Microplastics in Wastewater: Annual Trends and Biosolid Treatment Strategies.

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ABSTRACT

Microplastics in Wastewater: Annual Trends and Biosolid Treatment Strategies.

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This thesis determined the temporal dynamics of microplastics in the biosolid and final effluent of a WWTP for one year (October 2020–September 2021). The WWTP exported 354.1 ± 24.7 billion microplastic particles, or 296.7 ± 39.4 kg of microplastics; of this, 85.7% of counts and 86.6% of mass was exported via biosolids. As such, microplastic loads in biosolids need to be reduced before they are land applied. This thesis further examined the ability of settling treatments to liberate microplastics from biosolids under the effect of four variables (harvest method, stirring, settling time, and biosolid type). Across all treatments, average microplastic removal was 9.01 ± 5.82% in terms of count, and 6.91 ± 4.98% in terms of mass. Overall, this thesis contributed to our understanding of annual microplastic burden in a WWTP and set the foundation for the development of settling-based biosolids treatments to reduce microplastic emissions to the environment.

Keywords

Biosolid, Final Effluent, Treatment, Mass Concentration, Count Concentration, Polymer, Raman Spectroscopy, Density.

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Chapter 1: A Brief Introduction to Microplastics and Wastewater

Plastics have been mass produced for more than 60 years. Their durability, low-cost, and convenience have contributed to their unbridled rise in popularity (Wolff et al. 2019, Herrera et al. 2018). During the 1950s, plastic was produced at a rate of 1.7 million tonnes/year; however, by 2015, this figure had risen drastically to more than 400 million tonnes/year (Henry et al. 2019). As global production continues to increase, the mismanagement of plastic waste is considered one of the most prominent environmental threats posed by the Anthropocene (Brander et al. 2020). Improving waste management practices is essential to reduce plastic debris in the environment, as outlined in the concept of a circular economy (Payne 2019). Once in the environment, waste plastics degrade extremely slowly, and with no natural sink, plastics will continue to accumulate (Environment and Climate Change Canada 2020).

Typically, when thinking about plastic pollution, images of improperly discarded water bottles come to mind. Though this serves as an example of macroplastic pollution, it does not represent the full extent of plastic pollution. Microplastics, which are defined as plastic debris with a major axis less than 5 mm (Schmidt et al. 2020), have been observed in all environments globally. There are two key types of microplastics: primary and secondary (Simon et al. 2018); primary microplastics are directly manufactured (e.g., microbeads in cosmetics), whereas secondary microplastics form through the degradation of a parent material (e.g., fragmentation of a water bottle). Several mechanisms may contribute to the degradation of plastics, including UV radiation, microbial processes, extreme temperatures, mechanical stress, thermo-oxidation, and hydrolysis (Wolff et al. 2019, Anderson et al. 2016). Irrespective of the degradation process, the result is the same; the parent material is broken down into continually smaller plastic debris. As microplastics degrade, chemicals such as phthalates, nonylphenols, bisphenol A, heavy metals,

and polybrominated diphenyl ethers may be released into the surrounding environment (Driedger et al. 2015).

In general, microplastics are characterized by their size, shape, color, density, and polymer type (Bilgin et al. 2020). Microplastic size is particularly important when assessing environmental burden. For example, when comparing two cubes, one with a side length of 1 µm, and the other 1 mm, the latter has a volume that is greater by a factor of 1 billion (Simon et al. 2017). Though each of these particles would be counted as one microplastic, there is a clear difference in the quantity (burden) of plastic contributed by each particle. Microplastic shape (or morphology) and polymer type provide useful insight to the sources of microplastics and their potential transport pathways (Prata et al. 2019, Sui et al. 2020). Though shape categories are not universally standardized, shape is typically subdivided into categories such as fibres, films, fragments, beads, and foams (Talvitie et al. 2017, Allen et al. 2019). Plastics come in a wide array of polymer types (i.e., polyethylene terephthalate [PET], polypropylene [PP], polyethylene [PE], polyvinyl chloride [PVC], etc.), each of which has its own unique properties, like density (Anderson et al. 2016). By quantifying microplastic abundance (count), shape, size, and density, the mass of microplastics can be estimated (Simon et al. 2018, Rolsky et al. 2020, Roscher et al. 2022). Though microplastic quantities are typically communicated in terms of either microplastic counts, or masses, using both metrics provide a more comprehensive understanding of the impact of microplastics on the environment.

Microplastics have been identified in air, soil, water, and biota across the globe (Anderson et al. 2016). There are several sources of microplastics to the environment including, but not limited to: the mismanagement of waste, manufacturing, construction, wastewaters, and the production and use of cosmetics and textiles (Schmidt et al. 2020, Ziajahromi et al. 2017).

Once introduced to an ecosystem, microplastics are extremely persistent (Anderson et al. 2016). However, the characteristics of a microplastic can drastically influence its fate in the environment. Density is particularly important in aquatic environments, as it affects particle buoyancy. High density polymers tend to settle more readily, whereas low density polymers tend to float (Erni-Cassola et al. 2017). Other characteristics such as shape influence this as well, microplastics with high surface area to volume ratios, such as fibres and films, are more easily suspended than dense granular particles (He et al. 2019).

Microplastics have been reported to induce negative health effects at all trophic levels (Driedger et al. 2015, Simon et al. 2018). Microplastics can enter the body through inhalation, ingestion, and absorption (Koelmans et al. 2019). It is estimated that humans take in approximately 70,000 microplastics each year through eating, breathing, and drinking (Brander et al. 2017). Ingestion of microplastics has been linked to liver oxidative stress, retention in lymphatic tissues, metabolic changes, and altered gut microbiota (Environment and Climate Change Canada 2020). Inhalation on the other hand contributes to oxidative stress, cytotoxicity, lung inflammation, and the development of foreign body granulomas (Environment and Climate Change Canada 2020). Microplastics have been identified in a variety of tissues (e.g., placenta, blood, liver etc.), with their shape/size having the largest influence on their ability to traverse the body (Marfella et al. 2024). In smaller organisms (e.g., benthic macroinvertebrates) microplastics have been observed to completely block their gastrointestinal tract (Ziajahromi et al. 2017). Microplastics may also serve as a vector for pathogens, organic pollutants, and metals to enter the body (Driedger et al 2015). These contaminants bind to the surface of the microplastic leading to concentrations higher than the surrounding environment (Anderson et al. 2016, Wolff et al 2019).

Microplastics are an anthropogenic debris; as such, there is a tendency for environments near human centres to have higher microplastic concentrations (Driedger et al 2015). Terrestrial emissions account for the largest source of microplastics to the environment (Nizzetto et al. 2016). With the land-based application of biosolids as an agricultural amendment recognized as a major microplastics emissions source (Environment and Climate Change Canada 2020). Once in the terrestrial environment, microplastics may enter the aquatic environment via runoff, or the atmosphere via wind entrainment (Wolff et al. 2019). Microplastics are highly mobile in the aquatic environment, with rivers serving as a major transport pathway (Driedger et al 2015). In river systems, microplastics may settle into sediments or stay suspended in flows, with the ultimate destination being the marine environment (Schmidt et al. 2020). Since it is not feasible to remove microplastics from the environment after their introduction, it is crucial that source control is implemented.

Wastewater Treatment Plants (WWTPs) serve as a bottleneck between anthropogenically polluted waters and the environment. In this system, sewers are used to collect and transport contaminated water from its source to the WWTP. Sanitary sewer systems are responsible for collecting water from residential, industrial, and commercial waste producers, whereas storm sewer systems collect and channel urban runoff (Brander et al. 2020, Schmidt et al. 2020). The source of influent plays a major role in determining the microplastic profile observed (Schmidt et al 2020). Examples of residential microplastics include fibres from textiles and beads from cosmetics and cleaning products (Brander et al. 2020). Industrial and commercial sources tend to be more diverse, examples of which include air blasting particles, pre-production pellets, plastic dust/shavings, and synthetic fibres (Brander et al. 2020). Storm sewers typically contain tire

abrasion, fragments, and litter that was captured by runoff (Coppock et al. 2017, Schmidt et al. 2020).

There are two main waste streams that leave WWTPs: the biosolid, and the final effluent. Although limited, low frequency spot sampling has demonstrated that microplastics tend to accumulate in the biosolid. The effectiveness of microplastic removal depends on the WWTP's level of treatment. Systems with primary treatment alone were reported to remove 50–98% of microplastics, while secondary treatment systems were reported to remove 86–99.8% of microplastics, and tertiary treatment systems were reported to remove 98–99.8% of microplastics (Environment and Climate Change Canada 2020). The microplastics removed from wastewater are then concentrated in the biosolids, which are widely applied as an agricultural amendment, thus contributing to microplastic loading in terrestrial environments (Environment and Climate Change Canada 2020). As such, biosolids represent a critical pathway by which microplastics enter the environment, and so, a method of isolating microplastics from biosolid would help to reduce the environmental prevalence of microplastics.

