

# Characterization of microplastics and anthropogenic fibers in surface waters of the North Saskatchewan River, Alberta, Canada

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## Abstract

Microplastics are globally ubiquitous contaminants, but quantitative data on their presence in freshwater environments are sparse. This study investigates the occurrence, composition, and spatial trends of microplastic contamination in the North Saskatchewan River flowing through Edmonton, Alberta, the fifth largest city in Canada. Surface water samples were collected from seven sites throughout the city, upstream and downstream of the city, and near potential point sources (i.e., a wastewater treatment plant). Samples were spiked with fluorescent microbeads as internal standards and extracted by wet peroxide oxidation and density floatation. Microplastics were found in all samples, ranging in concentration from 4.6 to 88.3 particles·m<sup>-3</sup> (mean = 26.2 ± 18.4 particles·m<sup>-3</sup>). Fibers were the dominant morphology recovered, and most were of anthropogenic origin and chemically identified as dyed cotton or polyester by Raman microspectroscopy. The majority of fragments were identified as polyethylene or polypropylene. No upstream to downstream differences were found in concentration, size distribution, or morphological composition suggesting nonpoint sources of microplastics to the river. This study represents one of the first investigations into the occurrence of microplastics in the freshwater environment in western Canada and will provide a baseline for future studies.

**Key words:** microplastic, freshwater, fibers, Raman spectroscopy, internal standard, Canada

## Introduction

Over the past several decades, the prevalence of plastic usage in society has increased dramatically, with a >600% increase in the quantity of plastics produced since 1975 (Jambeck et al. 2015). Much of the plastic produced is destined for use as packaging and is disposed of within a year of manufacturing (Geyer et al. 2017), leading to an increase in the quantity of plastic in solid municipal waste (Jambeck et al. 2015). This increased production, usage, and mismanagement of plastic waste has led to widespread global contamination of plastic pollution. Plastic is now a ubiquitous global contaminant found at both poles (Bergmann et al. 2017; Waller et al. 2017), in the deepest reaches of the ocean (Van Cauwenberghe et al. 2013), and in remote mountain regions (Free et al. 2014).

Microplastics are typically defined as plastics less than 5 mm and in the environment have potential deleterious effects on biota and ecosystem function. In particular, aquatic organisms ingest



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macro- and microplastics (Thompson 2004; Cole et al. 2013; Cole and Galloway 2015), which are transferred within food webs (Provencher et al. 2019). Ingestion of microplastics may cause physical effects on organisms, including abrasions, lacerations, or blockages of an organism's digestive tract, resulting in a reduction in food intake, reduced growth, or death (Wright et al. 2013) as well as sub-lethal effects such as tissue inflammation, oxidative stress, and reduced locomotor behavior (Lu et al. 2016; Chen et al. 2017). Microplastics may also act as a vector for the transport and release of organic contaminants, such as pesticides, plasticizers, flame retardants, and additives, to organisms once ingested (Teuten et al. 2007, 2009). Because of the limited number of toxicology studies conducted using environmentally relevant exposures, the risk posed by microplastics to freshwater organisms and ecosystems is still unclear (Triebkorn et al. 2019).

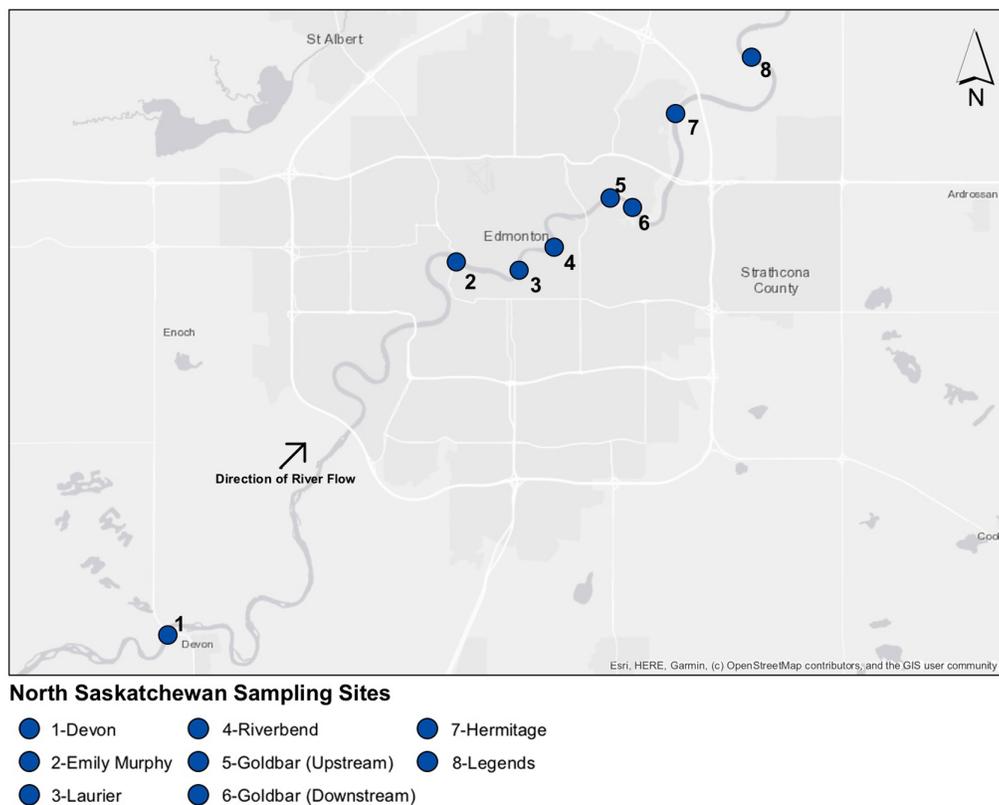
Although most studies have focused on characterizing quantities of microplastics in the oceans, recent studies demonstrate that freshwater ecosystems are contaminated at similar or greater levels than marine environments. For example, an average of 43 000 particles·km<sup>-2</sup> were found in the Great Lakes (Eriksen et al. 2013), which exceeds quantities found in open water marine environments (W.C. Li et al. 2016). Further, rivers are an important vector for transport of microplastics to the marine environment, and it is estimated that between 1.15 and 2.41 million tonnes of plastic is transported to the oceans via rivers annually (Lebreton et al. 2017). Globally, reported concentrations of microplastics in freshwater systems are variable and range over several orders of magnitude (Moore et al. 2011; Leslie et al. 2017; Lahens et al. 2018; Lin et al. 2018). Urbanization, in particular, is a key driver of microplastic pollution to freshwater systems (Peters and Bratton 2016; Kataoka et al. 2019). Important vectors for the introduction of microplastics to freshwater systems include the release of microplastics and synthetic fibers from wastewater treatment plants (WWTP; Mason et al. 2016; Gies et al. 2018), stormwater runoff (Grbić et al. 2020), runoff from agricultural fields with applied biosolids (Crossman et al. 2020), and atmospheric deposition (Dris et al. 2015a). Once introduced into the environment, little is unknown about the transport and fate of microplastics in freshwater systems.

Canada has approximately 20% of global surface freshwater, yet data on the occurrence of microplastics in freshwater ecosystems within Canada is lacking (Anderson et al. 2016). High levels of microplastic pollution have been reported in the Great Lakes (Eriksen et al. 2013; Corcoran et al. 2015; Dean et al. 2018; Mason et al. 2020) and Lake Winnipeg (Anderson et al. 2017), and in three river systems in Canada: the St. Lawrence river (Crew et al. 2020), the Ottawa River (Vermaire et al. 2017; Forrest et al. 2019), and the Red and Assiniboine Rivers (Warrack et al. 2017). Microplastic levels in these rivers are similar to those found in more populated river systems in Europe (Dris et al. 2015b). The occurrence of microplastic pollution in freshwater bodies in western Canada, however, is virtually unknown. We undertook the present study to address these knowledge gaps by quantifying and characterizing microplastics within an urbanized Canadian river. This study provides data on the prevalence of microplastic pollution within the Canadian freshwater environment needed to help understand the ecotoxicological risk posed by microplastics and provides baseline data of microplastic abundances on which future monitoring programs can be built.

## Materials and methods

### Study area

The North Saskatchewan River forms in the icefields of Banff and Jasper National Parks in Alberta and flows 1287 km eastward into Lake Winnipeg and eventually Hudson Bay. The total catchment area is approximately 122 800 km<sup>2</sup> and includes most of southern and central Alberta and Saskatchewan. Edmonton, with a population of 930 000, is the second largest city in Alberta, the fifth largest city in Canada, and the largest city within the North Saskatchewan River watershed. It is the



**Fig. 1.** Study area and sites of sample collection along the North Saskatchewan river.

first major urban area encountered by the river, although several smaller municipalities and wastewater treatment plants are located upstream of the city.

### Sample collection

Twenty-two surface water samples were collected in July and August 2017 from eight sites along an approximately 66 km stretch of the North Saskatchewan River (Fig. 1, Table S1). One site upstream of Edmonton (site 1) was included as a baseline site. Within the city limits, six sites were chosen, including sites in predominantly residential areas, throughout the downtown core, and one site each located immediately upstream (site 5) and downstream (site 6) of the municipal WWTP. A single sample was collected from each site in July, and duplicate samples were collected at each site in August, with the exception of site 3 (Emily Murphy) and site 7 (Hermitage), where only a single sample was collected in August.

Sampling occurred near the shore by wading into the river. All samples were collected using an 8-inch nylon plankton net with 53  $\mu\text{m}$  mesh (Wildco, Yulee, Florida, USA). Nets were fully submerged for 5 min into the flowing water just below the surface with the net opening facing the direction of water flow, enabling the collection of (on average) 5.5  $\text{m}^3$  of water (Table S1). The nets were rinsed from the outside with deionized water that had previously been filtered with 0.22  $\mu\text{m}$  filters to transfer the trapped particulates into the cod end of the net. The contents of the net were transferred into 1 L glass bottles and stored at 4  $^{\circ}\text{C}$  until analysis. At each site, river flow rates were measured using a PASPORT PS-2130 Flow Rate/Temperature Sensor (Pasco, Roseville, California,

USA) immediately prior to sample collection, and depth was determined at the point of sample collection using a meter stick.

Microplastic quantities in one sample (site 8—July) were excluded from concentration data set due to unreliable measurements of water flow rates. However, the morphological and chemical identification data from this site are included in the analysis as sampling volume will not impact these percentages of these categories.

## Sample processing

Prior to extraction, all samples and blanks were spiked with internal standards consisting of a known number of fluorescent polyethylene microbeads (Cospheric, Goleta, California, USA) of varying diameters and densities (Table S1) to assess the efficiency of the extraction procedure. We aimed to spike each sample at levels similar to the number of microplastics that may be expected; therefore, each sample was spiked with approximately 25–50 beads of each color. Following this, microplastics were isolated using density separation and wet peroxide oxidation (WPO) (Masura et al. 2015). All samples were filtered through a 37  $\mu\text{m}$  stainless steel mesh sieve to isolate the solid particulate matter. The collected material was transferred to a 250 mL Erlenmeyer flask, loosely covered with rinsed aluminum foil and placed overnight in an oven at 70  $^{\circ}\text{C}$  to remove any residual water. Plastics were separated from denser material using a solution of  $\text{ZnCl}_2$  ( $\rho = 1.55\text{--}1.60\text{ g}\cdot\text{cm}^{-3}$ ; VWR International, Mississauga, Ontario, Canada). Approximately 25 mL of the  $\text{ZnCl}_2$  solution was added to the flask and shaken on a shaker plate (Innova 44, New Brunswick Scientific, Edison, New Jersey, USA) at 125 rotations per minute (rpm) for 20 min to allow for mixing. The entire contents of the flask were then transferred to a density separation apparatus analogous to the one described by Masura et al. (2015). After settling overnight, the denser material was drained and discarded. The remaining material was filtered through a 37  $\mu\text{m}$  stainless steel mesh sieve, rinsed well with filtered deionized water to remove any residual  $\text{ZnCl}_2$ , and transferred to a clean Erlenmeyer flask. This flask was dried overnight at 70  $^{\circ}\text{C}$  to evaporate residual water. Samples then underwent digestion by WPO to remove organic materials and aid in the subsequent Raman analysis. Briefly, 20 mL of 30%  $\text{H}_2\text{O}_2$  (Sigma Aldrich, Oakville, Ontario, Canada) and 20 mL of 0.05 M  $\text{FeSO}_4$  (Sigma Aldrich, Oakville, Ontario, Canada) were added to the flask and shaken on a shaker plate at 90 rpm and 50  $^{\circ}\text{C}$  for 60 min. If any organic material remained at the end of this time, an additional 20 mL of  $\text{H}_2\text{O}_2$  was added, and reaction was continued for an additional 30 min.

Following removal of organic material, the contents of the flask were transferred to a sieve stack containing five stainless steel mesh sieves stacked on top of one another. The following mesh sizes were used: 37  $\mu\text{m}$ , 125  $\mu\text{m}$ , 250  $\mu\text{m}$ , 500  $\mu\text{m}$ , and 1 mm, resulting in separation of microplastics into the following size classes: >1 mm, 1 mm–500  $\mu\text{m}$ , 500–250  $\mu\text{m}$ , 250–125  $\mu\text{m}$ , and 125–53  $\mu\text{m}$  (due to the 53  $\mu\text{m}$  net used during sampling). The sieve stack was rinsed thoroughly with filtered deionized water to remove any residual WPO reagents and aid in separation. The contents of each sieve were transferred individually with filtered deionized water to 0.45  $\mu\text{m}$  gridded nitrocellulose filters (Ahlstrom-Munksjö, Helsinki, Finland), dried by vacuum filtration, and stored in an enclosed slide (Analyslide, Pall Laboratory, New York, USA) at 4  $^{\circ}\text{C}$  until enumeration and Raman analysis.

## Quality control

During sampling, extraction, and analysis, care was taken to minimize contamination from external sources. Analysts wore cotton lab coats, and all glassware and laboratory utensils were washed thoroughly with filtered deionized water prior to use. All glassware was covered with aluminum foil throughout to minimize contamination from airborne plastics.

Laboratory blanks ( $n = 4$ ), field blanks ( $n = 1$ ), and equipment blanks ( $n = 1$ ) were incorporated into the analysis scheme. Laboratory blanks consisted of 1 L of filtered (0.22  $\mu\text{m}$  filter) deionized water. Field blanks were employed during each separate day of sample collection and consisted of 0.22  $\mu\text{m}$  filtered deionized water in a 1 L glass bottle that was opened to the atmosphere during sample collection and subsequent transferring of the net contents. An equipment blank was used to assess potential contamination arising from carry-over between samples or from shedding of the nets themselves. The equipment blank was treated analogously to samples by thoroughly rinsing the outside of the net with filtered water, followed by transferring the contents of the net into 1 L glass bottles. All blanks were processed and analyzed identically to samples.

Suspected microplastics were found in all blanks, despite the precautions taken to minimize contamination. There were no differences in quantities of microplastic amongst blank types (i.e., field, net, and laboratory blanks), suggesting that the predominant source of contamination arose from the laboratory environment. Due to this lack of difference, all blanks were combined for further analyses. Following visual analysis, the number of identified microplastics in each sample was corrected for blank contamination by subtracting from each sample the average number of microplastics in each category (size and morphology) identified in the blanks. Uncorrected counts of microplastics identified in all samples and blanks can be found in [Tables S2](#) and [S3](#), respectively.

## Visual analysis

Identification and quantification of microplastics was carried out by brightfield microscopy using either a stereo microscope (Olympus SZ61) or a compound microscope (Olympus CX41) under magnification ranging from 2 $\times$  to 40 $\times$ . Microplastics were identified based on previously established criteria ([Hidalgo-Ruz et al. 2012](#)). Briefly, suspected microplastics were required to be free of any cellular structures, be homogenous in color throughout, and fibers should be of consistent diameter throughout their entire length. Based on structural characteristics, all identified microplastics were placed into one of four categories: spheres (round and regularly shaped), films (irregularly shaped with similar length and width, but thin and flexible), fragments (length and width similar; irregularly shaped), or fibers (length greater than diameter). The color of each suspected microplastic was also recorded for July samples only and placed into one of the following categories: blue, red, clear, black, green, or other.

## Chemical identification

Owing to time constraints, chemical identification of suspected microplastics was performed only on July samples. Raman microspectroscopy was carried out on 427 of the 773 visually identified microplastics (52.7%) across all sampling sites and blanks. Raman spectra of individual suspected microplastics and fibers were acquired with a SENTERRA I Raman microscope and Opus 6.5 software (Bruker Optics Ltd., Milton, Ontario, Canada). All spectra were acquired over the range of 45–3700  $\text{cm}^{-1}$  using a 785 nm laser to minimize fluorescence. Typically, spectra were acquired with a laser intensity of 10 mW, a 5 s integration time, and five co-additions; however, these parameters were modified for some samples (e.g., fibers) to acquire stronger spectra or prevent decomposition of the sample. Prior to library matching, spectra were preprocessed by cutting from 45 to 200  $\text{cm}^{-1}$  and 2500 to 3700  $\text{cm}^{-1}$  to remove noisy regions of the spectra that contained no spectral information but hindered library matching. Spectra were further baseline corrected using a 65-point rubber band correction and min–max normalized. All preprocessing and library matching were done using Opus 6.5 software. The chemical composition of individual microplastics was obtained by matching acquired spectra to spectra contained in the SLoPP and SLoPP-E microplastic spectral libraries ([Munno et al. 2020](#)). To be considered a match a hit quality value of greater than 750 was required (where HQ = 1000 is a perfect match and 0 indicates no correlation at all), and all matches were

visually confirmed to eliminate false positives. Samples that were not matched to any library spectra were classified as either “Unknown (Anthropogenic)” if they were colored (indicative of anthropogenic dye) or as “Unknown”.

## Data analysis

All statistical tests were performed using GraphPad Prism version 8.3.0 software (GraphPad Software, La Jolla, California, USA). Parametric statistical tests were used when the appropriate assumptions were met and included *t* tests, one-way ANOVA followed by Tukey’s multiple comparisons test, and two-way ANOVA. Nonparametric Kruskal–Wallis tests followed by Dunn’s Multiple were performed on data sets that did not pass normality tests or did not meet the requirements for parametric tests. For all analyses,  $\alpha = 0.05$  was set as the level of significance. All data are presented as mean  $\pm$  1 standard deviation.

## Results and discussion

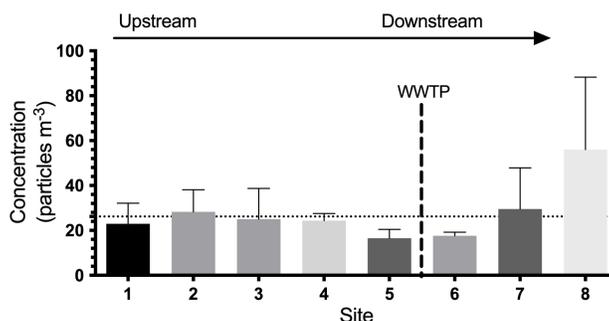
### Recoveries

To assess the efficiency of the extraction process, all samples and blanks were spiked with fluorescent microbeads in a range of sizes and densities (Table S4). The overall recovery of spiked fluorescent microbeads was  $67.9\% \pm 34.9\%$ , and mean recoveries ranging from 29.8% to 98.6%. Recoveries were dependent on size but not density (Table S4, Fig. S1) with larger microbeads ( $>150\ \mu\text{m}$ ) being extracted more efficiently than smaller beads ( $<150\ \mu\text{m}$ , Kruskal–Wallis Test,  $H = 116.2$ ,  $p < 0.001$ ); the mean recovery of larger microbeads was  $83.0\% \pm 26.5\%$  compared with  $44.7\% \pm 32.7\%$  for smaller microbeads. We also found considerable differences in variability between size classes, with smaller particles having a relative standard deviation (RSD) of 73.25% compared with an RSD of 31.85% for larger particles ( $>150\ \mu\text{m}$ ). The low recoveries and greater variability for the smaller microbeads is likely due to adhesion of beads to the sides of the extraction vessels, density separation device, and (or) vacuum filtration apparatus. Although care was taken to rinse all glassware multiple times when transferring contents from one container to another, the fluorescent beads made it possible to visualize where some microbeads were located during the extraction process. The use of internal standards is widely used in analytical chemistry, but we were only able to find a single other study that utilized internal standards for assessment of microplastic recoveries (Zobkov and Esiukova 2017); in many studies, recoveries are assessed by external validation and spike and recovery. The inclusion of internal standards enables more reliable microplastic quantification within chosen size ranges on a sample by sample basis, and we encourage their inclusion in future studies. In an effort to be consistent with previous studies, we chose not to correct reported concentrations for internal standard recoveries. As a first assessment of their use, more work is also necessary to determine whether microbead recoveries are representative of other morphologies, such as fibers.

### Total microplastic concentrations

Microplastics and anthropogenic fibers were identified in all surface water samples, ranging in concentration from 4.6 to 88.3 particles·m<sup>-3</sup>, with a mean concentration ( $\pm 1$  standard deviation) across all samples of  $26.2 \pm 18.4$  particles·m<sup>-3</sup> (Fig. 2). Across all sites, fibers accounted for a majority ( $67.5\% \pm 8.7\%$ ) of the total microplastics in the North Saskatchewan River, followed by fragments ( $21.8\% \pm 6.1\%$ ), films ( $8.7\% \pm 5.5\%$ ), and spheres ( $2.0\% \pm 1.5\%$ ) (Fig. 3a). Total concentrations in August were higher than in July (Mann–Whitney test,  $U = 8$ ,  $p = 0.0011$ ; Fig. S2), although there were no differences in distribution of morphologies or sizes between sampling times.

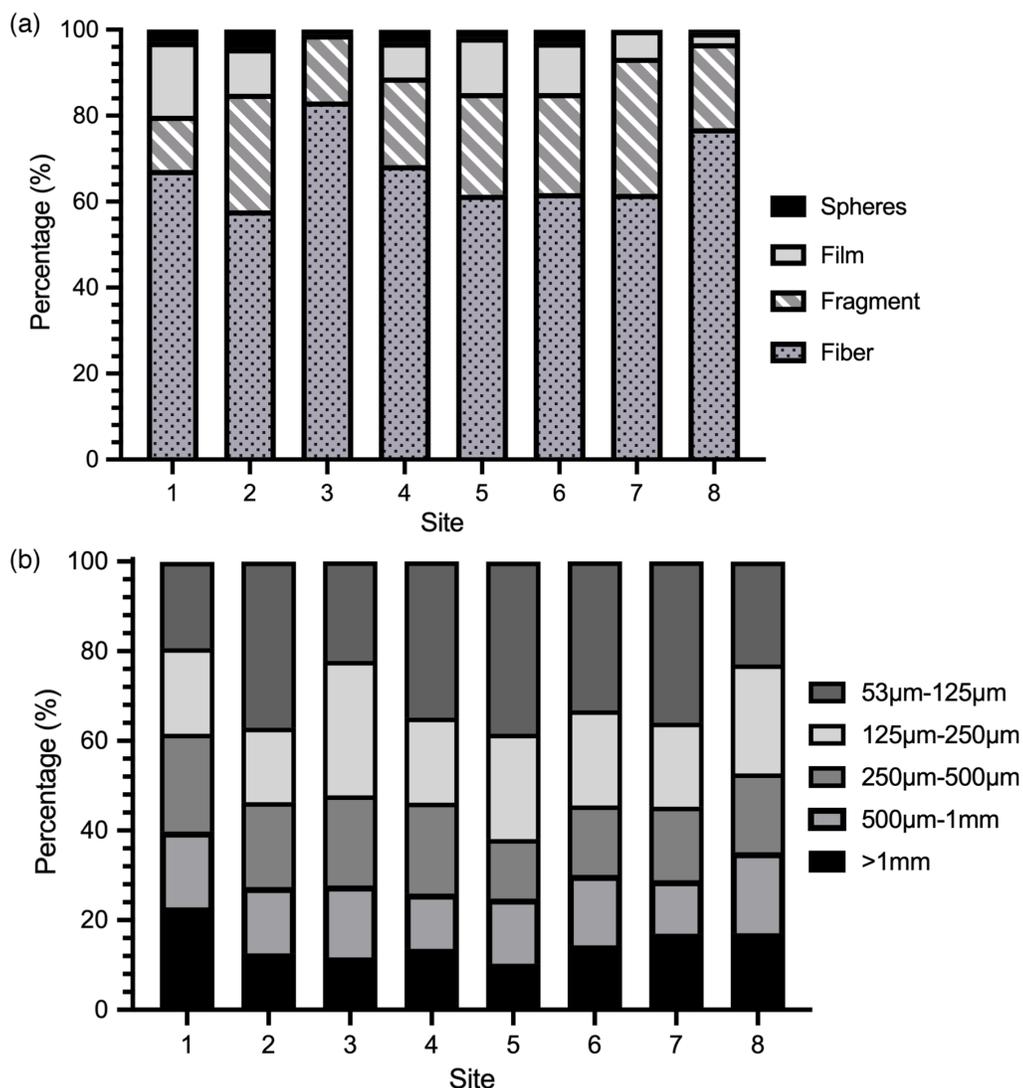
This is amongst the first studies to report on concentrations of microplastics in a western Canadian freshwater system and corroborates a multitude of previous findings demonstrating that microplastics



**Fig. 2.** Total concentrations (mean  $\pm$  standard deviation) of suspected microplastics in the North Saskatchewan River as it flows through Edmonton, Alberta. Dashed line represents the mean concentration of particles found in the North Saskatchewan River across all sites. WWTP, wastewater treatment plant.

are ubiquitous in freshwater environments. The microplastic concentrations reported herein are consistent with concentrations reported in other river systems in North America (McCormick et al. 2014; Baldwin et al. 2016; Estahbanati and Fahrenfeld 2016) and in particular, to concentrations of microplastics quantified in other river systems throughout Canada. Mean concentrations of microplastics in the North Saskatchewan River are higher than those reported in an urban creek downstream of Regina, Saskatchewan (population 229 000), (Campbell et al. 2017) and approximately five-fold higher than those reported for the Red, Assiniboine, and Nelson rivers, near Winnipeg, Manitoba (population 700 000), where microplastic concentrations averaged of 5.3 microplastics·m<sup>-3</sup> (Warrack et al. 2017). Similar concentrations of 12 particles·m<sup>-3</sup> and 16 particles·m<sup>-3</sup> were found upstream and downstream of wastewater treatment plants, respectively, in the St. Lawrence River, despite also reporting amongst the highest sediment concentrations thus far reported for freshwater systems (Crew et al. 2020). Concentrations in the North Saskatchewan River are approximately four-fold lower than those reported by Vermaire et al. (2017) and Forrest et al. (2019) for the Ottawa river and its tributaries, which flow through Ottawa, Ontario, a city of comparable size to Edmonton (1.23 million vs. 900 000). In this system, concentrations ranged from 20 to 410 particles·m<sup>-3</sup> based on 1 L grab samples. However, the concentrations reported herein are greater than the mean of 1.35 particles·m<sup>-3</sup> reported for the Ottawa River and tributaries when water was sampled using high-volume manta trawls (Vermaire et al. 2017).

Comparisons of microplastics concentrations amongst studies is difficult, however, given differences sampling and analytical methodology utilized amongst studies. In particular, differences in total concentrations may be attributable to the difference in mesh sizes used for sampling. In this study, a 53  $\mu$ m mesh size was chosen in an effort to capture the widest possible range of potential microplastics—smaller than the 333  $\mu$ m mesh size used by Warrack et al. (2017) or the 100  $\mu$ m filters used in studies of the Ottawa (Vermaire et al. 2017; Forrest et al. 2019) and St. Lawrence Rivers (Crew et al. 2020). Differences in mesh size have a substantial impact on the number of plastics recovered, as other studies have shown a 250-fold increase in the number of plastics captured following a decrease in mesh size from 333 to 80  $\mu$ m (Dris et al. 2018). Moreover, the most prevalent microplastics found in the North Saskatchewan River were those in the smallest size range investigated (53–125  $\mu$ m), which comprised 30.5%  $\pm$  7.7% of the total microplastics identified (Fig. 3b). This is consistent with other studies in both freshwater and marine environments that found concentrations of microplastics increase with decreasing particle size (Estahbanati and Fahrenfeld 2016; Lindeque et al. 2020). Although our findings are in line with these previous studies, it is germane to note that difficulty in positively identifying microplastics by light microscopy increases with decreasing size. At particles sizes <100  $\mu$ m, rates of positive identification for visual identification fall below 80%, leading to an



**Fig. 3.** Average percent composition of suspected microplastics in different size classes (a) and of each morphological category (b) in water samples collected from the North Saskatchewan River as it flows through Edmonton, Alberta. Site 1 represents the most upstream sampling location, and site 8 represents the most downstream sampling location.

overestimate of the true particle counts within smaller size fractions (Lenz et al. 2015). We were unable to attempt Raman microspectroscopy on all particles in this size fraction; however, 71.8% of the particles in this size fraction in which we were able to successfully acquire a Raman spectrum were identified as either plastics or unknown anthropogenic, which was consistent with other size fractions examined in this study.

Considerable spatial and temporal heterogeneity in microplastic concentrations exist within dynamic river systems, and microplastic concentrations may vary with sampling depth or sampled location within the cross-section of the river (Dris et al. 2018; Forrest et al. 2019; Lenaker et al. 2019), further complicating comparisons amongst studies. All samples were collected from the near-shore

environment, although other studies have sampled river channels from boats or bridges. Comparisons are also hindered by differences in sampling methodology, as grab sampling results in higher reported concentrations than bulk water sampling (Vermaire et al. 2017). Such reported differences makes clear the need for greater standardization in sampling methodology and (or) sampling under similar hydrogeomorphic river conditions to help to systematize microplastic sampling in rivers within riverine systems.

Overall concentrations varied temporally, with total concentrations across all sites being higher in August compared with July. Although mobilization and transport of land-based microplastics to the river following precipitation events have been identified as contributing to temporal changes in microplastic concentrations within rivers (Dris et al. 2015b), this is unlikely to be the cause for the trends observed here, as the total precipitation amounts in Edmonton were similar in the 7 d prior to each sampling day (July: 2.8 mm, August: 2 mm) and no precipitation within 3 d of either sampling day. Rather, we observed both decreased depth and increased flow velocity at sampling sites in August compared with July (Fig. S3). The increased velocity and decreased depth may have led to the resuspension of settled microplastics from sediments. However, in addition to urban runoff dynamics, hydrogeomorphic flow of the river would change with both local and upstream precipitation events. With a large and dynamic river such as the North Saskatchewan river, daily and seasonal fluctuations in discharge may lead to local variability at different time points. Further sampling is required to confirm and further elucidate any temporal trends within the North Saskatchewan River.

## Characterization of microplastics

Synthetic fibers have been widely reported as the primary microplastic morphology found in freshwater ecosystems globally (Dris et al. 2015b; Baldwin et al. 2016; Leslie et al. 2017; Lin et al. 2018), and the preponderance of fibers found in the North Saskatchewan River are consistent with these earlier reports. Moreover, this composition is similar to the percentage of fibers found in the Ottawa River and the Red and Assiniboine Rivers in Manitoba (Vermaire et al. 2017; Warrack et al. 2017; Forrest et al. 2019), Wascana Creek in Saskatchewan (Campbell et al. 2017), and Lake Winnipeg (Anderson et al. 2017).

Most of the fibers were either blue, clear, or black in color (Fig. S4) and were chemically identified as polyester or cotton (Fig. 4). We were unable to conclusively identify the chemical identity of 32% of the fibers due to the Raman spectrum of the polymer being obscured by that of the dyes, a common problem in Raman spectroscopy (Lenz et al. 2015). However, these unknown fibers were dyed and most likely of anthropogenic origin. Of these unknown fibers, most (62%) were blue in color and tentatively identified as containing indigo dyes, based on comparison with published spectra (Baran et al. 2010). Indigo dye is widely used in the dyeing of cotton fabric for the manufacturing of denim and use in blue jeans and has been identified as being major components of fiber pollution in wastewater treatment plant effluent (Mason et al. 2016). However, we found a dominance of fibers upstream of the WWTP, indicating the presence of other sources. These fibers may have originated from effluent discharged into the North Saskatchewan River from upstream WWTPs, although fibers also compose a large proportion of microplastics in stormwater runoff (Grbić et al. 2020) and urban air (Dris et al. 2015a; Wright et al. 2020), suggesting that these sources may also be contributing to the microplastic load to the North Saskatchewan River.

Most fragments found in this study were clear, with large proportions of blue, black, or red also present. Most (32.4%) were chemically identified as polyethylene or polypropylene (8.8%), consistent with other studies and with the high production volume of these two polymers (Geyer et al. 2017). Like fibers, however, a large proportion (32.4%) of fragments were identified as anthropogenic in

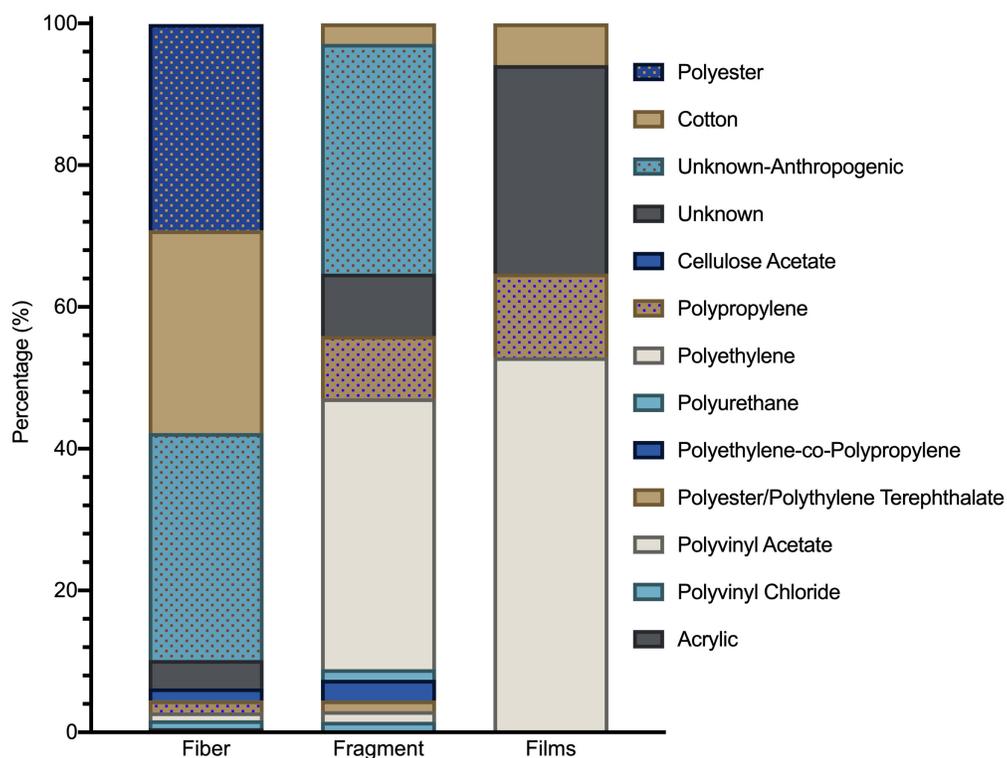


Fig. 4. Chemical composition of microplastics and anthropogenic fibers as determined by Raman microscopy.

origin, but the polymer type was not chemically identified due to a lack of matching spectrum in our library and the influence of dyes or additives on the Raman spectrum.

Changes in the predominant colors of microplastics found in samples collected from urban sites and those collected upstream and downstream of urban areas, along with a greater diversity of the colors identified in urban sites, suggests that urban areas contribute greater variability in plastics to rivers than nonurban areas (Peters and Bratton 2016). However, we were unable to find any differences between urban and nonurban sites with respect to predominant color or color variability, although this may be a consequence of collecting samples from only a single upstream nonurban site, as well as the constrained categories of colors that were identified. Further, changes in the discoloration and bleaching of plastics may occur due to weathering or due to our digestion procedure (J. Li et al. 2016), which may have contributed to the preponderance of clear fragments and fibers found. The reporting of color distributions is important for ecotoxicological risk assessments though, as a number of species of aquatic organisms likely selectively ingest microplastics based on color preference (Wright et al. 2013).

## Potential sources

Quantities of microplastics have been shown to be related to population densities and input from urban sources (Yonkos et al. 2014; Baldwin et al. 2016). We hypothesized, therefore, that microplastic concentrations would increase as the North Saskatchewan River flowed through the city of Edmonton. With the exception of site 8, the most downstream site, there were no significant differences in total concentrations of microplastics between sampling locations (Kruskal–Wallis test,

$H = 3.905$ ,  $p = 0.79$ ,  $df = 7$ ) and no identifiable upstream to downstream trends (Fig. 2). Although a single WWTP outfall is located within the stretch of river sampled, we found no difference in concentrations, or any other investigated characteristics, between sites immediately upstream of the WWTP (site 5) and samples collected immediately downstream of the WWTP (site 6) (Fig. 2), suggesting that the WWTP is not acting as a significant point source of microplastic pollution to the river. The higher observed concentration of microplastics at the furthest downstream site (site 8), with a total microplastic concentration approximately 3.5-fold higher than other sites, may be due to the lower river flow rates at this site. Although there was no relationship between flow rate and concentration ( $r^2 = 0.0199$ ,  $p = 0.6160$ ), flow rates at site 8 were the lowest of all of the sampling sites in this study (Table S1) and 30% lower than the next lowest site. Although point bars and regions of decreased river velocity lead to a depositional environment for microplastics and lower water column concentrations (Yonkos et al. 2014), the lower flow rate at site 8 may create a temporary accumulation zone for neutrally or positively buoyant microplastics prior to deposition. We intend to carry out further studies to elucidate the reason for the elevated concentrations at this site.

The finding that the WWTP is likely not acting as a point source for microplastic input to the river contrasts other studies, including studies in Canada, which found that local WWTPs were discharging microplastics to local waterways and that differences in concentration and profile were identifiable between upstream and downstream sampling locations (McCormick et al. 2014; Baldwin et al. 2016; Estahbanati and Fahrenfeld 2016; Vermaire et al. 2017; Warrack et al. 2017), although other studies have similarly reported a lack of influence from WWTPs (Hoellein et al. 2017; Crew et al. 2020). Such differences amongst studies may be a result of different treatment processes utilized at the various WWTPs (Talvitie et al. 2017) or sampled location relative to the outfall. The WWTP in Edmonton utilizes tertiary treatment (EPCOR 2020a), a process which removes >98% of the microplastic and fiber load that enters the treatment plant (Murphy et al. 2016). Thus, while microplastics and fibers are likely being discharged into the river, the total loadings to the river may not be sufficiently high to elicit a noticeable change in concentrations or morphological distribution at downstream sites. Alternatively, the choice of sampling location relative to the WWTP outfall may also impact reported concentrations. As microplastics will be carried toward the center of the river due to momentum from the WWTP effluent discharge, they will not be immediately mixed with the slower moving water near the shoreline. We sampled near the shore and within 100 m of the WWTP outfall, and at this close proximity to the outfall particles and fibers may not have mixed fully with the nearshore water (Pilechi et al. 2012). At the intermittent downstream site (site 7), samples were collected approximately 8.5 km downstream from the outfall and from the bank opposite from the WWTP outfall. At this distance downstream, the contaminant plume may not yet have fully transversed the river, or only the edges of the contaminant plume had reached the far bank (Pilechi et al. 2015). Thus, we may not have been able to detect any input from the WWTP. As discussed above, more work is necessary to standardize sampling within rivers, and the mixing of microplastics within a riverine system following discharge from WWTPs or stormwater outfalls requires additional study.

In addition to concentration, there was no significant variation in morphological composition (Fig. 3a), size distribution (Fig. 3b), color (Fig. S4), or chemical identity amongst sites (Fig. 4). Such similarity in characteristics amongst sites suggests that microplastics are being introduced to the North Saskatchewan River through diffuse, nonpoint sources. Microplastics and anthropogenic fibers found in the North Saskatchewan River likely originate from terrestrial sources and are introduced to the river via runoff or atmospheric transport and deposition. Like many jurisdictions located along rivers, stormwater runoff from the city of Edmonton enters the river untreated from multiple stormwater outflow pipes throughout the city. Stormwater runoff may contain concentrations of microplastics and fibers at levels comparable with or exceeding those found in treated WWTP effluent

(Grbić et al. 2020), and thus outfalls may act as discrete point sources of microplastic pollution (Horton et al. 2017). There are approximately 120 stormwater outfalls that discharge into the North Saskatchewan River between sites 1 and 8 (EPCOR 2020b) and, despite being discrete point sources, may act collectively as a diffuse nonpoint source of pollutants to the river. Additionally, much of the land area along the North Saskatchewan river upstream of Edmonton and in the greater Edmonton region is used for agriculture. In Alberta, biosolids, which are formed from the solids retained in WWTPs (e.g., Goldbar WWTP is Edmonton's largest and produces an average of 20 800 tonnes dry weight per year), are nutrient rich and are used as fertilizer for agricultural fields. Such biosolids retain the microplastics and fibers, which may be released to the environment through erosion and runoff (Crossman et al. 2020). Finally, microplastics are present in urban air and may be transported and deposited regionally in the environment surrounding urban areas (Wright et al. 2020). Morphological profiles were dominated by fibers (Wright et al. 2020), similar to our findings for North Saskatchewan River surface water, suggesting that atmospheric transport and deposition from the City of Edmonton may also be contributing to the microplastic and fiber load within the river.

## Conclusions

Although reports of microplastics in freshwater environments are increasing, the field is generally still in its infancy. This study adds to the growing knowledge of microplastic occurrence in Canadian freshwater ecosystems and is the first, to our knowledge, to characterize microplastic pollution in a western Canadian river. Microplastics and other anthropogenic fibers were found in every sample, with the overall composition dominated by fibers and smaller microplastics. Similarities in concentration, morphologies, and chemical compositions amongst sites suggest diffuse sources of microplastics to the river. In addition to vertical and horizontal hydrogeomorphic characteristics of lotic systems, wind, biofouling, and interaction with other materials may affect the behavior of microplastics in rivers, possibly biasing sampling and obfuscating sources. More research is needed to understand how different sampling, extraction, and analytical methodologies may bias quantitative results, along with a greater understanding of how urban sources and riverine flow dynamics impact observed profiles and concentrations of microplastics.

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## Author contributions

TB, DL, and MSR conceived and designed the study. TB, SK, and MSR performed the experiments/collected the data. TB, SK, and MSR analyzed and interpreted the data. DL and MSR contributed resources. TB, DL, and MSR drafted or revised the manuscript.

## Competing interests

The authors have declared that no competing interests exist.

## Data availability statement

All relevant data are within the paper and in the Supplementary Material.

## Supplementary materials

The following Supplementary Material is available with the article through the journal website at doi:[10.1139/facets-2020-0057](https://doi.org/10.1139/facets-2020-0057).

Supplementary Material 1

Supplementary Material 2

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