Received: 21 November 2022 | Accepted: 30 May 2023 | Published online: 26 June 2023

DOI: 10.1002/jeq2.20497

Journal of Environmental Quality

TECHNICAL REPORT

Emerging Contaminants

How many microplastic particles are present in Canadian biosolids?

Branaavan Sivarajah[1] | **David R. Lapen**[2] | **Sarah B. Gewurtz**[3] | **Shirley Anne Smyth**[3] | **Jennifer F. Provencher**[4] | **Jesse C. Vermaire**[1,5]

[1]Department of Geography and Environmental Studies, Carleton University, Ottawa, Ontario, Canada

[2]Ottawa Research Development Centre, Science and Technology Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

[3]Science and Risk Assessment Directorate, Science and Technology Branch, Environment and Climate Change Canada, Burlington, Ontario, Canada

[4]Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment and Climate Change Canada, Ottawa, Ontario, Canada

[5]Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Ontario, Canada

Correspondence

Branaavan Sivarajah, Department of Geography and Environmental Studies, Carleton University, Ottawa, Ontario, Canada.

Email: Branaavan.Sivarajah@carleton.ca

Assigned to Associate Editor Min Pan.

Funding information

Natural Sciences and Engineering Research Council of Canada; Environment and Climate Change Canada

Abstract

Application of treated sewage sludge (biosolids) from wastewater treatment plants (WWTPs) to farmlands is an important pathway through which microplastic particles (MPs) enter terrestrial ecosystems. Yet, microplastic concentrations in Canadian biosolids have only been estimated in samples from four WWTPs previously. We aimed to fill this knowledge gap by quantifying microplastics in biosolids from 22 WWTPs located in nine provinces and two commercial fertilizer producers in Canada. All samples had substantial microplastic concentrations ranging from 228 to 1353 particles per gram dry weight (median = 636 particles), which are orders of magnitude greater than MPs reported from earlier investigations of biosolids from other countries. Fibers (median: 86%) were the most common type of MPs observed, followed by fragments (median: 13%). There were no statistically significant differences in the amount of microplastics observed in the biosolids from different geographical regions, WWTP types, and sludge treatment processes. This suggests that diverse combinations of local sewershed characteristics, site-specific treatment approaches, and daily flow at WWTPs may be influencing concentrations of microplastics in biosolids. Our results indicate that microplastic concentrations in biosolids are substantially higher than they are in other environmental matrices, and this has important implications to managing microplastic pollution in terrestrial ecosystems.

1 | INTRODUCTION

Since 1950, over eight billion metric tons of plastic has been produced (Geyer et al., 2017). Not surprisingly, plastic waste is a global environmental problem, and governments around the world are taking steps to monitor and reduce plastic waste

---

Abbreviations: WWTP, wastewater treatment plant; MP, microplastic particle.

(e.g., AMAP, 2021; ECCC, 2019). Microplastic particles (MPs; small plastic particles <5 mm in size) are ubiquitous in the environment and are polluting ecosystems worldwide (Rochman et al., 2019). Momentum has been gaining to better understand the sources, transport, and fate of microplastic pollution in terrestrial environments (Baho et al., 2021; Machado et al., 2018; Möller et al., 2020).

Wastewater treatment plants (WWTPs) serve as important environmental conduits, where potentially harmful substances (e.g., contaminants, pathogens), nutrients, and suspended solids are removed from sewage influents. Although WWTPs have not been designed to remove MPs, studies have shown that 70%–99% of MPs are removed from the liquid stage of sewage influents (Carr et al., 2016; Gies et al., 2018; Iyare et al., 2020; Talvitie et al., 2017). These MPs, however, end up in the sludge, which, depending on the jurisdiction, may either be disposed at landfills, incinerated, or treated and applied to agricultural farmlands. The application of nutrient-rich–treated sewage sludge (biosolids) to agricultural soils to support crop growth and improve soil health is practiced extensively around the world. This approach has economic and environmental benefits as it helps to increase soil fertility in farmlands while reducing the amount of sludge that must be incinerated or sent to landfills (CCME, 2012a, 2012b). However, application of biosolids from WWTPs to agricultural fields has been identified as an important pathway of microplastics to terrestrial systems (Crossman et al., 2020; Ng et al., 2018; Nizzetto et al., 2016). Although biosolids applied to agricultural lands in Canada are highly regulated in terms of application rates, nutrient levels, metal(loid) content, location of application, and transportation at the federal and provincial levels (e.g., Alberta Environment, 2009; CCME, 2010, 2012a, 2012b; New Brunswick Environment & Local Government, 2014; Nova Scotia Environment, 2010), to date we are unaware of any regulatory constraints regarding MPs.

In order to properly monitor and assess the risk associated with MPs in the environment, we require an understanding of the sources, transport, and fate of MPs in specific environmental compartments. Biosolids are an important part of understanding the plastic cycle; however, to date, assessments of microplastic concentrations have been only carried out at a handful of Canadian WWTPs (Crossman et al., 2020; Gies et al., 2018; Lavoy & Crossman, 2021). Here, we present the first pan-Canadian assessment of microplastic concentrations in biosolids from 22 Canadian WWTPs and two commercial fertilizer producers. Samples for this study were obtained from WWTPs representing nine provinces. Three common WWTP types were sampled: primary, secondary, and advanced. Sludge treatment type included dewatering, alkaline stabilization, anaerobic digestion, and aerobic digestion. Given the range of geographies of Canada, the diverse types of WWTPs across the country, and the marked differences in sludge treatment processes used (Table 1), we expected to find notable variation in MP concentrations. Specifically, we hypothesized that the amount of MPs from the four geographical regions (Atlantic, Central, Prairies, and Pacific) would vary significantly. We predicted that the number of MPs observed in samples from WWTPs with different treatment approaches would vary substantially, as previous works have suggested that the amount of MP removed increases at secondary and advanced treatment plants (e.g., Iyare et al., 2020). We also predicted that a positive relationship would exist between the amount of sewage entering the WWTP (i.e., average daily flow) and the amount of MPs subsequently observed in biosolids.

Core ideas

- Biosolids are a key pathway through which microplastics enter terrestrial ecosystems.
- We quantified microplastics in 22 municipal biosolids and two fertilizer products from Canada.
- Microplastic concentrations ranged from 228 to 1353 particles per gram dry weight.
- Fibers were the most common microplastic particles followed by fragments.