For microplastics to be managed effectively, it is essential that their source-pathway relationship is understood (He et al. 2019). Wastewater Treatment Plants are a critical point in the chain between microplastic producers, and the environment; they represent a point at which microplastic inputs can be controlled (Talvitie et al. 2017). Removing microplastics from the environment is not yet feasible, and as such the development of a method to prevent their emission is crucial (He et al. 2019). This study had two main objectives: (a) to quantify and characterize annual microplastic export in the biosolid and final effluent streams, and (b) to investigate the feasibility of using a settling-based approach to liberate microplastics from the biosolids prior to land application. To accomplish objective (a), weekly samples of biosolid and

final effluent were collected from a WWTP in Southern Ontario, Canada, and were analysed as monthly composites for microplastic content. Objective (b) was addressed by conducting a settling experiment that evaluated the ability of four variables (Settling Time, Stirring, Harvest Method, and Biosolid Source) to promote the liberation of microplastics from biosolid. This thesis is written in manuscript style, where Chapter 2: Microplastic export from a Canadian wastewater treatment plant, addresses objective (a), and Chapter 3: Settling strategies to reduce microplastics loads in biosolids from wastewater treatment plants, addresses objective (b). Given the manuscript style, there is substantial overlap in the methods between the two chapters.

Chapter 2: Microplastic export from a Canadian wastewater treatment plant

2.1 Introduction

Microplastic contamination of the environment has been a topic of increasing concern in recent years. Microplastics may be intentionally produced (i.e., primary microplastics) or formed through the degradation of larger plastic materials (i.e., secondary microplastics), as described by Anderson et al. (2016) and Anger et al. (2018). Microplastics are defined as being less than 5 mm along any given axis. However, the lower size limit is often defined on a case-by-case basis (Schmidt et al. 2020, Bilgin et al. 2020). Further, microplastics are a diverse contaminant as they consist of various, shapes, sizes, and polymer types. To accommodate this diversity, microplastic attributes must be clearly described to accurately communicate true microplastic abundance. In general, it is common for microplastic abundance to be reported in terms of a microplastic count, or the number of microplastic particles per unit mass or volume of an environmental media. However, given the diversity of microplastics, counts are not suitable as a standalone metric (Simon et al. 2018, Rolsky et al. 2020, Kim et al. 2022, Roscher et al. 2022, Okoffo et al. 2023). Instead, microplastic abundance should also be described in terms of a mass of plastic to provide a more comprehensive understanding of their burden to the environment (Simon et al. 2018, Rolsky et al. 2020, Roscher et al. 2022).

Microplastics originate in areas of human activity before being dispersed across the globe (ECCC 2020, Brander et al. 2020, Schmidt et al 2020). From their source, microplastics may enter the natural environment through a variety of pathways; one of the most prominent being sewer systems and wastewater treatment plants (WWTPs). Sewers directly connect waste producers to the natural environment, with the only barrier prior to release being WWTPs (Kim and Park 2021). Therefore, WWTPs represent a critical junction where microplastic

contamination rates may be monitored and potentially controlled. Despite this, there is currently no legislation that requires WWTPs to monitor or control microplastic emissions (Anderson et al. 2016, Driedger et al. 2015, Mahon et al. 2017, Nizzetto et al. 2016).

Most studies have shown that WWTPs are effective in removing microplastics from the final effluent; that is, the treated wastewater released from the WWTP. In a Canadian context, WWTPs have demonstrated the ability to remove 50–98% of microplastics during primary treatment, 86–99.8% during secondary treatment, and 98–99.8% during tertiary treatment (ECCC 2020). As such, the majority of microplastics are diverted from the final effluent, which prevents their immediate discharge to the aquatic environment. Instead, these diverted microplastics are retained in the biosolids, with studies suggesting that 88–97% of microplastics entering a WWTP are captured and stored in biosolids (Brander et al. 2020). Nonetheless, these microplastics may still be introduced to the environment through land application of biosolids (Okoffo et al. 2023). In Canada, approximately 47% of biosolids are incinerated, 43% are land applied, and 4% are landfilled (Rolsky et al. 2020). A limitation within the literature surrounding microplastics and WWTPs, is that the majority of published studies look at microplastic behavior (abundance) at only one specific time. As such, longitudinal studies that consider seasonal variations, and other cofounding variables (e.g., water quantity or other water quality parameters) are needed to better assess microplastic exports to the environment (Rosher et al. 2022, Lares et al. 2018, Kim et al. 2022, Bhowmick and Sarmah 2022).

This study examined annual microplastic export from a WWTP with conventional activated sludge, and secondary sewage treatment serving a community of approximately 84,000 located in south-central Ontario, Canada. Monthly microplastic abundance (count and mass) was quantified for biosolid and final effluent between October 2020 and September 2021. When

combined with plant flows, these data were used to estimate microplastic flux (export), and microplastic treatment efficiencies; that is, the proportion of total microplastics that are retained in the biosolids. These metrics were compared with wastewater quality parameters and flows within the plant, as well as local precipitation and river flow data, to understand how microplastic loads in WWTPs behave in a broader context. This study is unique in the way microplastic count and mass concentrations are tracked and reported at monthly intervals for an entire year. This structure allows analysts to investigate the influence of seasonality, plant flows, and water quality metrics on microplastic loads and treatment efficiencies. In doing so, this study offers a comprehensive understanding of annual trends in the quantity and characteristics of microplastics discharged in the treated wastewater and biosolids. With a greater understanding of the variables that influence microplastic treatment, industry leaders and legislators will be empowered to address the problem of microplastics in wastewater.

2.2 Methods

2.2.1 Study Site

Samples of dewatered biosolid and final effluent were collected from a conventional style WWTP in Southern Ontario that services a population of approximately 84,000 people. Its influent is comprised of residential, commercial, and industrial wastewaters, as well as urban runoff and landfill leachate. The plant was designed to accommodate a peak flow rate of 190,900 m^3/day and is approved for an average daily flow of 68,200 m^3/day. The wastewater is treated using the following process: Influent → Bar Screening → Grit Removal → Primary Clarifiers → Aeration Tanks → Secondary Clarifiers → Ultraviolet (UV) Disinfection → Final Effluent (Figure 1). Biosolids are produced from settled material collected from the primary and

secondary clarifiers, as well as the grit removal tanks. The solids are then anaerobically digested prior to being dewatered using a large centrifuge (Figure 1).

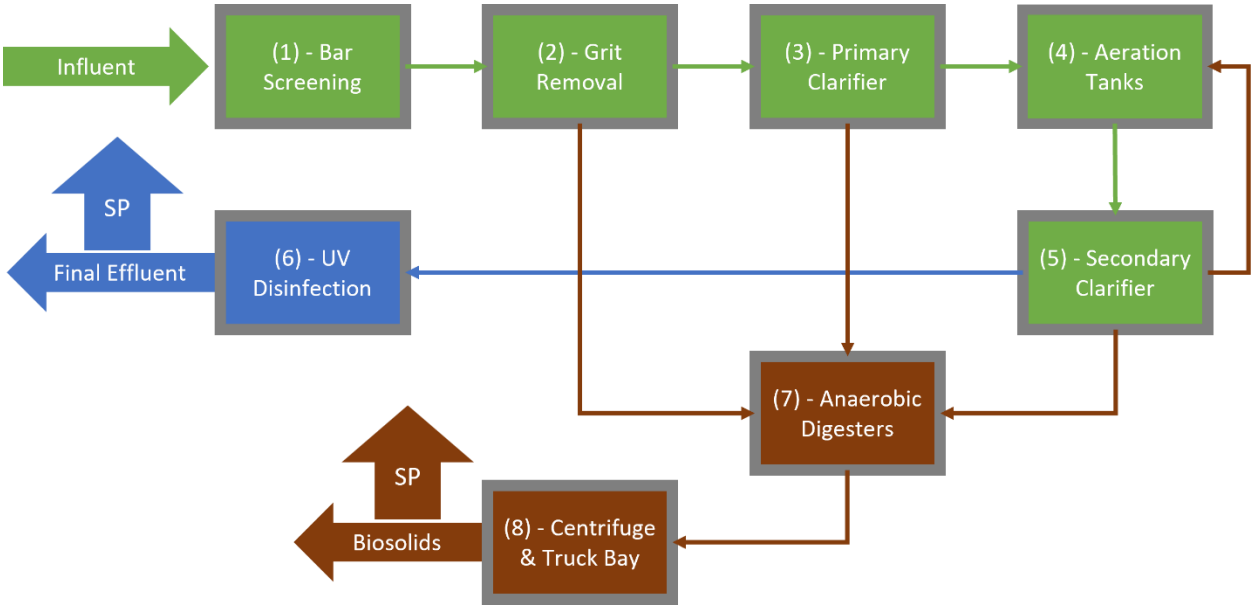

Figure 1: A simplified treatment schematic of the study's WWTP. Wastewater influent flows are green, final effluent flows are blue, and biosolids flows are brown. Note: The arrows designated "SP" represent the final effluent and biosolid sampling points used in this study.

2.2.2 Final Effluents

Weekly samples of final effluents (n = 51) were collected from the WWTP after UV disinfection and before discharge to the receiving waterbody between October 2020 and September 2021 (Figure 1). It should be noted that one week in June 2021 was missed due to sampler malfunction. The final effluents were collected using an ISCO composite autosampler (Model no. 6712) supplied by Teledyne Canada (Montreal, QC, Canada), which was programed to collect 100 mL every 15 minutes for 24 hours: the result being a 9.6 L, 96-part composite sample. It has been suggested that larger sample volumes may be more representative of plant flows (Carr et al. 2016). However, these methods have been shown to underestimate microplastic concentrations, particularly for fibres, due to the need to sieve the effluent during sample

collection (Sun et al. 2019). Using composite samples is beneficial, as they sample wastewater throughout the day, thus making them more representative of diurnal patterns in water usage (Simon et al. 2018, Brander et al. 2020, Talvitie et al. 2017, Bilgin et al. 2020). As such, composite samples are used by the wastewater treatment facility to conduct routine water quality monitoring of their final effluent.

After sample collection, a 1 L subsample of final effluent was poured into a glass jar and refrigerated prior to analysis. The weekly samples (n = 51) were composited to create monthly samples for the duration of the study period (n = 12), as documented in Appendix A1. In general, each monthly composite sample consisted of 4 × 250 mL weekly samples, to which 100 mL of hydrogen peroxide (30% by volume; VWR, Mississauga, ON, Canada. cat., 89097-776) was added to reduce organic material in the sample. Where five weekly samples were collected for the month, 5 × 200 mL aliquots were used. This 10:1 final effluent to hydrogen peroxide solution was then incubated at 40ºC for seven days. Afterward, the sample was filtered onto 4.2 cm glass fibre filter papers rated for 1.1 µm particle retention (VWR, Mississauga, ON, Canada., cat. 28297-504). Filter papers were stored in sealed Petri dishes prior to visual inspection for microplastics.

2.2.3 Biosolids

Just as with the final effluents, biosolids samples (n = 50) were collected weekly between October 2020 and September 2021. It should be noted that two biosolids sampling events were missed; one in December 2020, and one in March 2021, due to the misalignment of the sampling and centrifuge schedules. The biosolids (moisture content ~98%) are pumped from the anaerobic digesters into a centrifuge, where it is dewatered in batches to form biosolid cake (moisture

content ~ 75%). To sample the cake, approximately 100 g was collected as a grab sample, stored in a glass jar, and refrigerated. Monthly composite samples (n = 12) were then created using the weekly samples (n = 50), as documented in Appendix A1, where, in general 4 × 25 g samples were mixed thoroughly and dried at 50ºC for 48 hours to obtain dried samples. Where five weekly samples were collected for the month, 5 × 20 g samples were composited. As such, biosolid microplastic concentrations are reported in terms of dry weight (dw). It should be noted that the samples were not pulverized to avoid the fragmentation of microplastics (Simon et al. 2018).

Microplastics extraction from biosolids was conducted to isolate microplastics from organic and mineral materials. The procedure was outlined by Lavoy and Crossman (2021), which consisted of: Fenton's Digestion 1 → 20 µm sieve → Bioclean® Enzyme Digestion→ 20µm sieve → Fenton's Digestion 2 → Water Density Separation ×2 → Sodium Iodide Density Separation → Filtration. In this procedure, organic matter was targeted by the two Fenton's digestions and the Bioclean® enzyme digestion, whereas mineral materials were targeted by the density separation steps (Lavoy and Crossman 2021, Wang et al. 2021, Hurley et al. 2018, Blasing and Amelung 2018). The methods for removal of organic matter are described below.

2.2.3.1 Organic Matter Removal

To improve digestion efficiencies and reduce the counting intensity associated with high microplastic concentrations, dried 0.25 g samples of biosolids were used for analysis. Once weighed, the samples were transferred to 250 mL wide-mouth Erlenmeyer flasks using 50 mL of filtered DI water, then 20 mL of hydrogen peroxide (30% by volume; VWR, Mississauga, ON, Canada., cat. 89097-776) was added to the solution. The solution was allowed to sit overnight to promote the rehydration and disaggregation of the dried biosolid sample (Lavoy and Crossman

2021). Iron (II) (MP Biomedicals, Ottawa, ON, Canada., cat. 158048) was then introduced to the sample in 5 × 2 mL aliquots at 10–15-minute intervals. This helped to maintain a stable reaction rate, while meeting the 2:1 hydrogen peroxide to iron (II) ratio that is considered optimal for organic matter digestion (Lavoy and Crossman 2021). During the initial reaction, temperature was monitored to ensure the solution did not exceed 50ºC, since temperatures exceeding 70ºC have been shown to degrade some microplastics (Munno et al. 2018). In the case of high temperatures, a cold-water bath was kept on hand to cool the digestate (Munno et al. 2018, Simon et al. 2018). The reaction was allowed to proceed for up to 24-hours before being passed through a 20 µm metal sieve. The portion retained on the sieve was transferred back to the flask using 50 mL of filtered DI water, and the liquid portion was discarded (Lavoy and Crossman 2021).

Enzyme digestions have been shown to be an effective method of organic matter removal, especially when it comes to more complex organics, such as starches, fats, proteins, and cellulose (Wang et al. 2018, Lavoy and Crossman 2021). However, enzyme digestions are often considered costly, time consuming, and are not recommended as standalone digestion methods (Prata et al. 2019, Liu et al. 2022). Nonetheless, enzyme based septic tank cleaning products, such as Bioclean®, have been shown to remove 22% more organic matter compared to control treatments (Lavoy and Crossman 2021). Thus, Bioclean® was used due to its low cost, wide availability, and relatively fast reaction time (Lavoy and Crossman. 2021).

The Bioclean® solution was prepared by mixing 8 g of Bioclean® (Superior BioSolutions LLC, Grand Rapids, MI, USA., cat. SYNCHKG016874) for every 50 mL of water (Lavoy and Crossman 2021). After 30 minutes of stirring, the solution was sieved to 20 µm; the solids were discarded, and the Bioclean® solution was retained (Lavoy and Crossman 2021).

Then 50 mL of the Bioclean® solution was added to the Erlenmeyer flasks from the first Fenton's digestion (flasks should contain: 50 mL Bioclean®, 50 mL filtered DI, and solids >20 µm). The flasks were then incubated in an oven at 55ºC for 48-hours (Lavoy and Crossman 2021). Afterwards the samples were sieved again to 20 µm, the liquid portion was discarded, and the solids were flushed back into the Erlenmeyer flask with 50 mL of filtered DI water. Then, using the methods previously described, the second Fenton's digestion was performed (Lavoy and Crossman 2021).

2.2.3.2 Mineral Separation

A series of two water separations (1.0 g/cm^3) and one sodium iodide separation (1.6–1.8 g/cm^3) were used to isolate microplastics from the remaining mineral material (Lavoy and Crossman 2021, Wang et al. 2018). Typically, the density of plastic ranges from 0.8–1.6 g/cm^3, organic matter is between 1.0–1.4 g/cm^3, and mineral densities are ~2.65 g/cm^3 (Anger et al. 2018, Hurley et al. 2018, Prata et al. 2019). Sodium iodide separations have been shown to be capable of recovering both low and high-density polymers with >99% efficiency (Liu et al. 2022). This does not discount the importance of the water density separations, which not only target low density polymers, but are crucial to clean the sample and prevent the oxidation of the sodium iodide solution.

To conduct the initial water density separation, the flask containing the solution from the second Fenton's digestion (~100 mL) was diluted using an additional ~150 mL of pre-filtered DI water. The solution was stirred on a magnetic stir plate for ~1 minute and was left to settle for a minimum of 6 hours (Lavoy and Crossman 2021). After settling, the liquid fraction of the sample was decanted and filtered though a 4.2 cm glass fibre filter paper rated for 1.1 µm particle retention (VWR, Mississauga, ON, Canada., cat. 28297-504), which was retained for inspection.

The flask was then re-diluted with ~250 mL of pre-filtered DI water before stirring, settling, and filtering the sample in the same manner (Lavoy and Crossman 2021).

Lastly, the settled fraction from the second water density separation was rinsed thoroughly into a density separation unit using the sodium iodide solution (prepared to a density between 1.6–1.8 g/cm^3 using pre-filtered DI water; Sigma Aldrich Oakville, ON, Canada., cat. 746371). The density separation unit was constructed using PVC pipe, a ball valve, and a ceramic tile (Appendix A2; Coppock et al. 2017, Lavoy and Crossman 2021, Wang et al. 2018). The density separation unit containing the sample and sodium iodide solution was covered, stirred for ~1 minute, then left to settle for 24 hours. Though shorter settling times have been suggested, the separation quality increases with time (Lavoy and Crossman 2021). Afterward, the ball valve was turned to trap the settled portion in the bottom of the unit, while the top portion was filtered and rinsed onto a 1.1 µm glass fibre filter paper. (Lavoy and Crossman 2021). The filter paper was retained for visual inspection. The remaining sodium iodide was filtered, and density adjusted prior to it's reuse (Coppock et al. 2017, Lavoy and Crossman 2021).

2.2.4 Microplastic Identification and Characterization

Once the samples were isolated on filter papers, anthropogenic debris were manually counted using a stereomicroscope at 5–35× magnification (AmScope, Irvine, CA, USA., model. ZM-1 Series). This study quantified debris with a major axis between 20 µm and 5 mm. During counting, the debris were classified into the following shape-based categories: fibres, films, fragments, beads, and foams. Characteristics key to the identification of anthropogenic debris include: an unnatural colour, material homogeneity, particle resiliency, reflective surfaces, and limited fraying (Kosuth et al. 2018). Images of suspected microplastics were captured using a

Teledyne Lumenera Infinity 2 camera (Teledyne Lumenera, Ottawa, ON, Canada., model. INFINITY2-1R). To establish the proportion of microplastics in the anthropogenic debris counts, hot needle tests were performed (Brander et al. 2020, Prata et al. 2019). Only images of microplastics were retained, and the image analysis software Infinity Analyse was used to measure the major and minor axis of each microplastic particle.

After completing the counts, a subset of microplastics (n = 121) were randomly selected for polymer identification using Raman Spectroscopy (Oxford Instruments, Ulm, Germany., model. WITec alpha300R confocal Raman Microscope). In the case of fibres, 47 were selected from the biosolids (BS) and 31 from the final effluent (FE). The sample size for fragments (BS = 18, FE = 9), foams (BS = 9, FE = 0), films (BS = 4, FE = 2), and beads (BS = 1, FE = 0) was determined based on the relative proportions of each shape type. Raman scans were completed using lasers with wavelengths of 532 and 785 nm. All particles were first scanned using the 785 nm laser, and if a clean spectrum was not produced, the 532 nm laser was used (Lavoy and Crossman 2021). Several scanning variables can be altered to optimize spectral quality, such as the number of scans (accumulations), the length of each scan (integration time), the laser power/position, and the magnification level. Though these settings are adjusted on a case-by-case basis, scans were completed with a magnification between 20× and 100×, integration times between 0.05 and 50 seconds, the number of accumulations between 1 and 300, and the power level between 0.1 and 15 mW. Spectra analysis and polymer identification were conducted through the open access database *OpenSpecy* (Cowger et al. 2021).

2.2.5 Data Analysis

Mass based microplastic concentrations were calculated (Kalcikova et al. 2017, Simon et al. 2018) using particle counts, the average particle volumes, and the average polymer densities (Simon et al. 2018). Microplastic volumes were calculated using the major and minor axis measurements, where fibres were calculated as cylinders, films were calculated as rectangular prisms, fragments and foams were calculated as ellipsoids, and beads were calculated as spheres, as documented in Appendix A3. Then, using the relative abundance of polymers and their known densities, a weighted average density was determined for each microplastic shape category. Since foams typically contain 95–98% air, the weighted average density of foams was adjusted to account for a void fraction of 96.5%. The mass of plastic (g) in each shape category was calculated as the product of density (g/cm^3), volume (cm^3), and microplastic count (n) (Appendix A4), as described by Simon et al. (2018).

When comparing datasets, multiple statistical tests were used; all statistical tests were performed using Past v4.16c (Hammer et al. 2001). To evaluate the distribution of a dataset, the Shapiro-Wilk test for normality was used ($\alpha = 0.05$). The means of parametric datasets were compared using two-sample, equal-variance, t-tests at a significance level of $\alpha = 0.05$. In instances where non-normal distributions existed, the skew of the data was described visually using a violin plot (Hintze and Nelson 1998). When trying to determine whether a difference of medians existed between two non-parametric datasets, the Mann-Whitney U test was used at a significance level of $\alpha = 0.05$. However, if the test was comparing medians in three or more non-parametric datasets, the Kruskal-Wallis one-way analysis of variance was used ($\alpha = 0.05$). If a significant difference in medians existed, the Mann-Whitney pairwise post-hoc test was applied

with Bonferroni correction to rank the datasets ($\alpha = 0.05$). When comparing annual trends between different parameters, Pearson's correlation coefficient (r) was used.

Total monthly final effluent flows and total monthly biosolids export was obtained from WWTP annual performance reports from 2020 and 2021. This data was the used with corresponding microplastic concentration data to estimate monthly and annual microplastic flux, as described in Appendix A5. Note that the biosolids export data is reported as a wet weight of cake, and so dry weight microplastic concentrations were converted to wet weight concentrations prior to calculating microplastic flux. Treatment efficiencies were calculated monthly as the proportion of microplastics retained in the biosolids compared to the total number of microplastics in the biosolid and the final effluent [Treatment Efficiency % = ((biosolid + effluent) – effluent)/(biosolid + effluent) × 100%], as described in Appendix A6. Hydrometric data, including precipitation, and river flow were obtained from Environment Canada's historical weather and historical river discharge/water level databases. It should be noted that the river flow data comes from a waterbody that is adjacent to the waterbody that receives effluent from the WWTP being studied and is not from the receiving waterbody itself. The waterbody receiving effluent from the WWTP was not used because its flows were regulated by a dam system, and as such, they were not considered representative of unimpeded environmental conditions.

2.2.6 Quality Assurance and Control

Contamination can be introduced at several points through sample preparation and analysis. Analytical blanks (n = 7) were used to assess the degree to which contamination affected the results (Brander et al. 2020, Prata et al. 2019). During analysis, one analytical blank was included for every five samples analysed. Method detection limits (MDL) were calculated

for each method as the average blank value plus 3× it's standard deviation. The MDL for the final effluent was 2.9 microplastics, and the MDL for the biosolid was 9.5 microplastics. All samples were blank corrected by subtracting the average blank value from the analytical result, and then corrected for the proportion of non-plastic particles by multiplying by the hot needle correction factor. Analytical variability was monitored by including one set of triplicate samples (n = 3) for every ten samples analysed. Using these data, the relative standard deviation (RSD) for the final effluent was 29% while the RSD was 6% for the biosolid material.

While analysing samples, a series of best practices were employed. To reduce the impact of atmospheric contamination, all samples and beakers were covered with aluminum foil during analysis, and any time spent uncovered was recorded (Coppock et al. 2017, Prata et al. 2019). All solutions used in the analysis were filtered to remove microplastics prior to their use, and all glassware was washed thoroughly with soap and water, then rinsed 3× with filtered DI water (Brander et al. 2020). When interacting with samples, analysts wore laboratory coats and clothing made of cotton to avoid contamination from synthetic garments (Brander et al. 2020, Prata et al. 2019).

2.3 Results and Discussion

2.3.1 Biosolids and Final Effluent

Average annual microplastic count (mp) concentrations in the biosolids on a dry weight basis were 169.1 ± 25.7 mp/g dw (RSD = 15%) and were 3.7 ± 2.1 mp/L (RSD = 58%) in the final effluent (Table 1). Further, average annual microplastic mass concentrations were 143.3 ± 31.5 µg/g dw (RSD = 22%) in the biosolids, and 2.9 ± 2.0 µg/L (RSD = 70%) in the final effluent (Table 1). Overall, microplastic concentrations were more variable in the final effluent

compared to the biosolids owing to their lower concentrations. Microplastic concentrations in the biosolids were relatively consistent with previous studies. For instance, Lares et al. (2018) reported count concentrations of 170.9 ± 28.7 mp/g dw, and Talvitie et al. (2017) reported count concentrations of 186.7 ± 26.0 mp/g dw. Further, Rasmussen et al. (2021) reported a microplastic mass concentration of 305.3 ± 264.3 µg/g dw. Few studies have reported both count and mass concentrations. Nonetheless, the previous studies reported values similar to the data for biosolids in this study. In terms of the final effluent, a study that compared ten WWTPs in Denmark, reported that microplastic count concentrations in the final effluent ranged between 19 and 447 mp/L, with associated mass concentrations between 0.5 and 11.9 µg/L (Simon et al. 2017). Similarly, Roscher et al. (2022) reported count concentrations in a German WWTP at 30 mp/L, with an associated mass concentration of 3.8 µg/L. In comparison, microplastic count concentrations for the final effluent in the present study were relatively low, whereas the mass concentrations were similar (Table 1).

Table 1: Annual average microplastic content based on monthly biosolids and final effluent observations from October 2020 to September 2021. Average microplastic count concentration (dry weight [dw]), particle size, density, and mass concentration (dw) are described for fibres, films, fragments, beads, and foams. Variability is described as ± one standard deviation for the count and mass concentrations.

Shape Category	Biosolid							
	Count	*Size*				*Density*		*Mass*
	Count Conc. (mp/g dw)	Major Axis (µm)	Minor Axis (µm)	Avg. Particle Volume (cm^3)	Obs. (n)	Density (g/cm^3)	Obs. (n)	Mass Conc. (µg/g dw)
Fibres	113.2 ± 20.7	642.53	21.02	2.23 E-07	90	1.22	47	30.8 ± 5.6
Films	2.8 ± 1.5	547.01	294.44	3.39 E-06	10	1.03	4	9.6 ± 5.2
Fragments	42.1 ± 10.5	225.92	129.19	1.97 E-06	33	1.20	18	100.0 ± 24.8
Beads	1.6 ± 3.0	97.60	92.25	4.48 E-07	3	0.81	1	0.6 ± 1.1
Foams	9.4 ± 6.1	326.86	196.86	6.63 E-06	10	0.04	9	2.3 ± 1.5
Total	169.1 ± 25.7							143.3 ± 31.5
Shape Category	Final Effluent							
	Count	*Size*				*Density*		*Mass*
	Count Conc. (mp/L)	Major Axis (µm)	Minor Axis (µm)	Avg. Particle Volume (cm^3)	Obs. (n)	Density (g/cm^3)	Obs. (n)	Mass Conc. (µg/L)
Fibres	3.2 ± 1.6	1141.26	23.27	4.85 E-07	20	1.24	31	1.9 ± 0.9
Films	0.1 ± 0.1	467.65	242.02	2.63 E-06	10	1.29	2	0.4 ± 0.5
Fragments	0.4 ± 0.8	198.14	95.25	9.41 E-07	11	1.46	9	0.6 ± 1.0
Beads	0.0	–	–	–	–	–	–	0.0
Foams	0.0	–	–	–	–	–	–	0.0
Total	3.7 ± 2.1							2.9 ± 2.0

Note, beads and foams were not observed during analysis of the final effluent.

All five of the microplastic shape categories were identified in the biosolids samples (Table 1). In terms of count, fibres were by far the most common (66.8%), followed by fragments, foams, films, and beads (Figure 2). Despite discrepancies in shape categories between studies, fibres tend to be reported as the most abundant microplastic shape. For example, Li et al. (2018) found fibres accounted for 63% of microplastics in biosolids, while Lares et al. (2018) found fibres accounted for as much as 80%. However, the relative abundance of microplastic shapes by mass was not consistent with the count-based shape proportions. For mass, fragments were dominant (69.4%), followed by fibres, films, foams, and beads (Figure 2). Divergence in

shape based microplastic prevalence between microplastic counts and masses is explained by the inherent physical differences between the shape categories.

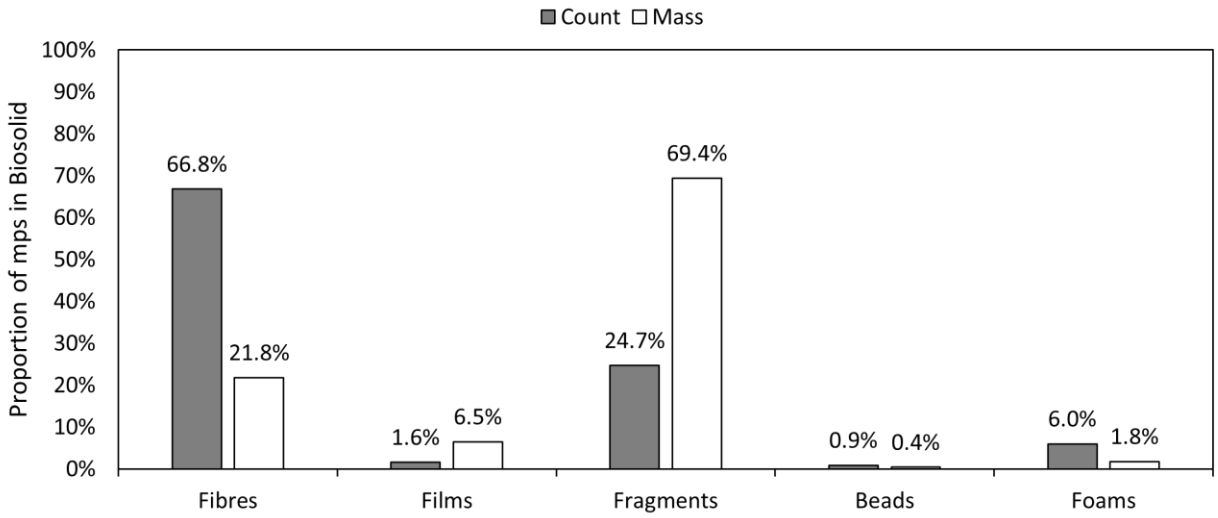

Figure 2: Average relative microplastic (mps) shape proportions (Fibres, Films, Fragments, Beads, and Foams) detected in the biosolids by count and mass between October of 2020 and September of 2021.

Microplastic size had the greatest influence on mass, as microplastic size ranged over several orders of magnitude, and though there was a vast range, the majority of microplastic observations came from small particles, as demonstrated in Appendix A7 (see also Hengstmann and Fischer 2019, Kalcikova et al. 2017, Mahon et al. 2017, Shahi et al. 2020). One study reported that 88% of microplastics in their influent and effluent samples had a major axis length between 20 and 200 µm (Kim et al. 2022). However, in the present study, only 27% of microplastics had a major axis between 37 and 200 µm, while 65% of microplastics had a major axis between 37 and 500 µm. We observed that count distributions tapered off towards the smallest particles (Appendix A7). Two factors contribute to this observation, firstly by using a 20 µm sieve, it is possible that small particles with major axis greater than 20 µm, but a minor axis less than 20 µm were able to pass through the sieve lengthwise, thereby reducing the abundance

of small particles (Sun et al. 2019). Secondly, analysis relied on the visual identification of microplastics, and small particles are more difficult to identify. The limits of the analytical method on the detection of small particles are supported by Löder and Gerdts (2015), who noted difficulty identifying and sorting microplastics <0.5 mm when using visual analysis.

Different microplastic shapes had inherent differences in volume (Appendix A8), based only on the major axis, fibres would be considered the largest microplastic shape. However, fibres generally have the smallest minor axis (Appendix A7). As such, the volume of plastic contributed by each fibre tends to be lower than other shape categories such as fragments, foams, or films (Appendix A8). In this study, on average, one foam was found to contribute the same volume of plastic as 30 fibres, one film contributed the same volume as 15 fibres, one fragment contributed the same volume as nine fibres, and one bead contributed the same volume as two fibres (Table 1). It should be noted that microplastics occupy three dimensions. However, since microplastics were measured using photographs, microplastic volumes were estimated based on two-dimensions (e.g., the major and minor axes), as described by Simon et al. (2018). Compared with microplastic size, the polymeric composition of microplastics had a lesser effect on the divergence between count and mass-based shape prevalence (Table 1). Many polymers have similar densities, which reduces the degree to which polymer type influences microplastic mass (Appendix A9). Further, when the weighted average density of each shape category was compared, there was no significant difference between fibres, films, or fragments (Kruskal-Wallis $p > 0.05$); note that beads were not included due to their low sample size (Table 1). However, there was a significant difference in the average density of foams when compared to the other shape categories (Kruskal-Wallis $p < 0.05$). This difference in density was not attributed to the polymer density but was instead attributed to the 96.5% void fraction used to account for

air space in the foams. This void fraction caused the shape-based prevalence of foams to decrease from counts to masses, despite the relatively large volume of foam particles, as shown in Figure 2 (Appendix A8).

In the final effluent samples, only three of the five shape categories were observed (Table 1), fibres were the most common, followed by fragments, and then films (Figure 3). In general, shape prevalence rankings were the same for both microplastic counts and masses. Nonetheless, there was a decrease in the proportion of fibres and an increase in the proportion of fragments and films in terms of masses compared with counts (Figure 3). Again, the decreased mass proportion of fibres is primarily explained by their tendency to be less voluminous than fragments and films (Appendix A8). However, given the overwhelming dominance of fibre counts, fibres remained the largest contributor to the total microplastic mass. In previous studies, the relative proportions of microplastic shapes in the final effluent were heavily reliant on the sampling methods employed. Studies that reported fibres as the predominant microplastic shape tended to employ 24-hour composite sampling techniques (Gundogdu et al. 2018, Conley et al. 2019) or grab samples, whereas studies that reported other shapes, such as fragments, as being dominant tended to use sieve stacks to filter large volumes of water (~1000 L) onsite during sample collection (Roscher et al. 2022, Ziajahromi et al. 2017). This discrepancy in shape proportions between studies may be attributed to the ability of fibres to pass through sieves lengthwise, reducing their retention rates, and thus resulting in their underestimation (Sun et al. 2019).

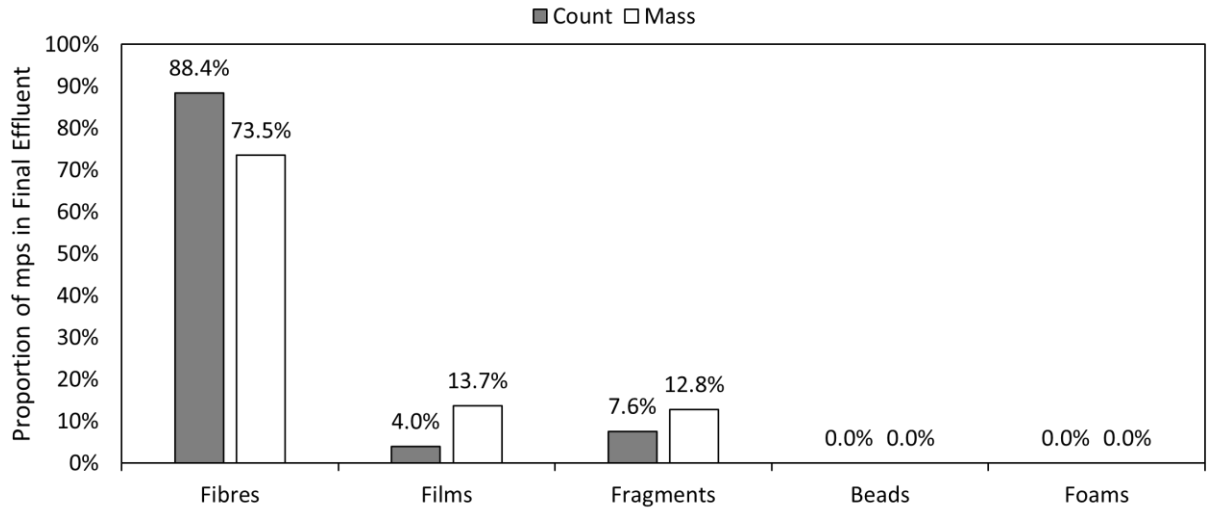

Figure 3: Relative microplastic (mps) shape proportions (Fibres, Films, Fragments, Beads, and Foams) detected in the final effluent by count and mass between October 2020 and September 2021.

Other than concentrations and microplastic shape proportions, the properties of microplastics between the biosolids, and the final effluents were somewhat similar. There were no significant differences in the average particle volumes of films or fragments (Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p > 0.05$). However, the average fibre volume in the biosolids was less than the average fibre volume in the final effluent (Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p < 0.05$). This was attributed to the fact that the average fibre length in the biosolids was less than in the final effluents (Table 1). In terms of average particle density, there was no significant difference in the average density of fibres (Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p > 0.05$), and the average density of films could not be compared due to their low sample size (Table 1). For fragments, the average particle density was less in the biosolids than in the final effluents (Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p < 0.05$). This was attributed to the high abundance of silicone that was observed in the final effluents (Appendix A9 and Appendix A10). Overall, there were no significant differences in the average particle masses of either films or fragments in the biosolids and final effluents (Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p > 0.05$; Appendix

A11). However, there was a significant difference in the average particle mass of fibres, as the fibres in the biosolids had a lesser average particle mass than in the final effluents (Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p < 0.05$; Appendix A11). This was attributed to the fact that the average volume of a fibre was greater in the final effluents, which caused the greater average mass of a fibre.

To summarize, the relative proportion of fibres was more dominant in the final effluents compared with the biosolids. Additionally, fibres in the final effluents were larger than in the biosolids. This indicates that large fibres have a greater likelihood of avoiding settling than small fibres. The relative proportion of films was also found to be higher in the final effluents compared with the biosolids, while there were no significant differences in their morphology. In contrast, the relative proportion of fragments was greater in the biosolids than in the final effluents, despite there being no significant difference in the size of fragments between the two waste streams. The relative proportions of both beads and foams were greater in the biosolids, since neither shape was detected in the final effluents. This indicates that granular microplastic shapes with centralized masses (e.g., fragments, beads, and foams) were preferentially retained in the biosolids, whereas linear and flat microplastics with a more dispersed mass (e.g., fibres and films) were more likely to avoid being retained in the biosolids, thus ending up in the final effluents. These treatment tendencies are likely related to the ability of each microplastic shape to settle. Bilgin et al. (2020), noted that the settling efficiency of dense granular microplastics (e.g., fragments, beads, and foams) was much greater than that of flat and linear shapes (e.g., films and fibres). As such, the ability of individual microplastics to settle is likely to have a strong influence on their fate in wastewater treatment.

2.3.2 Annual Trends

Microplastic concentrations in the biosolids were lowest in September (123.6 mp/g dw or 87.8 µg/g dw), and highest in February (212.1 mp/g dw or 217.6 µg/g dw), while concentrations in the final effluents were lowest in November (0.9 mp/L or 1.1 µg/L) and highest in April (7.8 mp/L or 7.2 µg/L; Figure 4). Overall, there was limited temporal variation in microplastic concentrations throughout the study period. Microplastics in the biosolids did not demonstrate a strong seasonal pattern, while microplastics in the final effluents were more concentrated in the spring/summer months than in the fall/winter (Figure 4). In a study by Li et al. (2018), microplastic count concentrations in biosolids were found to be high in spring (~25 mp/g dw), and low in fall (~10 mp/g dw). These results were not reflected in the present study as seasonality did not appear to have a strong influence on the microplastic concentrations in biosolids (Figure 4). Although significant differences were not observed in their final effluent samples, Kim et al. (2022) reported that the effluents collected in April and August had higher microplastic concentrations than effluents in October and December, which aligns well with the final effluent trends observed in the present study.

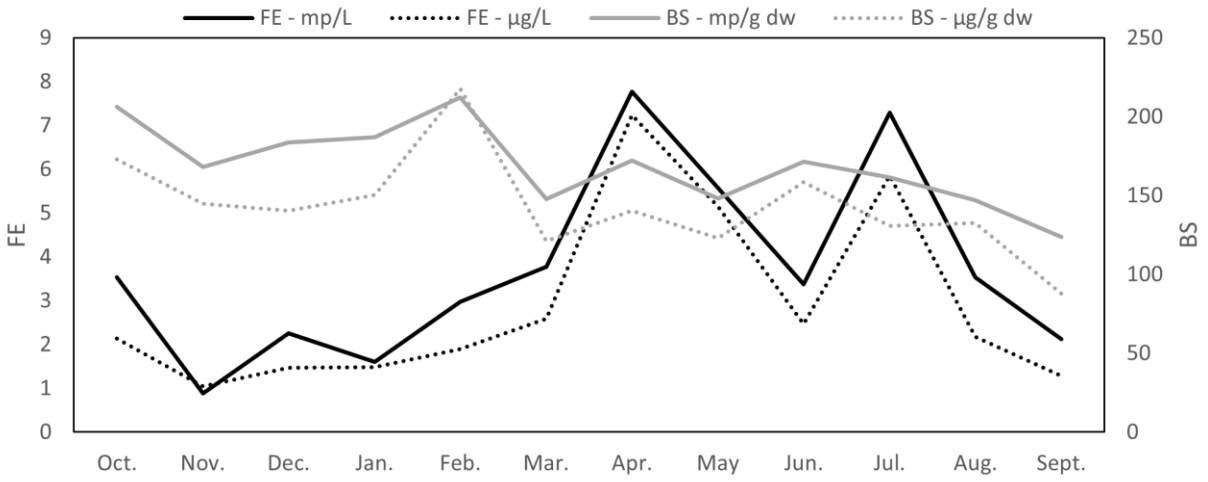

Figure 4: Monthly microplastic concentrations in the final effluents (FE, black), and biosolids (BS, grey) collected during the study period (October 2020–September 2021). The scale for final effluent concentrations (mp/L or µg/L) is on the primary axis, whereas the scale for biosolid concentrations (mp/g or µg/g) is on the secondary axis. Count concentrations are solid lines, whereas mass concentrations are the dotted lines.

The total annual microplastic export (biosolid plus final effluent) from the plant was 354.1 ± 24.7 billion microplastic particles, which equated to 296.7 ± 39.4 kg for the entire year (Appendix A12 and Appendix A13). Of this, 303.3 ± 19.8 billion particles were in the biosolids (85.7%), while 50.8 ± 14.8 billion particles were in the final effluents (14.3%). However, in terms of mass, 256.9 ± 34.9 kg were in the biosolids (86.6%), while 39.8 ± 18.3 kg were in the final effluents (13.4%). This means that the biosolids captured 6.0 times the count and 6.5 times the mass of microplastics exported via the final effluents. Monthly microplastic exports in the biosolid were lowest in September (19.7 billion microplastics at 14.0 kg), and highest in February (29.7 billion microplastics at 30.4 kg). In contrast, the monthly microplastic export in the final effluent was lowest in November (0.9 billion microplastics at 1.0 kg), and highest in April (9.7 billion microplastics at 9.1 kg). Interestingly, the extremes of total monthly export differed in terms of count and mass, where count exports were lowest in November (22.3 billion) and highest in April (35.8 billion), yet mass exports were lowest in September (15.6 kg) and

highest in February (32.1 kg). This indicates that total count exports were influenced to a greater degree by the final effluent, while total mass exports were influenced to a greater degree by the biosolid exports. In general, neither the biosolids, nor the total demonstrated a strong seasonal trend in microplastic exports (Figure 5). In contrast, microplastic exports in the final effluent were lowest in the fall and winter, and highest in the spring and summer (Figure 5). This variation in microplastic exports from the final effluent is best explained by the WWTP's ability to retain microplastics in the biosolid.

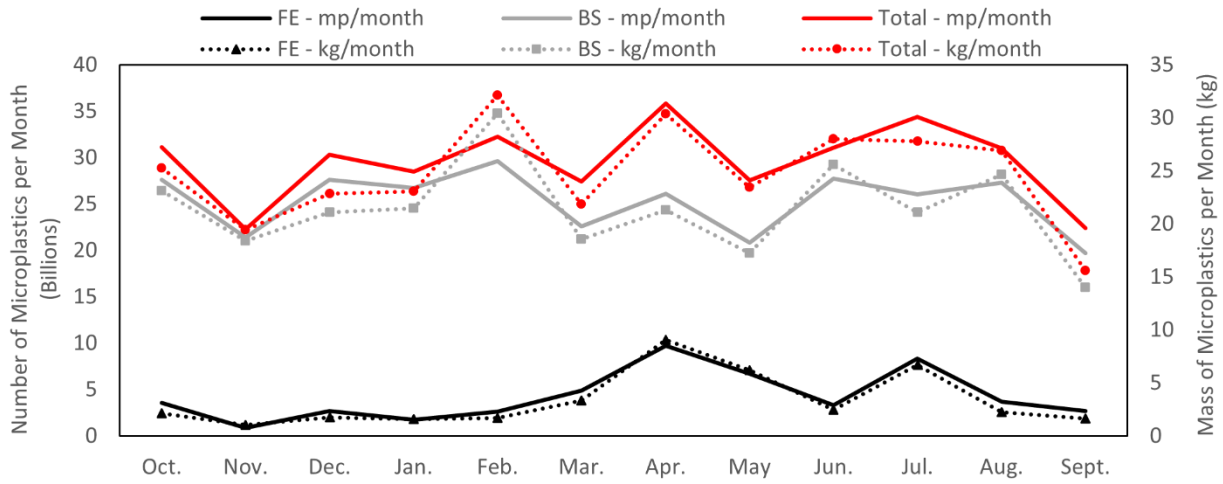

Figure 5: The count (mp/month), and mass (kg/month) of microplastics exported from the WWTP on a monthly basis during the study period (October 2020–September 2021). Data are presented for the final effluent (FE, black), biosolid (BS, grey), and the sum of the two waste streams (Total, red). Mass exports are shown as dotted lines, whereas count exports are solid lines.

The plant's ability to divert microplastics from the final effluents and retain them in the biosolids was described as treatment efficiency (Appendix A6). Throughout the study period, the microplastic mass treatment efficiency ranged between 70 and 95% with an average of 87% ± 9%, while the count treatment efficiency ranged between 73 and 96% with an average of 86% ± 8%. Similarly, Environment and Climate Change Canada (2020) reported that between 86–99.8% of microplastics are removed during secondary treatment. Many studies suggest that

microplastic treatment efficiencies are high, often greater than 98% (Carr et al. 2016, Lares et al. 2018, Simon et al. 2018). For example, Simon et al. (2018) reported microplastic treatment efficiencies of 99.3% by count, and 98.3% by mass. An explanation for the slight difference in treatment efficiencies may be attributed to the method of calculation. In studies where influent and effluent loads are calculated, treatment efficiencies are often calculated as the inverse proportion of microplastics in the influent compared to the effluent [Treatment Efficiency % = (influent – effluent)/(influent) × 100%] (Talvitie et al. 2017), whereas in the present study, treatment efficiencies were calculated as the inverse proportion of microplastics in the effluent compared with the sum of microplastics in the influent and the biosolids [Treatment Efficiency % = ((biosolid + effluent) – effluent)/(biosolid + effluent) × 100%]. Calculating treatment efficiencies in relation to the influent microplastic load is likely to produce different treatment efficiencies than using the sum of the final effluents and biosolids. This highlights the importance of considering how treatment efficiencies were calculated when comparing studies.

In general, the monthly trends in treatment were very similar between microplastic mass and count. Treatment was most efficient from October through February, and then it decreased from March through July, with a spike in June, before again increasing in August and September (Figure 6). Further, there was an inverse relationship between treatment efficiency and plant flows (Mass: $r = –0.62$, Count: $r = –0.62$). This suggests that as the flow increased, the WWTPs ability to remove microplastics decreased. That is, high wastewater flows reduced the retention time of wastewater, which reduced treatment efficiency, resulting in a higher fraction of microplastics in the final effluent. When compared to monthly precipitation, treatment efficiencies had a weak negative correlation (Mass: $r = –0.16$, Count: $r = –0.21$), while plant flows had a weak positive correlation with precipitation (Plant Flow: $r = 0.19$). However, flow in

an adjacent river (not the receiving waterbody) had a stronger negative correlation with treatment efficiency (Mass: r = –0.43, Count: r = –0.44), and a strong positive correlation with plant flows (Plant Flow: r = 0.82). This suggests that plant flows are heightened during periods of increased precipitation and river flow, which in turn reduces microplastic treatment efficiency by reducing retention times.

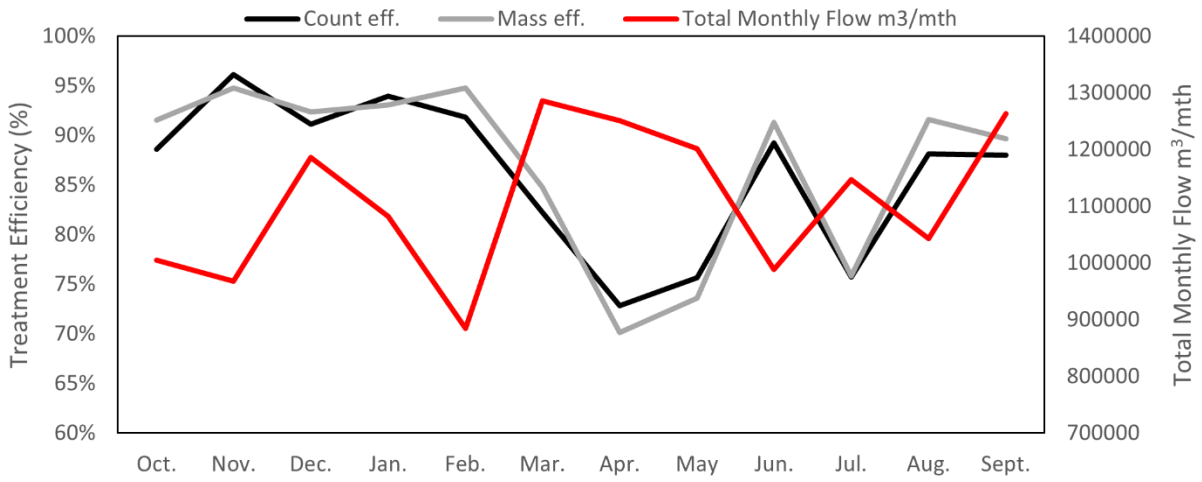

Figure 6: Treatment efficiency (%) calculated on a monthly basis in terms of the count and mass of microplastics diverted from the final effluent and stored in the biosolids. These treatment efficiencies are compared to the total volume of water processed by the plant each month.

The influent water quality parameters provide an indication of the degree to which wastewaters are contaminated. As such, there is reason to believe that increased microplastic loads at the WWTP would be associated with increasingly contaminated wastewaters. Though many negative correlations were identified, there were no significant correlations between the total microplastic load received at the plant (sum of the final effluent and biosolids) and the influent water quality parameters measured by WWTP lab staff (Appendix A14). The Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Phosphorous (TP), and Total Kjeldahl Nitrogen (TKN) were not significantly correlated with microplastic mass (BOD: r = –0.33, COD: r = –0.48, TSS: r = –0.25, TP: r = –0.67, TKN: r

= –0.46) or count (BOD: r = –0.21, COD: r = –0.32, TSS: r = –0.22, TP: r = –0.53, TKN: r = –0.23). Similarly, when microplastic loads in the final effluent were compared to the other water quality parameters observed in the final effluent, many negative correlations were identified (Appendix A15). The trends observed for Carbonaceous Biological Oxygen Demand (cBOD) (Mass: r = –0.55, Count: r = –0.56), and TSS (Mass: r = –0.58, Count: r = –0.56) were more clearly inverse to microplastic loads than the trends observed for TP (Mass: r = 0.05, Count: r = –0.01), and TKN (Mass: r = –0.36, Count: r = –0.30). The only significant correlation was between the TSS and the microplastic mass in the final effluent ($r = –0.58$, $p < 0.05$). This is an especially surprising observation, since intuition suggests that TSS and microplastic loads should be positively correlated. This is because as suspended particles, microplastics are a part of TSS. In their study, Roscher at al. (2022) compared microplastic loads to other effluent quality parameters in two German WWTPs. In one WWTP, they found an insignificant, positive correlation between microplastic loads and Suspended Matter (SPM) (Kendall-Tau-b correlation, Count: $\tau = 0.033$, Mass: $\tau = 0.114$), while in the other plant, an insignificant negative correlation was identified (Kendall-Tau-b correlation, Count: $\tau = –0.032$; Roscher et al. 2022). What appears to distinguish microplastics from other suspended matter, is their non biologic nature. Since there is no evidence of microplastics being degraded by the aerobic digestion process, it is possible that microplastic treatment is minimally affected by the digestion efficiencies of biologic suspended matter. Since overall microplastic loads in the plant were observed to be highest in the spring/summer months, a period when aerobic digester efficiency typically improves, it is possible that microplastics begin to represent a larger proportion of effluent TSS. Conversely, in the fall/winter when microplastic loads were reduced, there tends to be a marginal reduction in aerobic digestion efficiency due to reduced temperatures. This would cause microplastics to

represent a smaller proportion of effluent TSS, essentially driving the negative correlation observed between microplastic and suspended matter loads.

2.4 Conclusion

Since sewer systems are a major pathway for microplastics to enter the environment, it is critical to recognize WWTPs as a point where these contaminants can be controlled. This study evaluated annual microplastic trends in a Canadian WWTP between October 2020 and September 2021. By doing so, this study addresses the need for continued long term evaluation of microplastic exports and treatment efficiencies, in consideration of seasonality and water quality/quantity. Additionally, this is the first study to track microplastic burden in the biosolids and the final effluent of a wastewater treatment plant at monthly intervals for an entire year. In this study, total microplastic count exports were highest in April 2021, and lowest in November 2020, while mass exports were highest in February and lowest in September. Despite minor variation, total monthly microplastic export stayed relatively consistent across the study period; however, the treatment efficiency was more variable. High flow periods were observed to reduce the WWTP's microplastic treatment efficiency, while low flow periods increased microplastic treatment efficiency. This suggests that the reduced retention times associated with high wastewater flows influenced the ability of microplastics to settle, thereby allowing a greater proportion of microplastics to exit the WWTP via the final effluent. This is supported by the types of microplastics identified in the final effluents and the biosolids. The microplastic shapes in the biosolid were very diverse, including fibres, films, fragments, beads, and foams. In contrast, the microplastics in the final effluent were less diverse, consisting of only fibres, films, and fragments. This indicates that granular particles had greater treatment efficiencies due to their improved settling tendencies when compared with more dispersed shapes like fibres and

films. Since the majority of microplastics that enter the WWTP will exit via the biosolids (85.7% of microplastic counts, and 86.6% of microplastic mass), the focus should be placed on biosolids to reduce microplastic emissions. The retention of microplastics in biosolids does not represent true environmental diversions, as approximately 43% of all biosolids are land applied in Canada. Therefore, a method to reduce the quantity of microplastics in biosolids should be developed to mitigate the environmental burden associated with the land application of biosolids. In addition, further research on the temporal dynamics of microplastics is needed, especially in context of other environmental and water quality parameters.

Chapter 3: Settling strategies to reduce microplastic loads in biosolids from wastewater treatment plants

3.1 Introduction

Microplastics, the particulate form of plastic pollution, are ubiquitous within the environment, which has led to rising concern about their impacts on organisms and human health (Environment and Climate Change Canada 2020, Marfella et al. 2024). Microplastics are synthetic organic polymers derived from petroleum (Anderson et al. 2016, Brander et al. 2020), and are defined as having a major axis with a length less than 5 mm (Schmidt et al. 2020). In general, there are two classifications of microplastics, primary, and secondary (Simon et al. 2018). Primary microplastics are manufactured (e.g., microbeads used in cosmetics, or pre-production pellets in manufacturing), whereas secondary microplastics form from the degradation of larger plastic materials (e.g., microfibres in textiles, or fragmented plastic debris) as described by Brander et al. (2020), Simon et al. (2018), and Ziajahromi et al. (2017).

Microplastics are very diverse in terms of their shape, size, polymer type, and abundance (Anger et al. 2018, Simon et al. 2018). Common shape categories used in microplastic analysis include fibres, films, fragments, beads, and foams (Allen et al. 2019; He et al. 2019). Additionally, microplastic shapes can provide a basic indication of their source (Liu et al. 2022). Their size has been found to range over several orders of magnitude, which directly influences the mass of plastic in each particle (Anger et al. 2018). Polymer information helps to identify microplastic sources, as well as provide a greater understanding of the plastic densities, which influence particle mass (Anger et al. 2019, Ziajahromi et al. 2017). When quantifying microplastics, there are two metrics that are generally used: count concentrations (e.g., mp/g, mp/L) and mass concentrations (e.g., µg/g, µg/L), as discussed by Kalcikova et al. (2017), and

Simon et al. (2018). While count concentrations are fundamental to microplastics analysis, mass concentrations communicate plastic quantities more objectively since they take shape, size, and polymer type into account (Kalcikova et al. 2017, Simon et al. 2018).

Wastewater Treatment Plants (WWTPs) are a major source of microplastics to the environment. Microplastics in treated effluent are discharged directly to aquatic environments, while microplastics entrained in the biosolids can be introduced to terrestrial environments through land application (Environment and Climate Change Canada 2020). Despite this, microplastics in WWTPs go largely unmonitored (Anderson et al. 2016, Driedger et al. 2015). In general, WWTPs are effective in removing microplastics from wastewater through the use of screening and settling processes. In a study that examined microplastics in primary settling tanks, 96% of microplastics were found to be removed in the settled sludge, with only 4% being removed in the floating scum, which left 0% in the effluent (Lofty et al. 2022). However, not all studies have reported the same treatment efficiencies. In Canada, WWTPs have been reported to remove 50–98% of microplastics during primary treatment, 86–99.8% during secondary treatment, and 98–99.8% during tertiary treatment (Environment and Climate Change Canada 2020). With this, it is common for 88–97% of microplastics present in untreated wastewater (i.e., influent) to be diverted from the effluent to the biosolids (Brander et al. 2020).

Though these ‘diverted’ microplastics are redirected from entering the aquatic environment via the effluent, they may still enter the terrestrial environment through the land application of biosolids (Environment and Climate Change Canada 2020). It is estimated that land application is responsible for contributing between 44,000 and 300,000 tons of microplastics to farmlands in North America each year (Crossman et al. 2020, Nizzetto et al. 2016). Once land applied, these microplastics may be transported by wind and runoff, with an

estimated 99% of these microplastics potentially ending up in the aquatic environment (Crossman et al. 2020).

Despite having high microplastic loads, biosolids still represent a valuable resource. Therefore, their land application should be maintained, so long as an appropriate microplastic treatment method is implemented (Coalition Clean Baltic, 2017). In Ontario, it is common for biosolids from WWTPs to be processed by a third party prior to land application. Here the biosolids are often stored in tanks, and allowed to settle before the supernatant is removed; thus, thickening the biosolids prior to its application to fields (Crossman et al. 2020). One study suggested that the thickened biosolids that underwent this additional settling period had lower microplastic concentrations compared with biosolids without settling (Crossman et al. 2020). As a result, storage and settling has been proposed as a potential method to reduce microplastic loads in biosolids (Crossman et al. 2020).

This study examined the potential of low energy settling treatments as a method to reduce microplastic loads in biosolids. To do this, biosolid samples were collected from two WWTPs located in south-central Ontario and were subjected to settling treatments with four key variables (biosolid type, settling time, stirring, and harvesting method) to assess the efficiency of the treatments to liberate microplastics from biosolids. Treatment efficiency was expressed as the percentage of microplastics removed from biosolids. It was also important that the treatments minimized the removal of organic matter given its value as an agricultural soil amendment. It should be noted that the complete removal of microplastics is not feasible as it would require the complete removal of biosolids. Instead, the treatments were assessed in terms of their ability to promote (increase) the concentration of microplastics and minimize the concentration of

biosolids present in the upper layer of the settling tank. With the goal of maximizing the fractional removal of microplastics per gram of biosolid.

3.2 Methods

3.2.1 Study Sites

Biosolid samples were collected from two WWTPs serving communities in south-central Ontario. Plant 1 was a slightly larger plant, servicing a population of ~145,000, whereas Plant 2 services ~84,000. Despite differences in the population serviced, both plants were approved for an average daily flow of 68.2 ML/day. In terms of peak flow capacity, Plant 1 was rated for 180 ML/day, whereas Plant 2 was rated for 190.9 ML/day. Subtle differences existed in the treatment processes between the two plants, but both employed secondary treatment methods with return of activated sludge. Raw sewage received at the plant was passed through screens and grit tanks to remove large debris and dense granules. The wastewater was then dosed with a coagulant and allowed to settle in the primary clarification tanks. Subsequently, the wastewater was digested in aeration tanks before being allowed to settle again in the secondary clarification tanks. The clarified water was then disinfected before being released to an adjacent surface water body. The effluent in Plant 1 was disinfected using chlorination, whereas Plant 2 used UV radiation. Sludge collected from the grits, primary, and secondary settling tanks was anaerobically digested to produce biosolids. Plant 2 de-watered the biosolids using a centrifuge to make “cake” (moisture content ~75%), whereas in Plant 1, the biosolids were not de-watered (moisture content ~98%).

3.2.2 Experimental Design

Using samples of biosolids collected from both WWTPs, preliminary tests were performed to investigate how the moisture content of biosolids influences the settling of

microplastics and biosolids (Appendix B1). The results indicated that a moisture content ~98% was optimal for creating a distinct separation of biosolid and water layers during settling without adversely affecting the biosolid quality. Subsequently, the effects of four variables on microplastic and biosolids settling were assessed using an experimental block design (Appendix B2). The variables were the biosolids type (liquid vs. cake), stirring (stirred vs. un-stirred), settling time (short vs. long), and harvest method (scoop vs. syphon). The liquid biosolids received from Plant 1 did not require any moisture adjustments to reach the target moisture content of 98%, whereas the cake from Plant 2 needed to be rehydrated. The cake was rehydrated by mixing biosolid cake with tap water to achieve the desired moisture content which was verified by drying subsamples to calculate moisture content. To rehydrate enough cake for 8 settling tanks (160 L), approximately 12.7 kg of biosolid cake was mixed into 147.3 L of water (1.6 kg of cake, and 18.4 L of water per tank). Once the moisture content was adjusted, 20 L sub-samples were poured into a series of 16 settling tanks (51.4 cm Length × 26.7 cm Width × 32.0 cm Height). Samples from each treatment were collected every three days for the duration of the experiment (9–21 days). The stirring events occurred directly after the 3-day sampling event. The tanks were stirred first with a spatula, taking 6 strokes lengthwise down the tank, and then the perimeter of the tank was traced using an electronic hand mixer for 1 minute. Conversely, the un-stirred tests were not agitated for the entirety of the settling period. Settling time, was conducted as short (9 days) vs. long (21 days). The biosolids were added to the tanks on the same day, and 250 mL subsamples were collected from the surface of the tank every three days until the allotted time expired. It should be noted that since the sample volume was kept consistent (v = 250 mL), and the long duration tests had more sampling events (n = 7) than the short (n = 3), a larger volume (v = 1750 mL) was collected from the long duration tests relative to the short duration

tests (v = 750 mL). Samples were harvested either by scooping or syphoning. Scooping used a large metal spoon to scrape the surface layer of the tank (similar to a surface skim). This method made little distinction between floating solids, and the supernatant liquid. Whereas syphoning used a rubber head pipette (44 mL) to focus on the liquid supernatant below the solid floating surface layer (if present). Samples were stored in 1 L glass jars and refrigerated until analysis.

3.2.3 Microplastic Extraction

Prior to analysis, samples were homogenized, poured into aluminum baking trays, covered with tinfoil, and dried at 50^{o}C for 48h. The wet and dry weights (dw) were then used to calculate the moisture content of each sample. It should be noted that the samples were not pulverized to avoid fragmentation of existing microplastics (Simon et al. 2018). The procedure for microplastic extraction from biosolids (isolation of plastic from organic and mineral material) followed the methods described by Lavoy and Crossman (2021). These methods remove organic matter using two Fenton's digestions and a Bioclean® enzyme digestion, and remove mineral materials using two water density separations, and one sodium iodide density separation (Lavoy and Crossman 2021). Note that it is important to remove the organic matter prior to density separation since biofilms can influence particle density and therefore settling tendencies (Driedger et al. 2015, Blasing et al. 2018).

3.2.3.1 Organic Matter Removal

To improve digestion efficiencies and reduce the counting intensity associated with high microplastic concentrations, dried 0.25 g samples of biosolids were used for analysis. