |
| **MP-16** | Black fragment | HDPE | - | - | -66.5 | 14.2 |
| **MP-17** | Blue fragment | PP | - | - | -102.0 | 13.9 |
| **MP-18** | Orange fragment | PS | - | - | -83.5 | 7.0 |

FTIR analysis was performed on each of the microplastics to see how well the identification of their polymer type using that method correlated with our identification using the carbon and hydrogen concentration and isotopic composition. One microplastic was identified using FTIR as PVC, three were identified as PS, three were identified as chlorinated PE (PE(Cl)), four were identified as HDPE, and seven were identified as PP. The identifications of all except for one of the microplastics using their $\delta^{13}C$, $\delta^{2}H$, %C, and %H values correlated with the identification made using FTIR. The sample MP-7 was initially identified as being from the PP 1 polymer type, however the FTIR analysis identified it as being composed of PS. The sample had a higher %H than what was observed in the commercial PS products, instead fitting in with the range expected for PP 1. Because the %H of the microplastic is not typical for PS, it is possible that the FTIR incorrectly identified this microplastic. The FTIR identification relied on a qualitative matching of the microplastics' spectra to those present within the spectral library used. This represents a potential source of errors. The FTIR spectra can be affected by weathering, making it possible that weathered microplastics can be misidentified. However, the isotopic and elemental composition provides a quantitative result, meaning that the potential for misidentification due to improper qualitative interpretation is eliminated. Most of the microplastics composed of the HDPE, LDPE, and PP polymer types could only be identified as coming from one of the three types, as they were too similar to be distinguished. However, they were clearly separated from PET, PVC, and PS, so are still distinct from the other polymer types.

We plotted $\delta^{2}H$ and %H values of the microplastic samples for comparison with the $\delta^{2}H$ and %H values characterized from the commercial plastic samples in our database of polymer types (Figure 2). As discussed, there is a cluster of microplastics that closely match the $\delta^{2}H$ and %H values of HDPE, LDPE, and PP 1, which are difficult to further identify due to their similar molecular structure. The microplastics identified as PP 1 are distinct due to their more negative $\delta^{2}H$ values, and differences in the %H allow the PVC and PS microplastics to be easily distinguished.

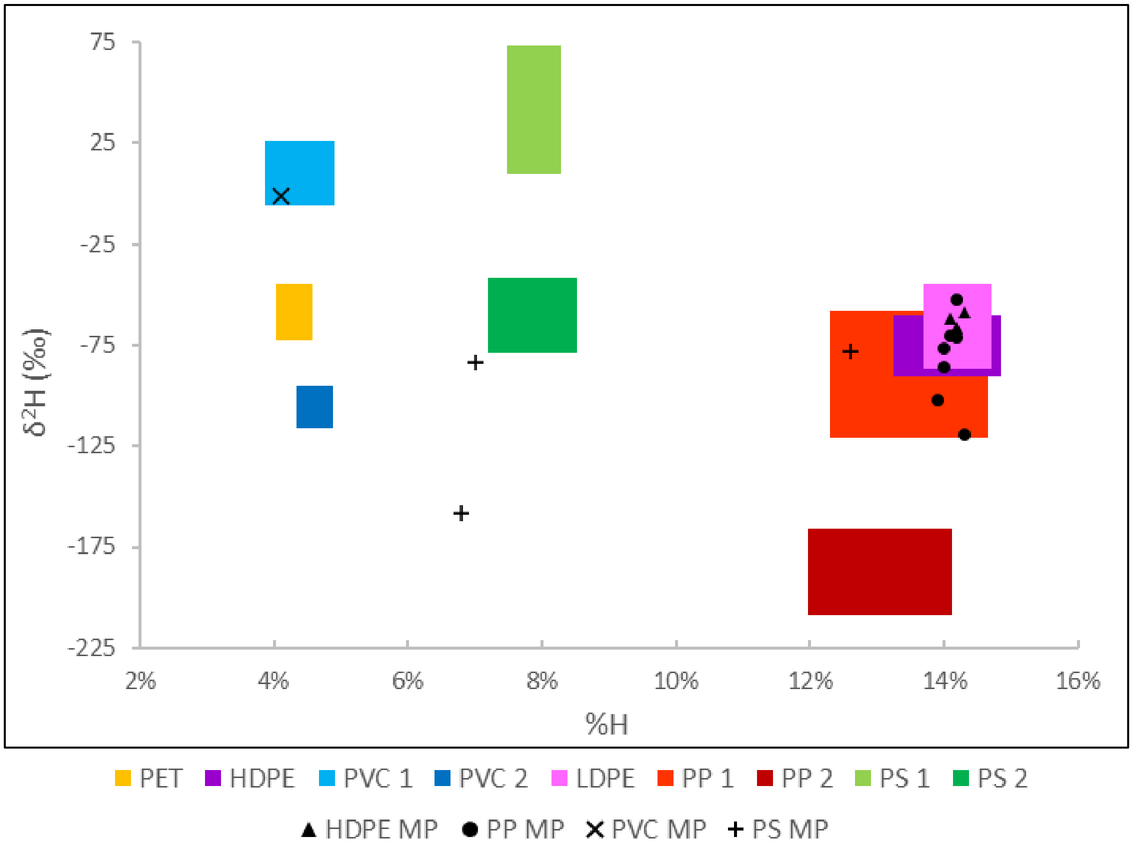

***Figure 2** Microplastic samples compared with the mean ± 1 SD of $\delta^2$H and %H*

The mean $\delta^{13}$C values of the microplastics were -30.2‰ ± 1.6, -29.3‰ ± 1.2, -29.1‰ ± 0, and -29.6‰ ± 0 for HDPE, PP, PS, and PE(Cl), respectively. The $\delta^{13}$C values of the microplastics are generally similar to those observed in the commercial plastic products in this study and reported in others (Berto et al., 2017; Birch et al., 2021; Rogers et al., 2021). Aged plastics have been reported to have slightly higher δ13C values than pristine plastics (Battulga et al., 2023; Berto et al., 2017; Kuznetsova et al., 2023). Changes to the hydrogen isotopic composition have also been observed but have been less studied than the carbon isotopic composition (Kuznetsova et al., 2023). Our results also indicate that microplastics collected from the environment have slightly higher $\delta^{13}$C values than commercial plastics not weathered after exposure to the environment. The exact degradation process that results in the changes to the isotopic composition of the plastic has not yet been determined (Kuznetsova et al., 2023).