2 | MATERIALS AND METHODS

2.1 | Samples

Biosolids samples from 22 WWTPs located across nine Canadian provinces were collected between 2014 and 2021 by Environment and Climate Change Canada (ECCC) and Agriculture and Agri-Food Canada (AAFC). Most of the samples analyzed in this study were obtained from the Canadian Biosolids Archive maintained by ECCC as part of the national wastewater monitoring program (described in Lakshminarasimman et al., 2021). Biosolid-based fertilizer pellets produced by two commercial suppliers were also included in this survey to increase representation of the biosolid-based products that are used in Canada. To facilitate participation from diverse WWTPs and fertilizer providers, identifying information (names and specific locations) is not included as many provided samples for this study on the condition of anonymity. We have grouped the WWTPs into four geographic regions, Atlantic ($n = 5$; which includes Newfoundland and Labrador, Nova Scotia, New Brunswick, and Prince Edward Island), Central ($n = 10$; Quebec and Ontario), Prairies ($n = 3$; Manitoba, and Alberta), and Pacific ($n = 4$; British Columbia) (Figure 1).

Information about each WWTP was provided by the plant as part of the monitoring program. The average flows at the

TABLE 1 Descriptions of the environmental characteristics associated with each sample are presented.

Sample code	Year sample collected	Region	WWTP type	Average flow on sampling dates (m^3/day)	Residential inputs (%)	Industrial-commercial-institutional inputs (%)	Sludge treatment type	Water content
Sample 17	2019	Atlantic	Advanced	9708	90	10	Alkaline stabilization	40.08
Sample 13	2018	Atlantic	Primary	95,171	90	10	Alkaline stabilization	49.18
Sample 23	2021	Atlantic	Primary	134,800	NA	NA	Anaerobic digestion and dewatered solids are landfilled	66.61
Sample 5	2014	Atlantic	Secondary	15,777	90	10	Dewatering, potassium permanganate	77.64
Sample 14	2019	Atlantic	Secondary	17,574	90	10	Biopasteurization, mesophilic anaerobic digestion, Dewatering	79.41
Sample 21	NA	Central	Secondary	NA	75	25	Pelletization	8.38
Sample 2	2019	Central	Secondary	29,308	45	55	Anaerobic digestion, mesophilic	89.47
Sample 8	2019	Central	Primary	270,139	95	5	Dewatering	68.69
Sample 6	2019	Central	Secondary	2271	90	10	Aerobic digestion	92.43
Sample 11	2014	Central	Secondary	259,657	50	50	Anaerobic digestion, mesophilic	73.01
Sample 3	2016	Central	Secondary	2580	100	0	Aerobic digestion	91.30
Sample 22	NA	Central	Secondary	175,000	NA	NA	Pelletization	7.03
Sample 24	2021	Central	Secondary	436,000	NA	NA	Anaerobic digestion, mesophilic	71.52
Sample 18	2019	Central	Secondary	5071	80	20	Aerobic digestion, dewatering	79.69
Sample 15	2017	Central	Secondary	22,941	60	40	Anaerobic digestion, mesophilic	92.52
Sample 12	2016	Prairies	Secondary	177,890	90	10	Anaerobic digestion, mesophilic	75.08
Sample 9	2016	Prairies	Advanced	98,710	85	15	Anaerobic digestion, mesophilic	93.95
Sample 16	2016	Prairies	Advanced	338,123	85	15	Anaerobic digestion, mesophilic	91.84
Sample 4	2018	Pacific	Advanced	36,730	80	20	Dewatering	81.47
Sample 10	2018	Pacific	Advanced	11,723	90	10	Dewatering	81.06
Sample 7	2019	Pacific	Primary	408,000	95	5	Anaerobic digestion, mesophilic	92.48
Sample 1	2019	Pacific	Secondary	526,667	95	5	Anaerobic digestion, thermophilic	73.56
Sample 20	NA	Fertilizer	NA	NA	NA	NA	NA	6.85
Sample 19	NA	Fertilizer	NA	NA	NA	NA	NA	4.93

Note: The biosolid sample collection year, region of Canada where the wastewater treatment plant (WWTP) is located, type of WWTP, average influent flow rate (m^3/day), amount of residential and industrial activities (%) in the sewershed, along with the type of sludge treatment used at the WWTP are provided. The amount of water (%) present in each sample are also included.

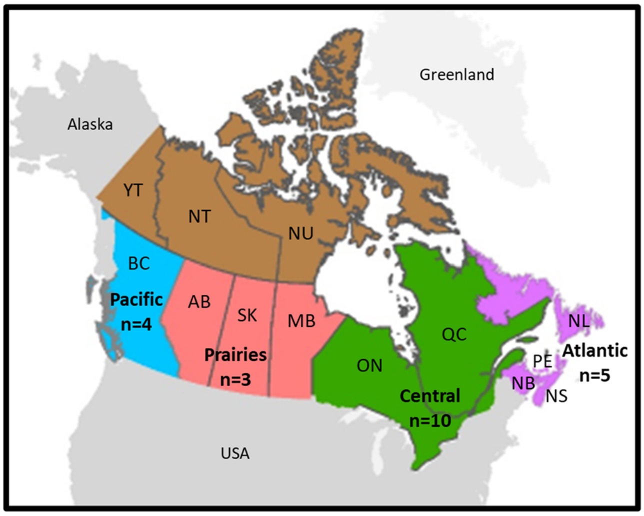

FIGURE 1 The location of the 22 wastewater treatment plants located across Canada are presented by geographical groups (Atlantic, Central, Prairies, and Pacific).

WWTPs were between 2271 and 526,667 m^3/day (Table 1). The WWTPs represented the three common wastewater treatment types in Canada (primary: $n = 4$; secondary: $n = 13$; advanced: $n = 5$). Primary treatment of wastewater provides physical removal of suspended solids and associated oxygen demand by settling. In some cases, settling is enhanced by the addition of polymeric and/or metal salt coagulants. Secondary treatment is usually (but not always) preceded by primary treatment and is designed to substantially degrade the biological content of the waste through aerobic biological processes. Advanced treatment includes anoxic and anaerobic microbial regimes for nitrogen and phosphorus removal. Sludge treatment approach also varied among sites. Anaerobic digestion was the most common ($n = 11$), followed by dewatering ($n = 4$), pelletization ($n = 4$), aerobic digestion ($n = 3$), and alkaline stabilization ($n = 2$). Dewatering is a physical operation used to reduce the moisture content of sludge. Pelletization is a physical process where the raw sludge is thermally dried and compacted into pellets. Alkaline treatment involves the addition of an alkaline material to sludge for pathogen reduction. Aerobic digestion accomplishes stabilization through biological mineralization in the presence of oxygen. For anaerobic digestion, sludge is biologically mineralized in the absence of oxygen to stabilize the solids and generate biogas. The WWTPs in this study received inputs primarily from residential areas (Table 1).

2.2 | Analytical approach

Biosolids are complex matrices and the composition of samples from different WWTPs varied greatly. A common analytical approach that can be used for all samples was determined after exploring a series of alternatives (e.g., potassium hydroxide digestion, density separation; Crossman et al., 2020; Hurley et al., 2018). First, the water content of the biosolids was determined by calculating change in weight before and after freeze drying the samples. Next, ∼1 g dry weight equivalent of biosolid was subsampled from a homogenized larger volume of sample. The subsampled biosolids were treated with 125–300 mL of 30% hydrogen peroxide (H_2O_2) to oxidize organic matter in the samples. The exothermic reaction was monitored using a thermometer, and a cold bath was used to cool down samples when temperatures began to increase rapidly to keep the samples under 50˚C. Then, samples were placed in a water bath set at 50˚C for at least 16 h to accelerate the breakdown of organic material. Following oxidation with H_2O_2, the digested samples were filtered entirely onto pre-cleaned and inspected 80 µm nylon filters (MS® Nylon Mesh Filter, Membrane Solutions) using a vacuum filtration unit. By using 80 µm nylon filters, we retained microplastic particles that were at least 80 µm long or wide. We filtered the entire sample (both floating and settled fractions) to maximize recovery rates of MPs in the samples. The filters were placed into petri dishes for visual inspection and MP enumeration.

The filters with processed biosolid samples were inspected under a stereo microscope (LeicaS9, Leica Microsystems) that was housed inside a clean hood with positive laminar flow. Generally, MPs are colorful and come in diverse shapes and sizes (Masura et al., 2015; Miller et al., 2021; Rochman et al., 2019). Therefore, we followed the classification approach described in Rochman et al. (2019) and enumerated MPs based on shape (fibers, fiber bundles, fragments, films, foams, and glitter) and color. Care should be taken when enumerating MPs in environmental matrices as some natural fibers and non-plastic microlitter could visually resemble MPs. Hence, suspected MPs were prodded with fine tipped metal tweezers and teasing needle to assess breakability and malleability as these features can be used to differentiate MPs from natural/organic material (Lusher et al., 2020). This approach aided in the exclusion of fibers that were likely cellulose material. The remaining particles that were then counted fit the definition of microplastics through their physical characteristics.

2.3 | Quality control

Microfibers are present in airborne dust; hence, several steps were taken to prevent potential microfiber contamination in the laboratory. A white laboratory coat was worn when handling samples, and it was regularly cleaned with a lint roller to remove microfibers. All equipment (e.g., glass beakers, petri dish, thermometer, stir rods, scapula, filtration units) were cleaned with Sparkleen (Fisherbrand) and triple rinsed with deionized water to minimize contamination. The triple rinsed beakers and filtering units were covered with aluminum foil when not in use. Sample preparation (i.e., triple rinsing equipment, subsampling biosolids for analysis) and inspection of filters were done inside a clean hood with positive laminar flow to minimize microfiber contamination. The clean hood with positive laminar flow was fitted with a high efficiency particulate air filter. The H_2O_2 solution used for analyses was filtered through a glass fiber filter to remove potential microfibers. The temperature of the samples was controlled at or around 50˚C to prevent severe degradation of MPs in the samples (Munno et al., 2018). We chose 80 µm nylon filters for this study, as particles smaller than this size fraction can be challenging to identify under stereo microscope. The 80 µm nylon filters were precleaned with deionized water and inspected under a stereo scope for potential contamination before filtering samples onto them. Laboratory blanks were run with each batch of samples to measure potential contamination during sample processing (Miller et al., 2021; Rochman et al., 2019). The blanks were exposed to the same treatments as the biosolids and were filtered through 80 µm nylon filters. All suspected MPs observed in the seven laboratory blanks were recorded and subtracted from the corresponding biosolid samples. The number of MPs observed in laboratory blanks varied between 0 and 8 particles with fibers (clear, black, grey, blue) being the most common type of MP observed.

2.4 | Numerical analysis

The number of MPs observed in each sample was standardized to 1 g of biosolid (dry weight) by dividing the total number of encountered MPs by the amount of biosolids processed. This standardization approach helped to compare results across samples. Because the data did not meet the assumptions of parametric tests, non-parametric Kruskal–Wallis tests were conducted to assess if the number of suspected MPs found from each geographic region, WWTP type, and sludge treatment type varied significantly. Further, the examination of sample medians can be useful data exploratory tools to qualitatively assess patterns in environmental data. Therefore, the differences in medians were used to examine potential patterns in the number of suspected MPs from the various geographic regions, WWTP types, and sludge treatment types. The relationship between the number of MPs observed and average daily flow at the WWTP was assessed using Spearman rank correlation analysis. Prior to conducting the correlation analysis, a Shapiro Wilk test was conducted to assess normality in the variables. Since the MP data were not normally distributed, Spearman rank correlation analysis was deemed appropriate. The statistical analyses were conducted in R Studio using the stats package (R Core Team, 2019). The relative abundances of different shapes of suspected MPs (fibers, fiber bundles, fragments, films, foams, and glitter) were calculated for each sample by dividing the number of a specific type by the total number of MPs observed in the sample.

3 | RESULTS

Microplastic particles were present in all 24 samples analyzed in this study with concentrations varying between 228 and 1353 particles per gram dry weight (Figure 2). The median was 636 particles per gram dry weight across all samples. Several different types of suspected MPs were observed in the biosolid samples (Figure 3). Fibers were the most common type of suspected MPs present across all samples, and the frequency of occurrence (FO) ranged between 73% and 92%, with a median FO of 86% (Figure 4). Fragments were the second most common type of suspected MPs (range: 6%–26%, median: 13%; Figure 4). Black colored fibers were the most common (range: 20%–38%) type of MP in this dataset, followed by clear fibers (range: 0%–35%), gray fibers (range: 7%–13%), blue fibers (range: 4%–24%), pink fibers (range: 5%–11%), light blue fibers (range: 1%–6%), and purple fibers (range: 1%–4%). Among fragments, blue colored particles (range: 0.7%–5%) were the most common, followed by green (range: 0.5%–4%) and black (range: 0.2%–6%) fragments.

Despite the large variation in the number of MPs observed at the sites, we observed no significant difference in MP counts based on region ($p = 0.077$), WWTP type ($p = 0.535$), or sludge treatment type ($p = 0.080$; Figure 5). Some patterns were apparent when the medians of the different groups were compared. However, these differences should be viewed with caution as the number of samples within each category was not equal and some groups had small sample sizes (e.g., Prairies, $n = 3$). Generally, higher numbers of MPs were present in biosolids from the Pacific region (median: 914), followed by the Prairies (median: 812), Central (median: 588), and Atlantic (median: 454) regions (Figure 5). The median number of MPs was greater in biosolids samples from advanced (693) and secondary (609) treatment facilities compared to primary (460) WWTPs (Figure 5). The greatest amount of microplastics were present in biosolids that underwent anaerobic digestion (median: 812), followed by dewatering (median: 633), aerobic digestion (median: 589), pelletization (median: 541), and alkaline treatment (median: 299) (Figure 5). Although a positive relationship was observed between flow and number of MPs, this was not statistically significant (rho = 0.268 $p = 0.237$; Figure 6a). The weak positive correlation was likely influenced by Sample 14, which, despite receiving relatively low flow, recorded the greatest concentration of MPs in 1 g of biosolids. To explore

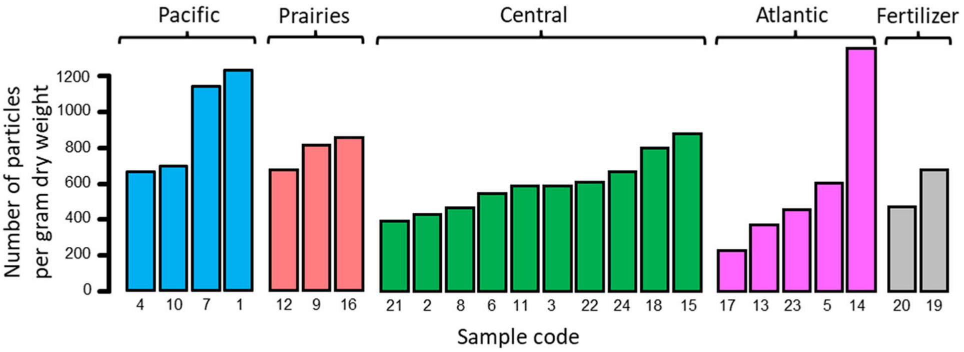

FIGURE 2 The number of microplastic particles in a gram (dry weight) of biosolids, and the sites are clustered based on geographical groups.

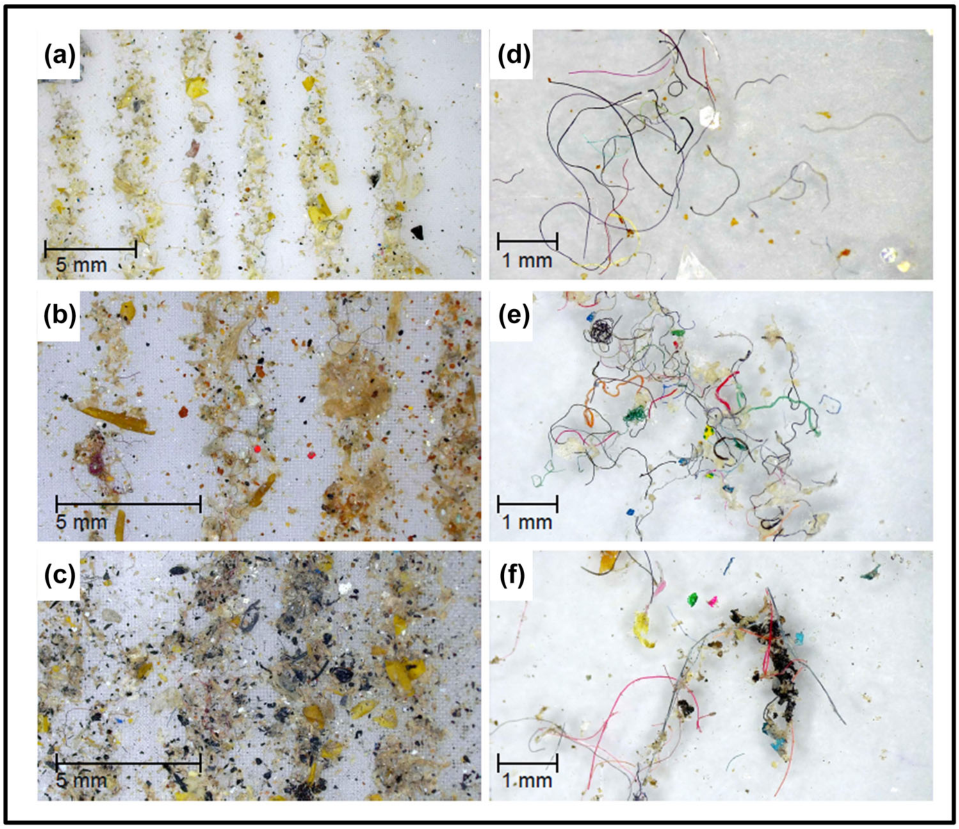

FIGURE 3 (a–c) Images of processed samples on 80 μm nylon filters for microplastic enumerations. (d–f) Images of plastic particles from various samples.

further, a correlation analysis was conducted after removing Sample 14. A stronger positive relationship was observed (rho = 0.359); however, it too was not statistically significant ($p = 0.120$) (Figure 6b).

4 | DISCUSSION

Microplastic particles were present in biosolid samples from the 22 Canadian WWTPs and two commercial fertilizer suppliers, which are representative of typical Canadian WWTPs. This is consistent with earlier investigations of biosolids from four WWTPs in Canada (Crossman et al., 2020; Gies et al., 2018; Lavoy & Crossman, 2021), and several studies completed elsewhere around the world (Edo et al., 2020; Li et al., 2018; Lusher et al., 2017; Magni et al., 2019). Fibers were the most common type observed in all samples in our study as has been reported in earlier investigations from Australia, China, Finland, and Ireland (Lares et al., 2018; Li et al., 2018; Mahon et al., 2017; Ziajahromi et al., 2021). We observed octagonal

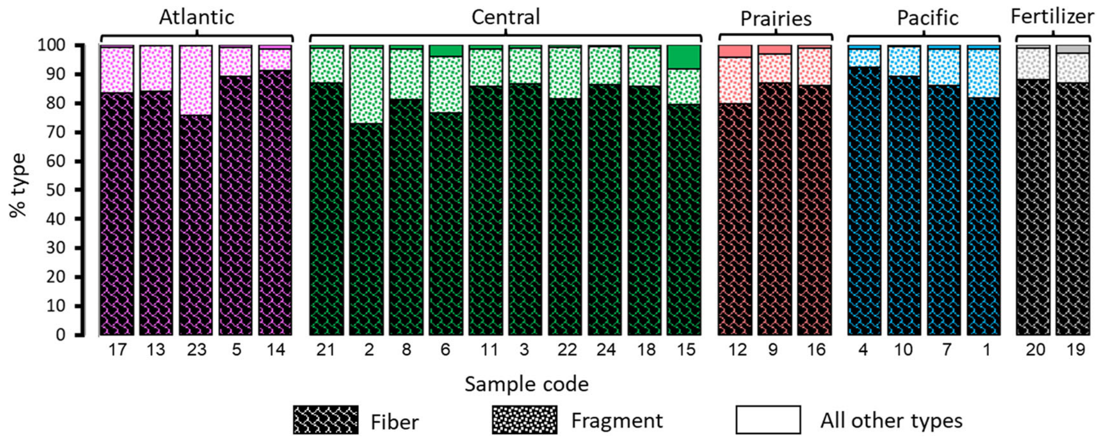

FIGURE 4 The percentage of the most common type of microplastic particles (fibers and fragments) in the 24 samples. The all other types category includes the rare forms such as film, foam, glitter, and spheres.

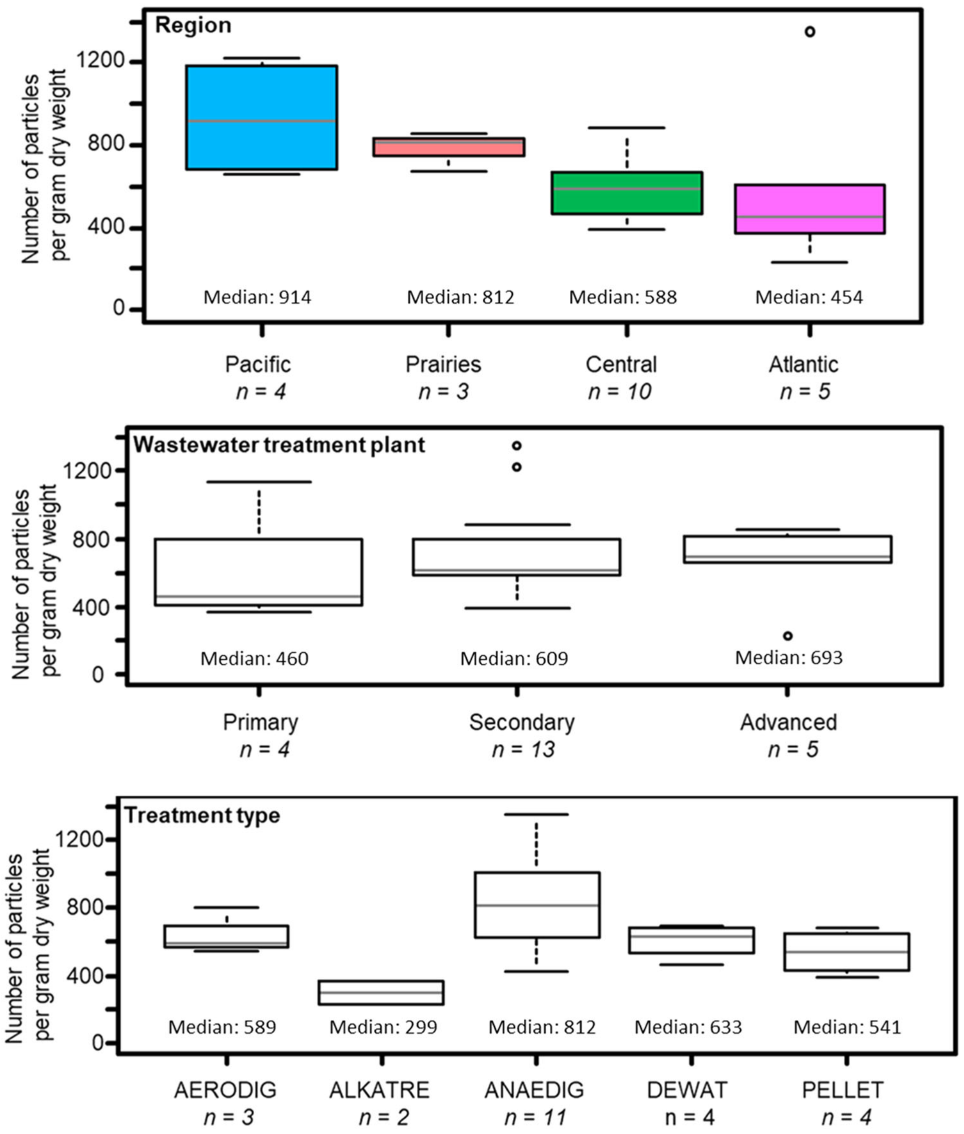

FIGURE 5 Boxplots showing the differences in the number of microplastic particles in a gram (dry weight) of biosolids based on the region (top), wastewater treatment plant type (middle), and sludge treatment approach (bottom). AERODIG, aerobic digestion; ALKATRE, alkaline treatment; ANAEDIG, anaerobic digestion; DEWAT, dewatering; PELLET, pelletization.

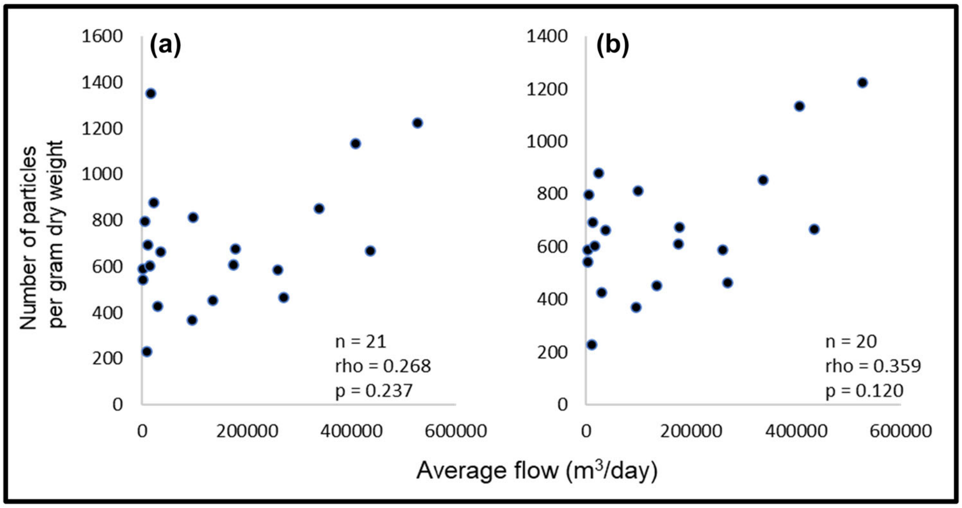

FIGURE 6 Scatter plot highlighting the relationship between average daily flow (m^3/day) and the number of microplastic particles in a gram (dry weight) of biosolid. (a) Plot with all samples ($n = 21$). (b) Plot with the outlier sample removed ($n = 20$).

shiny MPs (Figure 3d) in some samples. The source of these fragments cannot be confirmed, but given their physical morphology found across various samples, they may be types of glitter or sparkles that are commercially available.

The number of MPs present in biosolid samples varied greatly across the country; however, we did not detect a statistically significant difference between the four geographical groups (Atlantic, Central, Prairies, Pacific) as we had predicted. Interestingly, we observed substantial variation within each region (Figure 2). For instance, the lowest and highest estimates of suspected MPs were observed in the Atlantic region. The lack of statistically significant difference among the a priori defined geographical groups and high variability within each group suggest that factors other than geographic location could be influencing the MP content in biosolids from various WWTPs in this study. For example, the treatment plant type, sludge treatment approach, and amount of sewage received (i.e., average daily flow) by WWTPs could influence the amount of MPs ending up in biosolids.

Previous studies have investigated the efficacy of WWTP type (i.e., primary, secondary, advanced) in removing MPs from influents (e.g., Carr et al., 2016; Iyare et al., 2020). While much of the microplastics are removed during primary treatment, secondary and advanced treatments also play roles in removing additional MPs (Iyare et al., 2020). In this study, statistically significant differences were not observed among the three types of WWTPs. This may be due to differences in the sewersheds and small sample sizes within each group. However, if we were to assume that there is relatively equal input of MPs per m^3/day into the WWTPs included in this study, it could suggest that using current technology, the different types of WWTP do not differ in their ability to remove MPs from sewage that enters the system. The median number of MPs were lowest in samples that underwent alkaline treatment, which involves diluting sludge with alkaline substances that can dilute the concentration of MPs. As we had predicted, a weak positive correlation between the number of MPs in biosolids and the average flow at the WWTPs was observed. Although this relationship was not statistically significant, it suggests that the amount of raw sewage received by WWTPs may influence the number of MPs present in biosolids. Specifically, sites with higher average daily flow rates, representing larger sewersheds and diverse industrial, commercial and residential inputs, could be receiving more MPs, which may be concentrated in the biosolids.

In this study, we found 228–1353 of MP particles per gram dry weight of biosolids, with a median of 636. This is higher than most previously reported values. Globally, the amount of MPs reported in a gram of biosolid (dry weight) range between 1 and 171 in Australia, China, Ireland, Italy, Spain, and the United Kingdom (Edo et al., 2020; Iyare et al., 2020; Li et al., 2018; Lofty et al., 2022; Magni et al., 2019; Mahon et al.,2017; Ziajahromi et al., 2021). Lares et al. (2018) have reported levels >200 particles of microplastics in a gram (dry weight) of biosolids from a Finnish WWTP. In Canada, previous investigations have estimated 8–15 particles per gram dry weight in biosolids (Crossman et al., 2020) and 4–15 particles per gram of sludge (Gies et al., 2018). However, in a recent investigation, Lavoy and Crossman (2021) reported more than 500 suspected MPs per sample (~1 g of biosolid was processed in each sample) in biosolids from a WWTP in Ontario, Canada. Clearly, there is a large range in the number of MPs reported in biosolids from Canada and around the world, and our estimates are higher than previous reports. There are several reasons that could be leading to these differences. In a recent synthesis, Koutnik et al. (2021) estimated that more than 90% of MPs in sludge are not accounted in studies due to limitations in processing approaches. Since biosolids are complex environmental matrices, previous studies have employed a series of analytical steps to isolate MPs from organic and silt-rich samples. Since the density of most MPs is lower than other materials present in biosolids, density-based techniques are commonly used to separate MPs from raw (e.g., Li et al., 2018) or processed (e.g., Crossman et al., 2020; Edo et al., 2020) samples. While density separation using heavier liquids (e.g., sodium iodide $\rho = 1.8$ g/cm^3; calcium chloride $\rho = 1.3$ g/cm^3) can be useful techniques, sometimes MPs bound to organic or silt material could not be separated, thus leading to potential underestimation of suspected MPs in samples. During the initial exploratory phase of this study, we processed a few biosolid samples with H_2O_2 and then used calcium chloride solution ($\rho = 1.3$ g/cm^3) to isolate MPs. After filtering the supernatant to the filters for enumeration, we examined the residue sediments under a stereomicroscope and observed several microplastics (fibers, fragments) bound to organic and inorganic material. Hence, for our final analyses, we elected to examine all of the processed material for suspected MPs without the density separation step. Our approach also allowed us to limit the number of analytical steps, which likely helped to reduce potential recovery losses and airborne contamination that could happen when multiple steps are involved (e.g., multiple digestions with H_2O_2 and sequential density separation).

We recognize that this study provides a preliminary survey and evidence of potential relationships and patterns of MPs in biosolids in Canada. Our goal was to examine the main attributes of WWTP to explore patterns in their entrapment of MPs in biosolids that may enter terrestrial ecosystems. There are many other attributes of sewage and WWTP that may also influence their ability to capture and retain MPs. For example, there may be seasonal differences as WWTP capacity and function are influenced by the amount of water in the systems. Further analysis using multiple samples collected throughout the year at multiple WWTPs are necessary to confirm and establish trends. The low sample size in certain a priori defined groups could also potentially influence the outcome of statistical analyses. Hence, future studies should consider expanding the number of sites for various geographic regions and WWTP treatment plants, which can help to discern complex interactive relationships between WWTP plants and variables such as flow. Similarly, the efficacy of individual WWTPs at removing MPs from raw sewage can be assessed by examining influents, effluents, and biosolids.

Microplastic analysis in biosolids is a labor-intensive process, and new methodologies to enhance the efficiency of this process would greatly benefit monitoring programs and assessments of microplastic variability from diverse geographic regions and across different time periods. The composition of biosolids from different WWTPs varies greatly due to site-specific treatment methods. Hence, a variety of analytical/laboratory approaches have been proposed to quantify MP content in biosolids, and each method has benefits and challenges. For example, the approach used in this study has fewer analytical steps and is relatively accessible. However, the samples processed with H_2O_2 alone often contain undigested organic particles that result in filters with large amounts of material, which had to be carefully inspected to enumerate MPs (Figure 3A–C). Since this is a time-intensive approach, it limits the number of unique and replicate samples that can be analyzed. Additionally, the presence of cellulose material impeded the enumeration of clear and white fibers, and this is a challenge that has been reported in other studies too (Harley-Nyang et al., 2022). Recently, pressurized liquid extraction combined with double-shot pyrolysis gas chromatography–mass spectrometry (PLE with Pyr-GC-MS) has also been used to quantify the mass of MPs (reported in mg/g dry weight) in biosolids (Okoffo et al., 2020, 2021). These complementary techniques can be helpful to advance our understanding of MPs in biosolids and develop standardized approaches to enable comparison between different regions. Importantly, the development of these techniques will be critical for widescale monitoring of biosolids so that samples from WWTPs and fertilizer companies can be processed quickly using methods that are comparable across time and space.

Our survey of biosolids from several regions of Canada, in combination with previous studies around the world, has shown that hundreds of MPs are present in a gram of biosolid (e.g., Iyaro et al., 2020; Lares et al., 2018; Magni et al., 2019). The quantity of microplastic particles observed in the biosolids is several magnitudes higher than other environmental matrixes such as water, soil, and river sediments from Canada (Crossman et al., 2020; Labaj et al., 2022; Vermaire et al., 2017). These findings are important in understanding the transport and fate of MPs in the environment, and in assessing what ecosystems may be the most vulnerable to MP pollution where more effects assessments should be undertaken. Some studies have estimated that billions of MPs are entering agricultural lands through annual biosolid applications (Crossman et al., 2020; Nizzeto et al., 2016). Indeed, Crossman et al. (2020) demonstrated that biosolid application to agricultural farmlands is an important and substantial pathway through which MPs enter terrestrial ecosystems. Collectively, these studies highlight the need to monitor and assess the risk associated with MP content in biosolids.

Despite the large amount of MPs present in biosolids, we acknowledge that carefully managed application of treated sludge has documented benefits to agricultural landscapes (CCME, 2012a, 2012b). Most importantly, biosolids help to enrich soil nutrient levels, limit the use of commercial fertilizers, and reduce landfill wastes. The presence of large quantities of MPs in biosolids presents an important opportunity to innovate and develop engineered solutions at WWTPs that can help to reduce MPs entering agricultural landscapes.

However, effective management strategies should first consider upstream source control, which can substantially limit the amount of MPs entering WWTPs. For instance, pilot studies have shown that installing washing machine filters can substantially reduce the amount of synthetic microfibers leaving households and eventually ending up at WWTPs (Erdle et al., 2021).

5 | CONCLUSION

Quantifying MPs in biosolid samples is an important first step that will help to inform evidence-based policies to curb terrestrial microplastic pollution. Further, developing analytical approaches that can be applied to diverse samples is necessary as the composition of biosolids varies greatly among WWTPs. In this study, we showed that Canadian biosolids contain hundreds of microplastics per gram dry weight. Biosolids are likely one of the most concentrated environmental matrices rich in MPs and play a key role in global plastic cycle. WWTPs serve as important environmental conduits and concentrators of microplastics as they a capture large amount of plastic material from sewage and stormwater. Therefore, WWTPs are an excellent location for monitoring microplastic concentrations in the environment. Future studies should evaluate the risk associated with microplastic concentrations in biosolids reported here, although any risk management would need to target upstream sources. The data provided in this baseline assessment can be used to build future monitoring programs and evaluate if plastic reduction measures implemented at various levels of government can be effective in reducing microplastic pollution.

AUTHOR CONTRIBUTIONS

Branaavan Sivarajah: Conceptualization; data curation; formal analysis; visualization; writing—original draft. **David R. Lapen**: Conceptualization; resources; supervision; funding acquisition; writing—review and editing. **Sarah B. Gewurtz**: Conceptualization; investigation; resources; writing—review and editing. **Shirley Anne Smyth**: Conceptualization; resources; funding acquisition; writing—review and editing. **Jennifer F. Provencher**: Conceptualization; resources; funding acquisition; writing—review and editing. **Jesse C. Vermaire**: Supervision; conceptualization; resources; funding acquisition; visualization; writing—review and editing.

ACKNOWLEDGMENTS

We are grateful to the 22 WWTPs and two fertilizer companies for providing samples for this study. Many thanks are also due to Madelaine Bourdages for providing recommendations about microplastic analytical approaches and to Ramon Pineda for assisting with laboratory procedures. We thank two anonymous reviewers and the Associate Editor (Dr. Min Pan) for providing constructive feedback on an earlier version of this manuscript. Funding from the Environment Canada and Climate Change Increasing Knowledge on Plastic Pollution program, Agriculture and Agri-Foods Canada, and Natural Sciences and Engineering Research Council of Canada are gratefully acknowledged.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data will be made available upon request.

ORCID

Branaavan Sivarajah https://orcid.org/0000-0002-3739-4299

REFERENCES

Alberta Environment. (2009). *Guidelines for the application of municipal wastewater sludges to agricultural lands*. Alberta Environment, Government of Alberta.

Arctic Monitoring Assessment Programme (AMAP). (2021). *AMAP litter and microplastics monitoring guidelines*. Version 1.0. Arctic Monitoring and Assessment Programme (AMAP).

Baho, D. L., Bundschuh, M., & Futter, M. N. (2021). Microplastics in terrestrial ecosystems: Moving beyond the state of the art to minimize the risk of ecological surprises. *Global Change Biology*, *27*, 3969–3986. https://doi.org/10.1111/gcb.15724

Canadian Council of Ministers of the Environment (CCME). (2010). *A review of the current Canadian legislative framework for wastewater biosolids*. Canadian Council of the Ministers of the Environment.

Canadian Council of Ministers of the Environment (CCME). (2012a). *Canada-wide approach for the management of wastewater biosolids*. Canadian Council of the Ministers of the Environment.

Canadian Council of Ministers of the Environment (CCME). (2012b). *Guidance document for the beneficial use of municipal biosolids, municipal sludge and treated septage*. Canadian Council of the Ministers of the Environment.

Carr, S. A., Liu, J., & Tesoro, A. G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. *Water Research*, *91*, 174–182. https://doi.org/10.1016/j.watres.2016.01.002

Crossman, J., Hurley, R. R., Futter, M., & Nizzetto, L. (2020). Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment. *Science of the Total Environment*, *724*, 138334. https://doi.org/10.1016/j.scitotenv.2020.138334

Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., & Rosal, R. (2020). Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. *Environmental Pollution*, *259*, 113837. https://doi.org/10.1016/j.envpol.2019.113837

Environment and Climate Change Canada (ECCC). (2019). *Canada's plastics science agenda*. Environment and Climate Change Canada.

Erdle, L. M., Nouri Parto, D., Sweetnam, D., & Rochman, C. M. (2021). Washing machine filters reduce microfiber emissions: Evidence from a community-scale pilot in Parry Sound, Ontario. *Frontiers in Marine Science*, *8*, 777865. https://doi.org/10.3389/fmars.2021.777865

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, *3*, e1700782. https://doi.org/10.1126/sciadv.1700782

Gies, E. A., Lenoble, J. L., Noël, M., Etemadifar, A., Bishay, F., Hall, E. R., & Ross, P. S. (2018). Retention of microplsatics in a major secondary wastewater treatment plant in Vancouver, Canada. *Marine Pollution Bulletin*, *133*, 553–561. https://doi.org/10.1016/j.marpolbul.2018.06.006

Harley-Nyang, D., Memon, F. A., Jones, N., & Galloway, T. (2022). Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK. *Science of the Total Environment*, *823*, 153735. https://doi.org/10.1016/j.scitotenv.2022.153735

Hurley, R. R., Lusher, A. L., Olsen, M., & Nizzetto, L. (2018). Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. *Environmental Science and Technology*, *52*, 7409–7417. https://doi.org/10.1021/acs.est.8b01517

Iyare, P. U., Ouki, S. K., & Bond, T. (2020). Microplastic removal in wastewater treatment plants: A critical review. *Environmental Science: Water Research and Technology*, *6*, 2664.

Koutnik, V. S., Alkidim, S., Leonard, J., DePrima, F., Cao, S., Hoek, E. M. V., & Mohanty, S. K. (2021). Unaccounted microplastics in wastewater sludge: Where do they go? *Environmental Science and Technology Water*, *1*, 1086–1097.

Labaj, A. L., Leblanc, A. D., Doucet, C. V., Tardif, C. E., & Kurek, J. (2022). Microlitter in the water, sediments, and mussels of the Saint John River (Wolastoq) watershed, Atlantic Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *79*, 566–575. https://doi.org/10.1139/cjfas-2021-0121

Lakshminarasimman, N., Gewurtz, S. B., Parker, W. J., & Smyth, S. A. (2021). Removal and formation of perfluroalkyl substances in Canadian sludge treatment systems—A mass balance approach. *Science of the Total Environment*, *754*, 142431. https://doi.org/10.1016/j.scitotenv.2020.142431

Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Research*, *133*, 236–246. https://doi.org/10.1016/j.watres.2018.01.049

Lavoy, M., & Crossman, J. (2021). A novel method for organic matter removal from samples containing microplastics. *Environmental Pollution*, *286*, 117357. https://doi.org/10.1016/j.envpol.2021.117357

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., & Zeng, E. Y. (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. *Water Research*, *142*, 75–85. https://doi.org/10.1016/j.watres.2018.05.034

Lofty, J., Muhawenimana, V., Wilson, C. A. M. E., & Ouro, P. (2022). Microplastics removal from a primary settler tank in a wastewater treatment plant and estimations of contamination onto European agricultural land via sewage sludge recycling. *Environmental Pollution*, *304*, 119198. https://doi.org/10.1016/j.envpol.2022.119198

Lusher, A. L., Bråte, I. L. N., Munno, K., Hurley, R. R., & Welden, N. A. (2017). Is it or isn't it: The importance of visual classification in microplastic characterization. *Applied Spectroscopy*, *74*, 1139–1153. https://doi.org/10.1177/0003702820930733

Lusher, A. L., Hurley, R., Vogelsang, C., Nizzetto, L., & Olsen, M. (2020). *Mapping microplastics in sludge*. Norwegian Institute for Water Research.

Magni, S., Binelli, A., Pittura, L., Avio, C. G., Della Torre, C., Parenti, C. C., Gorbi, S., & Regoli, F. (2019). The fate of microplastics in an Italian wastewater treatment plant. *Science of the Total Environment*, *652*, 602–610. https://doi.org/10.1016/j.scitotenv.2018.10.269

Mahon, A. M., O'connell, B., Healy, M. G., O'connor, I., Officer, R., Nash, R., & Morrison, L. (2017). Microplastics in sewage sludge: Effects pf treatment. *Environmental Science Technology*, *51*, 810–818. https://doi.org/10.1021/acs.est.6b04048

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). *Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments*. Siver Spring, MD, NOAA Marine Debris Division.

Miller, E., Sedlak, M., Lin, D., Box, C., Holleman, C., Rochman, C. M., & Sutton, R. (2021). Recommended best practices for collecting, analyzing, and reporting microplastics in environmental media: Lessons learned from comprehensive monitoring of San Francisco Bay. *Journal of Hazardous Materials*, *409*, 124770. https://doi.org/10.1016/j.jhazmat.2020.124770

Möller, J. N., Löder, M. G. J., & Laforsch, C. (2020). Finding microplastics in soils: A review of analytical methods. *Environmental Science Technology*, *54*, 2078–2090. https://doi.org/10.1021/acs.est.9b04618

Munno, K., Helm, P. A., Jackson, D. A., Rochman, C., & Sims, A. (2018). Impacts of temperature and selected chemical digestion methods on microplastic particles. *Environmental Toxicology and Chemistry*, *37*, 91–98. https://doi.org/10.1002/etc.3935

New Brunswick Environment and Local Government. (2014). *Guidelines for the beneficial use of industrial by-products as soil amendments*. The Interdepartmental Committee of Environment and Local Government and Agriculture, Aquaculture and Fisheries.

Ng, E.-L., Huerta Lwanga, E., Eldridge, S. M., Johnston, P., Hu, H.-W., Geissen, V., & Chen, D. (2018). An overview of microplastic and nanplastic pollution in agroecosystems. *Science of the Total Environment*, *627*, 1377–1388. https://doi.org/10.1016/j.scitotenv.2018.01.341

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are agricultural soils dumps for microplastics of urban origin? *Environmental Science Technology*, *50*, 10777–10779. https://doi.org/10.1021/acs.est.6b04140

Nova Scotia Environment. (2010). *Guidelines for land application and storage of municipal biosolids in Nova Scotia*. Nova Scotia Environment.

Okoffo, E. D., Donner, E., Mcgrath, S. P., Tscharke, B. J., O'brien, J. W., O'brien, S., Ribeiro, F., Burrows, S. D., Toapanta, T., Rauert, C., Samanipour, S., Mueller, J. F., & Thomas, K. V. (2021). Plastics in biosolids from 1950 to 2016: A function of global plastic production and consumption. *Water Research*, *201*, 117367. https://doi.org/10.1016/j.watres.2021.117367

Okoffo, E. D., Ribeiro, F., O'brien, J. W., O'brien, S., Tscharke, B. J., Gallen, M., Samanipour, S., Mueller, J. F., & Thomas, K. V. (2020). Identification and quantification of selected plastics in biosolids by pressurized liquid extraction combined with double-shot pyrolysis gas chromatography-mass spectrometry. *Science of the Total Environment*, *715*, 136924. https://doi.org/10.1016/j.scitotenv.2020.136924

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for statistical computing.

Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., Mcilwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S. B., Wu, T., Santoro, S., Werbowski, L. M., … Hung, C. (2019).

Rethinking microplastics as a diverse contaminant suite. *Environmental Toxicology and Chemistry*, *38*, 703–711. https://doi.org/10.1002/etc.4371

Souza Machado, A. A., Kloas, W., Zarfl, C., Hempel, S., & Rillig, M. C. (2018). Microplastics as an emerging threat to terrestrial ecosystems. *Global Change Biology*, *24*, 1405–1416. https://doi.org/10.1111/gcb.14020

Talvitie, J., Mikola, A., Setälä, O., Heinonen, M., & Koistinen, A. (2017). How well is microliter purified from wastewater?—A detailed study on the stepwise removal of microliter in a tertiary level wastewater treatment plant. *Water Research*, *109*, 164–172. https://doi.org/10.1016/j.watres.2016.11.046

Vermaire, J. C., Pomeroy, C., Herczegh, S. M., Haggart, O., & Murphy, M. (2017). Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. *FACETS*, *2*, 301–314. https://doi.org/10.1139/facets-2016-0070

Ziajahromi, S., Neale, P. A., Telles Silveira, I., Chua, A., & Leusch, F. D. L. (2021). An audit of microplastic abundance throughout three Australian wastewater treatment plants. *Chemosphere*, *263*, 128294. https://doi.org/10.1016/j.chemosphere.2020.128294

How to cite this article: Sivarajah, B., Lapen, D. R., Gewurtz, S. B., Smyth, S. A., Provencher, J. F., & Vermaire, J. C. (2023). How many microplastic particles are present in Canadian biosolids? *Journal of Environmental Quality*, *52,* 1037–1048. https://doi.org/10.1002/jeq2.20497