The mean $\delta^2$H values of the microplastics were -63.4‰ ± 3.7, -1.5‰ ± 0, -82.6‰ ± 22.3, -106.6‰ ± 44.9, and -321.8‰ ± 98.4 for HDPE, PVC, PP, PS, and PE(Cl), respectively. The $\delta^2$H values of the microplastics generally fit within the ranges of the $\delta^2$H values observed in the commercial plastic products in this study and reported in others (e.g., Rogers et al., 2021; Jones et al., 2018; Kuznetsova et al., 2023). The PE(Cl) microplastics had much more negative $\delta^2$H values than all the other polymers, however PE(Cl) commercial plastic products were not included in this study, so it is unknown at this time whether these values are characteristic for this polymer type. The mean %C values of the microplastics were 83.2% ± 2.2, 84.7 ± 1.6, 78.4% ± 0, and 65.0% ± 0 for HDPE, PP, PS, and PE(Cl), respectively. The %C values reported for the microplastics are consistent with those reported for commercial plastic products in this study and others (e.g., Rogers et al., 2021; Zhang et al., 2023). The mean %H values reported for the microplastics were 14.2% ± 0.1, 4.1% ± 0, 14.1% ± 0.1, 8.8% ± 3.3, and 5.1% ± 2.7 for HDPE, PVC, PP, PS, and PE(Cl), respectively, which are consistent with the %H values reported in this study.

All of the microplastics included in this study were collected from surface water samples. The ones most commonly identified were HDPE and PP. The densities of both HDPE (0.93-0.97 g/cm$^3$) and PP (0.90-0.92 g/cm$^3$) are lower than water and will float. Therefore, these polymer types were more likely to be collected than other polymer types using surface water sampling techniques. It is expected that the amounts of polymer types found in the environment will be proportional to type of polymer that is produced and used. Both HDPE and PP are among the most commonly produced polymer types, representing 12.2% and 19.3% of global plastic demand respectively (Plastics Europe, 2019). Information on the polymer types of microplastics found in the environment is essential to understanding the effects that they may have on organisms. HDPE and PP are produced from some of the least hazardous monomers, while the monomers and chemicals used in the production of PVC and PS are known to have negative effects on organisms (Lithner et al., 2011; Rochman, 2015). Importantly, PVC and PS were able to be distinguished from all other polymer types in this study by their $\delta^2$H and %H values. Our method is able to distinguish more harmful polymer types from less harmful ones, providing valuable information for assessing the risk of microplastics in the environment.

## 2.5. Conclusion

Using a combination of % C vs. % H, and $\delta^2$H vs. %H we are able to uniquely characterize most of the polymer types. The development of new techniques to identify the polymer type of microplastics will have significant implications for the study of microplastics, allowing for the identification of polymer type regardless of the presence of dyes in the plastic or the effects of weathering. Our results demonstrate that while the carbon isotopic composition does not vary between different polymer types, the variation present in the hydrogen isotopic composition and the hydrogen and carbon concentrations between different polymer types can be used distinguish between polymer types, representing a novel method for identifying the polymer type of microplastics. As well, this study represents the first characterization of the %H of plastics. While other methods, such as FTIR, rely on qualitatively matching a spectra from a microplastic to the best fit in a spectra library, using the isotopic and elemental composition provides a quantitative number, thus eliminating some bias and uncertainty in the identification, which makes it an attractive method for identifying the polymer type of microplastics.

Information on the specific polymers that are contributing to microplastic pollution in the environment is key to understanding their potential effects on organisms. Our method is able to distinguish PVC and PS, some of the most harmful common plastic polymers, from other polymers, thereby providing information on the risk that microplastics in the environment may pose. In addition, characterizing the polymer types that are the major contributors to microplastic pollution is critical because this type of information can be used by government agencies to create policy regarding the use of plastics in society.

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# 3. Seasonal Variations in Microplastic Concentrations in the Red River, Manitoba

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## 3.1. Abstract

Microplastic concentrations were investigated in the Red River in Manitoba, Canada. Surface water samples were collected using a pump-based sampling system from nine sites along the river in the spring, summer, and fall to evaluate spatial and temporal trends in microplastic concentrations. Microplastics were found in all samples (n = 19), with concentrations ranging from 70.0 to 268.7 particles m-3. Significant differences were observed between the seasons, with the spring having the highest average microplastic concentrations (147.2 ± 55.1 particles m-3), followed by the summer (102.6 ± 26.9 particles m-3) and fall (90.8 ± 16.2 particles m-3). Increased flow rates and stormwater runoff in the spring likely influenced this seasonal variation. Fibers were the most common morphology found. No differences were observed between microplastic concentrations upstream and downstream of a wastewater treatment plant, suggesting that it is not a major point source of microplastics to the Red River. Stormwater runoff, combined sewage overflows, and agricultural runoff, especially from land where biosolids had been applied, are potential sources of microplastics to the Red River.

## 3.2. Introduction

Since the 1950s, our use of plastic materials has increased dramatically. Plastic has many positive qualities, such as its light weight, durability, low cost to produce, and versatility as a material (Andrady and Neal, 2009). In 2021, 390.7 million tonnes of plastic were produced worldwide (Plastics Europe, 2022). Improper waste management has resulted in 19-23 million metric tonnes of plastic entering aquatic ecosystems annually (Borrelle et al., 2020). While the durability of plastic is a positive quality for commercial applications, it results in the persistence of plastic in the environment. Plastics do not completely decompose, instead fragmenting into smaller particles through physical abrasion and exposure to UV radiation (Andrady, 2003; Barnes et al., 2009). Pieces of plastic <5 mm in diameter are known as microplastics (Frias and Nash, 2019). Microplastics are ubiquitous in waters and sediments around the world: they have been found in every ocean (Cole et al., 2011), in remote areas (Allen et al., 2019; Aves et al., 2022; Free et al., 2014), in deep sea sediments (Van Cauwenberghe et al., 2013), in the air (Gasperi et al., 2018; Prata, 2018), and even in human blood (Leslie et al., 2022).

Because of their density, some of the most prevalent plastics found as microplastics reside in the parts of the water column where they are available to more organisms, and their small size makes them easily confused for food and ingested (Andrady, 2017). Zooplankton (Cole et al., 2013; Setälä et al., 2014), invertebrates (Goldstein and Goodwin, 2013; Naji et al., 2018; Song et al., 2019), fish (Neves et al., 2015; Ory et al., 2017), sea birds (Bessa et al., 2019; De Pascalis et al., 2022), and marine mammals (Besseling et al., 2015; Fossi et al., 2016) have all been reported to ingest microplastics. Many chemical additives are incorporated during the production of plastics (Lithner et al., 2011). These additives, which can include plasticizers, flame retardants, UV stabilizers, and dyes, can leach from the microplastics into the environment and the tissues of organisms after ingestion (Campanale et al., 2020; Hahladakis et al., 2018; Teuten et al., 2009). Additionally, microplastics can accumulate persistent, bioaccumulative, and toxic substances (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and pesticides) on their surfaces, which can also transfer to organisms upon ingestion (Engler, 2012; Mato et al., 2001; Rochman et al., 2013). Exposure to these chemicals can have negative consequences for the health of organisms (Botterell et al., 2019; Guzzetti et al., 2018; Rochman, 2015). Information on the concentrations and sources of microplastics in aquatic ecosystems is essential to understanding their potential effects.

While the presence of microplastics in marine environments has been well documented, it is only in recent years that more work has been done focusing on microplastics in freshwaters. Rivers are now known to be an important pathway for plastics to enter into the oceans (Klein et al., 2015; Rodrigues et al., 2018; Wagner et al., 2019). It is estimated that 80% of marine microplastics originate from land-based sources and are carried to the oceans through fluvial transport (Andrady, 2011; Cole et al., 2011). Sources of microplastics in rivers include effluent discharge from wastewater treatment plants, combined sewage overflows, storm events, and atmospheric deposition (Dris et al., 2016; Forrest et al., 2022; Talbot and Chang, 2022).

Microplastics have been reported in several lakes and rivers in Canada (e.g., Anderson et al., 2017; Bujaczek et al., 2021; Crew et al., 2020; Eriksen et al., 2013; Forrest and Vermaire, 2023), including the Red River in Manitoba (Warrack et al., 2017). The results from Warrack et al (2017) study suggested that seasonal trends in microplastic concentrations may exist in the Red River. It is important to examine microplastic concentrations at different sampling locations as well as at different times of year, as both factors can influence the abundance of microplastics in freshwaters (Stanton et al., 2020). In this study, we characterize microplastics collected from surface waters of the Red River in the spring, summer, and fall of 2022 to evaluate spatial and temporal trends in microplastic concentrations and identify potential sources. This will show how seasonality affects microplastic concentrations in the Red River, which will contribute to our understanding of the prevalence of microplastics in Canadian freshwaters and their sources.

## 3.3. Methodology

### 3.3.1. Study Area

The Red River originates at the confluence of the Bois de Sioux and Otter Tail rivers in Minnesota and North Dakota and flows northward for 885 km before draining into Lake Winnipeg. It passes through Fargo (population of 127,000) and Grand Forks (population of 59,000) in North Dakota, and Winnipeg in Manitoba (population of 872,000), and has a total drainage area of about 290,000 $km^2$. As it flows north, the Red River is joined by the Pembina River at the border of Canada and the United States, the Roseau River, and the Assiniboine River in the city of Winnipeg.

### 3.3.2. Sample Collection

Surface water samples were collected from nine sites along the Red River during the spring, summer, and fall of 2022 (Figure 1). One site along the Canadian-American border (site 1) was included to evaluate the abundance of microplastics in the Red River as it enters Manitoba from Minnesota and North Dakota. One site located upstream of the city of Winnipeg (site 2) was included as a baseline site before the city. Five sites were chosen within the city limits, including one site upstream (site 6) and one site downstream (site 7) of the North End Water Pollution Control Centre, a municipal wastewater treatment plant. Finally, one site just before where the Red River joins Lake Winnipeg (site 9) was also included to represent near-final inputs into the lake. Sample were collected from each site in May, July, and October of 2022, except for sites 8 and 9, which were only sampled in May and July.

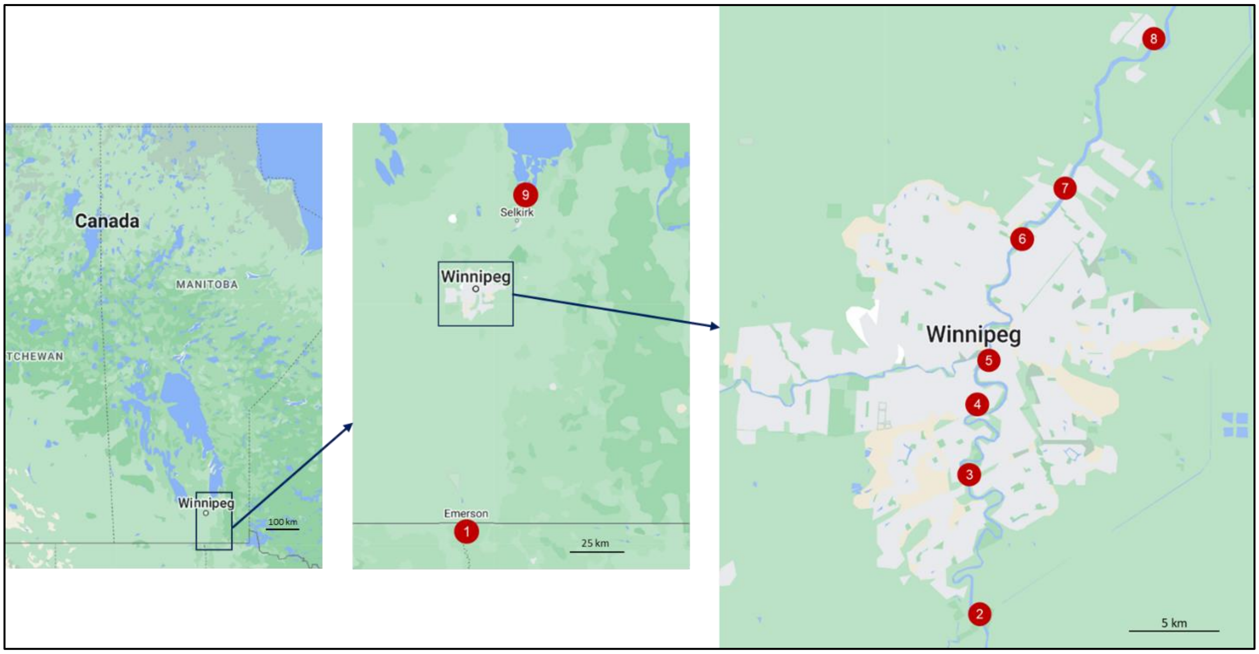

***Figure 1*** *Study area and sample collection sites on the Red River*

Surface water samples were collected using a custom-made mobile pump-based sampling device following the design of Donovan et al., (2019). The device consisted of a polyvinyl chloride (PVC) hose connected to a 12V water transfer pump, a filter housing (polycarbonate) with a PVC lid, and a 35 µm stainless steel cartridge filter (Figure 2). Prior to sampling, the pump was primed for five minutes with river water. Samples were collected from 10 cm below the water surface. A sample volume of 1.5 $m^3$ was filtered. After collection, filters were transferred to glass jars and stored before counting and analysis.

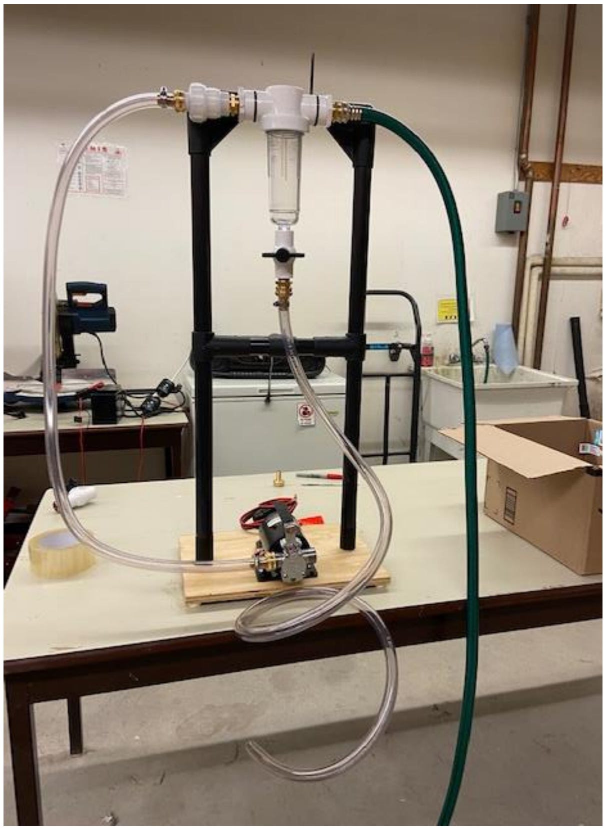

***Figure 2*** *Sampling device, following the design of Donovan et al. (2019)*

### 3.3.3. Sample Processing and Quantification

Samples were processed using a wet peroxide oxidation treatment following the protocol outlined by Masura et al., (2015) to remove organic materials from the samples. The collected filters were rinsed into glass beakers with deionized (DI) water to reconstitute the sample to 100 ml. To facilitate the digestion of organic material, 20 mL of a 0.05 M Fe (II) solution and 20 mL of 30% hydrogen peroxide ($H_2O_2$) was added to the sample. The 0.05 M Fe (II) solution was prepared by adding 7.5 g of $FeSO_4$ to 500 mL of water and 3 mL of concentrated sulfuric acid. The mixture was left to stand on the lab bench at room temperature for five minutes. Then, a stir bar was added to the beaker, and it was covered with a watch glass. The beaker was then placed on a hot plate and heated to 75°C. The sample was left to digest for 30 minutes on the hot plate. At 30-

minute intervals, samples were re-examined and an additional 20 mL of $H_2O_2$ was added if organic material was still present. This process was repeated until all visible organic material was digested. Samples were then covered and left for 24 hours to allow for complete digestion. Samples were then rinsed with DI water to remove the $H_2O_2$ and were stored in glass containers before counting. The filters were visually examined using a stereoscopic microscope and the number, type, and colour of the microplastics were recorded. Microplastics were categorized into five types based on their shape: fragments, fibres, foams, films, and pellets. Fragments are irregularly shaped microplastics, fibres are thin and elongated microplastics, foams are soft and porous, films are thin and transparent microplastics, and pellets are globular in shape and hard microplastics.

### 3.3.4. Quality Control

During sampling, sample processing, and analysis, care was taken to minimize contamination with microplastics from external sources. Working spaces and equipment were cleaned thoroughly prior to use and were rinsed with filtered (0.45 µm filter) DI water. Plastic materials were avoided as much as possible, and cotton lab coats were worn to mitigate contamination from synthetic fibres from clothing and glassware was covered with aluminum foil when not in use to minimize contamination from airborne microplastics. Lab and field blanks were used to evaluate possible contamination from the air. One lab blank was deployed, which consisted of a 0.45 µm filter left exposed to the laboratory air where microplastics analysis took place for the duration of sample processing and analysis. Field blanks were collected at each sample site and consisted of 1 L glass bottles of filtered (0.45 µm filter) DI water left exposed to the air during sample collection. All blanks were processed and analyzed identically to samples. The final counts of identified microplastics in each sample were corrected for blank contamination by subtracting the number of microplastics of each size and shape found in the blanks. Uncorrected counts of microplastics from each site can be found in Table S1.

### 3.3.5. Data Analysis

Concentrations of microplastics were expressed as the number of particles $m^{-3}$ to allow for comparison with other studies. Statistical analyses were performed using XLSTAT software version 2022.4.1 (Addinsoft, New York, USA). The normality of the data was assessed using a Shapiro-Wilk normality test. Comparisons between seasons were done using ANOVA with a Bonferroni post hoc test for multiple pairwise comparisons. To compare the mean concentrations between upstream and downstream of the wastewater treatment plant, Student's *t* test was used. Results were only considered statistically significant when the p value was ≤ 0.05.

## 3.4. Results

Microplastic particles were identified in all surface water samples, with concentrations ranging from 70.0 to 268.7 particles $m^{-3}$. Mean concentrations (± 1 standard deviation) from all sites in the Spring, Summer, and Fall were 147.2 ± 55.1 particles $m^{-3}$, 102.6 ± 26.9 particles $m^{-3}$, and 90.8 ± 16.2 particles $m^{-3}$ respectively (Figure 3). Significant differences were observed between microplastic concentrations in the different seasons ($P = 0.016$), with Spring having significantly higher concentrations than in the Fall ($P = 0.022$) and Summer ($P = 0.05$). There were no differences in the distribution of microplastic morphologies between the sampling seasons ($P > 0.05$). Fibres overwhelmingly represented the majority of particles identified (77.36% ± 4.52%), followed by fragments (18.64% ± 3.96%), films (2.82% ±2.69%), foams (0.79% ± 1.44%), and pellets (0.40% ± 0.79%) (Fig. 4).

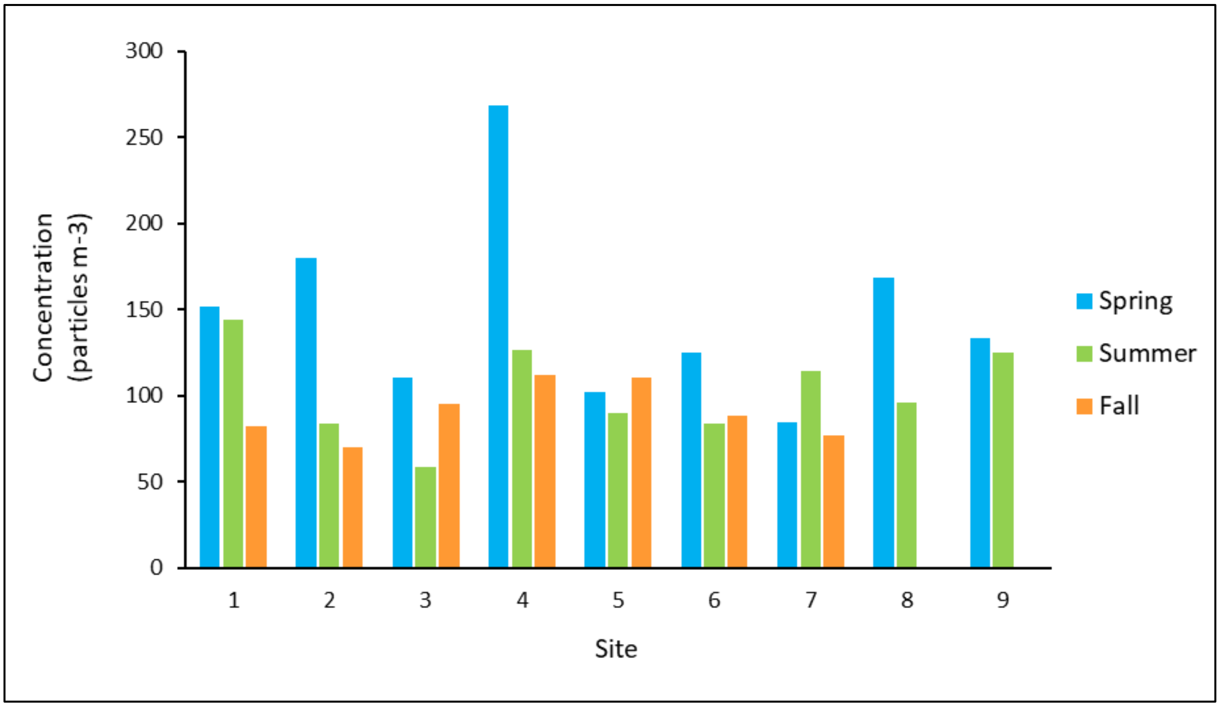

***Figure 4*** *Total concentrations of microplastics in the Red River at sampling sites in the Spring, Summer, and Fall*

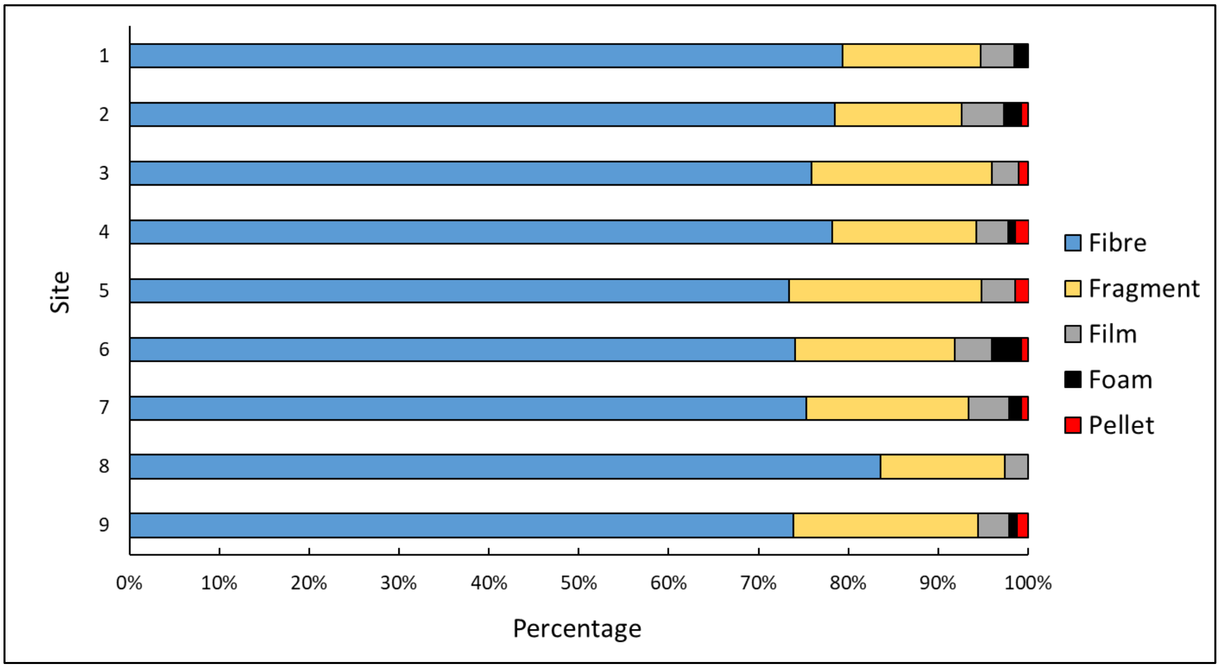

***Figure 3*** *Average percent composition of microplastics of each morphology at sites along the Red River*

## 3.5. Discussion

Concentrations of microplastics reported here are similar to or greater than those reported in surface waters of other river systems across Canada and North America. In the Ottawa River, microplastic concentrations ranged from 20 to 410 particles $m^{-3}$ with an average of 120 particles $m^{-3}$ (Forrest et al., 2019). Mean concentrations of microplastics upstream and downstream of wastewater treatment plants in the St. Lawrence River were 120 and 160 particles $m^{-3}$ respectively (Crew et al., 2020). Estahbanati and Fahrenfeld (2016) reported concentrations of microplastics upstream and downstream of wastewater treatment plants in the Raritan River, with mean concentrations of 24 particles $m^{-3}$ upstream and 71.7 particles $m^{-3}$ downstream of the plants (Estahbanati and Fahrenfeld, 2016). Microplastic concentrations were higher than in the North Saskatchewan River, (Bujaczek et al., 2021), Wascana Creek (Campbell et al., 2017), and the Great Lakes tributaries (Baldwin et al., 2016). In this study, microplastic concentrations in the Red River were higher than those reported by Warrack et al. (2017), which found an average microplastic concentration of 5.3 particles $m^{-3}$ (Warrack et al., 2017). The higher concentrations observed in this study are likely due to differences in the mesh size used during sampling. Warrack et al. (2017) sampled surface water of the Red River using a manta trawl with a mesh size of 333 µm, while in this study, a 35 µm filter was used. Mesh size used influences the amount of microplastics collected during sampling. There are higher concentrations of smaller particles, which means that significant increases in the number of microplastics are observed when mesh size decreases as more microplastics are captured (Dris et al., 2018; Estahbanati and Fahrenfeld, 2016). Differences in sampling methodologies (i.e., grab sampling vs. bulk sampling), sampling location (i.e., depth, distance to shore), and mesh size can affect the amount of microplastics reported. Studies assessing microplastic concentrations in freshwaters are further complicated by factors such as season, river flow, precipitation, presence of suspended matter, etc., making comparisons between studies difficult (Dris et al., 2015). There is a need for standardized sampling methods and reporting to allow for better comparisons between studies and reliable risk assessments. Without standardized methods, it is almost impossible to accurately compare microplastic concentrations in different areas and makes it incredibly difficult to understand the risk that the presence of microplastics may pose in the environment.

Microplastic concentrations in rivers can be influenced by storm events with high amounts of precipitation and increased flow rates. Several studies have reported higher concentrations of microplastics after storms, with as much as a 40-fold increase in concentrations following rainfall (Hitchcock, 2020). In Winnipeg, 2022 was the wettest year on record, with the city receiving over 750 mm of precipitation and much of southern Manitoba experiencing extreme flooding in the spring. Concentrations of microplastics in the Red River changed temporally, with the Spring having higher concentrations than in Summer or Fall. This trend is not likely explained by increased precipitation in the days prior to sampling as average precipitation amounts in the week prior to sampling days were similar in each season (spring: 22.77 mm, summer: 24.66 mm, fall: 20.72 mm). However, flow rate has also been identified as a factor influencing microplastic concentrations. Dense microplastics that had settled in sediments can become resuspended during high energy flows, exacerbating the number of microplastics in the river (Horton and Dixon, 2018; Nizzetto et al., 2016a). The Red River had much higher flow rates in the 7 days prior to sampling in the Spring compared with the other seasons (spring: 2534.5 $m^3/s$, summer: 647.3 $m^3/s$, fall: 110.2 $m^3/s$). This increase in flow could explain the seasonal trends in microplastic concentrations observed here. Further sampling at a high frequency during high flow and storm events should be done to confirm and elaborate on trends in microplastic concentration related to flow rates in the Red River.

Proximity to urban areas has been found to positively influence concentrations of microplastics in freshwaters (André-Marie et al., 2023; Grbić et al., 2020). Our sampling did not find any significant differences in microplastic concentrations between each of the sampling sites ($P > 0.05$) and no trends were observed regarding the relationship between microplastic concentrations and proximity to the city of Winnipeg. The city of Winnipeg releases the treated wastewater from its three wastewater treatment plants into the Red River and the Assiniboine River (a major tributary of the Red River). Previous work by Warrack et al. (2017) found significant differences in microplastic densities upstream and downstream of wastewater treatment plants and identified a correlation between the volume of effluent discharge and microplastic densities near wastewater treatment plants in Winnipeg (Warrack et al., 2017). However, we did not observe any significant differences ($P > 0.05$) between microplastic concentrations at sites immediately upstream and downstream of the North End Water Pollution Control Centre, which processes 70% of the wastewater from Winnipeg, suggesting that the wastewater effluent is not a significant source of microplastics to the Red River. This finding is in contrast to several studies, which reported an increase in microplastics downstream of wastewater treatment plants (Estahbanati and Fahrenfeld, 2016; Leslie et al., 2017; Murphy et al., 2016), but similar to other studies that have found no differences between sites upstream and downstream of wastewater treatment plants (Bujaczek et al., 2021; Crew et al., 2020; Hoellein et al., 2017). Further work should be done to determine the impact that wastewater treatment plants have on microplastic loads in freshwater systems and elaborate on the factors governing their microplastic contributions.

Globally, fibers are often reported as the most prevalent morphology of microplastics in freshwaters (Baldwin et al., 2016; Dris et al., 2015; Wright et al., 2020). In the Red River, the vast majority of collected microplastics were fibers, which is consistent with microplastic morphologies identified in other studies of Canadian rivers (Baldwin et al., 2016; Bujaczek et al., 2021; Campbell et al., 2017; Forrest et al., 2019; Warrack et al., 2017). Microplastic fibers are often derived from the breakdown of larger plastic items and from synthetic materials for clothing. Fibers make up about 90% of microplastics found in wastewater effluent, and a significant source of those fibers is laundry (Grbić et al., 2020). Washing machines can release over 700,000 fibers from an average load of laundry (Napper and Thompson, 2016). Additionally, microplastics in stormwater runoff and airborne microplastics are primarily fibers (Dris et al., 2016; Grbić et al., 2020; Wright et al., 2020).

Fibers and fragments exhibited the greatest diversity of colours compared to films, foams, and pellets (Table S2). The most common colours of fibers were clear and black, although other colours, such as blue, red, brown, and yellow were also identified. Black, red, and white were the most prevalent colours of fragments, with orange, blue, yellow, green, and other colours also identified. The majority of films were clear, while most foams and pellets were white. Information on the range of colours observed in microplastics is important for assessing the risks they may pose to organisms in freshwaters. Some colours of microplastics have posed different levels of risk because some organisms preferentially ingest microplastics that are coloured similarly to their food (Ory et al., 2017; Wright et al., 2013; Xiong et al., 2019). Discoloration and lightening of plastics can occur from weathering and can be indicative of residence time in the environment (Hidalgo-Ruz et al., 2012; Martí et al., 2020), potentially explaining the abundance of clear and lighter colours observed in the Red River.

No significant variation in the distribution of morphologies and colours was observed between seasons or sampling sites on the Red River. Bujaczek et al., (2021) suggested that homogeneity in microplastic concentrations between sampling sites indicates that the microplastics are being introduced to rivers through diffuse, nonpoint sources rather than at discrete locations like wastewater treatment plants (Bujaczek et al., 2021). Stormwater runoff, combined sewage overflows, and agricultural runoff are possibly introducing microplastics to the Red River. Runoff from high precipitation events can transport land-based microplastics into rivers as the stormwater is released. (Forrest et al., 2022; Grbić et al., 2020; Xia et al., 2020). Stormwater runoff in the city of Winnipeg enters the river through several land drainage outfalls located throughout the city. Combined sewer systems convey both sewage and stormwater through the same pipe to a treatment facility. During high precipitation events, treatment facilities can become overwhelmed, and a combined sewage overflow can occur, releasing both stormwater and untreated sewage into the river. Older parts of the city of Winnipeg have a combined sewer system, with 76 combined sewer outfalls located along the Red and Assiniboine Rivers. On average, 22 combined sewer overflows occur at each outfall annually. Normally, the majority of microplastics would have been removed during treatment, but during combined sewer overflows the untreated sewage gets released into the river and with it, the microplastics. (Forrest et al., 2022).

The microplastics removed during wastewater treatment remain suspended in the biosolids (Sivarajah et al., 2023). In many places, including the city of Winnipeg, the nutrient-rich biosolids are applied to agricultural land. This use of biosolids as a fertilizer has been identified as an important pathway for microplastics to enter terrestrial ecosystems (Crossman et al., 2020; Nizzetto et al., 2016b). Biosolids being applied to land in Manitoba possibly represent another source of microplastics to the Red River. Microplastics in sewage sludge may persist in the environment, with synthetic fibers being found in soils up to 15 years after being fertilized with biosolid (Zubris and Richards, 2005). They may also be directly transferred to the river through runoff from the agricultural lands.

## 3.6. Conclusion

Our research shows that microplastic pollution is prevalent in the Red River, at levels similar to or greater than other Canadian rivers. Microplastics were present in every water sample. The majority of microplastics were fibers, which is consistent with other rivers across Canada. This study found more microplastics in the Red River than a previous study, highlighting how differences in sampling methods can impact microplastic concentrations. Seasonal variations in microplastic concentrations were observed, with the spring having significantly higher concentrations than the summer and fall. Higher flow rates in the river and increased stormwater runoff potentially explain these findings. The lack of significant differences between microplastic concentrations at sites upstream and downstream of a wastewater treatment plant suggest that the plant is not acting as a point source for microplastics to the Red River. Atmospheric deposition, stormwater runoff, combined sewer overflows, and biosolids application are possibly significant sources of microplastics to the Red River. The separation of existing combined sewer systems could reduce the amount of microplastics entering rivers through overflow events. The application of biosolids enhances soil health while reducing reliance on synthetic fertilizers and diverting waste from landfills, however it also represents an important source of microplastics to the environment. Existing legislation on the management of pollutants in biosolids should be revised to incorporate microplastics as a pollutant of concern. The results from this study can be used to guide local policy and can inform best management practices for microplastic waste reduction and mitigation in Manitoba.

## 3.7. Supplementary Tables

***Table S1** Uncorrected counts of microplastics in all samples investigated in this study*

| Site Number | Sampling Season | Fragment | Fibre | Film | Pellet | Foam | Overall Total |
|---|---|---|---|---|---|---|---|
| 1 | Spring | 35 | 205 | 4 | 0 | 0 | 244 |
| 2 | Spring | 47 | 240 | 11 | 2 | 1 | 301 |
| 3 | Spring | 37 | 152 | 3 | 0 | 0 | 192 |
| 4 | Spring | 51 | 371 | 9 | 0 | 0 | 431 |
| 5 | Spring | 39 | 130 | 11 | 0 | 0 | 180 |
| 6 | Spring | 41 | 174 | 9 | 0 | 7 | 231 |
| 7 | Spring | 26 | 114 | 8 | 1 | 1 | 150 |
| 8 | Spring | 35 | 236 | 6 | 0 | 0 | 277 |
| 9 | Spring | 38 | 178 | 11 | 1 | 1 | 229 |
| 1 | Summer | 41 | 187 | 2 | 0 | 3 | 233 |
| 2 | Summer | 21 | 132 | 0 | 0 | 4 | 157 |
| 3 | Summer | 23 | 84 | 6 | 1 | 0 | 114 |
| 4 | Summer | 38 | 167 | 8 | 5 | 0 | 218 |
| 5 | Summer | 33 | 120 | 5 | 4 | 0 | 162 |
| 6 | Summer | 32 | 130 | 5 | 1 | 1 | 169 |
| 7 | Summer | 31 | 161 | 3 | 0 | 0 | 195 |
| 8 | Summer | 25 | 142 | 1 | 0 | 0 | 168 |
| 9 | Summer | 53 | 161 | 2 | 1 | 0 | 217 |
| 1 | Fall | 20 | 108 | 10 | 0 | 2 | 140 |
| 2 | Fall | 18 | 106 | 12 | 0 | 0 | 136 |
| 3 | Fall | 35 | 134 | 0 | 0 | 0 | 169 |
| 4 | Fall | 37 | 152 | 6 | 0 | 1 | 196 |
| 5 | Fall | 43 | 149 | 1 | 0 | 0 | 193 |
| 6 | Fall | 29 | 133 | 6 | 0 | 8 | 176 |
| 7 | Fall | 29 | 101 | 7 | 0 | 1 | 138 |

*Table S2 Corrected microplastic counts separated by shape and colour*

| | Clear | Black | Red | White | Gray | Brown | Blue | Green | Pink | Yellow | Orange | Purple |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fragment** | 14 | 273 | 231 | 150 | 31 | 13 | 64 | 27 | 2 | 46 | 67 | 1 |
| **Fibre** | 1648 | 1230 | 277 | 11 | 117 | 192 | 278 | 29 | 9 | 86 | 41 | 5 |
| **Film** | 135 | | | 7 | | 2 | 1 | | | | 1 | |
| **Pellet** | | | 1 | 14 | | | | | | | | |
| **Foam** | | 7 | | 23 | | | | | | | | |

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# 4. Conclusion

## 4.1. Summary of Research Findings

In this thesis, the overarching focus of my research was on the identification and characterization of microplastics. The first objective of my work was investigating the use of carbon and hydrogen stable isotope and elemental composition as a technique to distinguish petroleum-based plastic polymers and the application of this technique to microplastics identification. This was the first characterization of the hydrogen concentration of plastic polymers and expanded on previous works that had characterized the carbon concentration and the carbon and hydrogen stable isotope composition of plastics. I hypothesized that:

1. *The carbon stable isotope composition of the different polymer types will overlap, as observed in previous studies*
2. *The hydrogen stable isotope composition of the different polymer types will exhibit wider ranges than the carbon stable isotope composition*
3. *The different processes involved in manufacturing different polymers will result in distinct hydrogen isotopic compositions for each polymer type*
4. *The carbon and hydrogen elemental composition of HDPE, LDPE, and PP will be the same, but PET, PVC, and PS will be distinct*
5. *It will be possible to identify the polymer type of the unknown microplastics by their carbon and hydrogen isotopic and elemental composition*

As hypothesized, the carbon stable isotope composition of the different polymer types overlapped, reflecting their similar petroleum feedstocks. The hydrogen stable isotope composition of the plastics did exhibit wider ranges than the results for carbon, however not every polymer type had distinct hydrogen isotopic compositions. Two pairings, HDPE and PP, and PVC and PS were not significantly different from each other, while all other polymer types were distinct. However, the hydrogen isotopic composition characterized in the PVC, PP, and PS samples resulted in two distinct groupings of samples from each of those polymer types, which further distinguished PVC and PS samples from each other and some PP samples from HDPE. As expected, the carbon and hydrogen concentration were able to distinguish between all polymers except for HDPE, LDPE, and PP, which were indistinct from each other because of their similar chemical compositions. Using the combination of the hydrogen isotopic and elemental composition it is possible to uniquely characterize most of the polymer types. The hydrogen isotopic and elemental composition of the environmental microplastics characterized in this thesis matched the values of the polymers characterized in our database, allowing for their identification. The isotopic composition is a quantitative result rather than a qualitative result that must be identified subjectively, which eliminates some bias that is present with other existing methods used to identify microplastics. This makes the use of the hydrogen isotopic and elemental composition of the microplastics an appealing method to use to identify their polymer type.

The second objective of my work was to examine microplastic pollution in the Red River, Manitoba to improve the understanding of the spatial and temporal variations in microplastic concentrations in this freshwater environment. I hypothesized that:

1. *Microplastic concentrations will be lower upstream of the city of Winnipeg compared to concentrations downstream of and within the city*
2. *Microplastic concentrations will be higher downstream of wastewater treatment plants, as wastewater effluent has been identified as a significant source of microplastics to rivers*
3. *In the spring, microplastic concentrations will be higher than during the summer or fall due to increased flow rates and surface runoff*

Contrary to what was expected, microplastic concentrations did not increase within and downstream of the city of Winnipeg compared to upstream concentrations. As well, microplastic concentrations were not significantly different between sites upstream and downstream of wastewater treatment plants. The homogeneity of microplastic concentrations and distribution of morphologies and colours between sites suggests that the microplastics are being introduced through diffuse, nonpoint sources rather than at discrete locations like wastewater treatment plants. Stormwater runoff, combined sewer overflows, agricultural runoff, and atmospheric deposition possibly are significant sources of microplastics to the Red River. As hypothesized, microplastic concentrations were higher in the Spring than in the Summer and Fall. Increased flow rates in the river likely resuspended microplastics that had settled in sediments along the river bottom, and the increased stormwater runoff from precipitation would have contributed more microplastics to the river and led to higher incidences of combined sewer overflows, further increasing microplastic concentrations.

This research has demonstrated that hydrogen isotopic composition and elemental concentration can be used as a novel method to identify plastic polymer types. As a complementary technique for microplastics identification, using hydrogen stable isotope and concentration analysis has several advantages, including that analyses are automated, and, unlike FTIR and Raman spectroscopy, the presence of colourants does not interfere with analysis. As mentioned previously, it eliminates biases that are inherently present in other methods of identification (i.e., which polymer library is used as a reference, individual researcher differences in interpreting spectra, etc.) However, the technique is destructive, and sample mass may be a limiting factor as many microplastics are simply too small for the minimum weight of material required for analysis. To accurately identify microplastics from environmental samples, multiple analytical techniques are required, and innovation in methodologies is essential as the field develops. This new combination of hydrogen isotopic composition and concentration technique can be used in tandem with existing methodologies to provide critical information on the polymer types that are contributing to microplastic pollution.

Understanding the sources of microplastics to the Red River as well as how the concentrations change throughout the year is essential for guiding local policy decisions and best management practices for plastic waste.

## 4.2 Future Work

Further work should investigate the effects that plastic additives and the source of petroleum used as feedstock for the plastics may have on the isotopic composition of the polymer. As well, investigating how different environmental conditions affect the isotopic composition of plastics should be a priority for future research, as changes to the isotopic signature of the plastic could be a valuable tool for tracking microplastics in the environment. Expanding the number of polymer types that have had their carbon and hydrogen isotopic and elemental composition characterized will create a larger reference database for comparison and will refine the identification of the polymer type of unknown microplastics.

Future research in freshwater microplastics should continue to examine different sources of microplastics in the environment, including the impact that wastewater treatment plants have on microplastic loads in freshwater systems. As well, further sampling at high frequency during high flow and storm events should be done to better understand how storms and river flow affect microplastic concentrations.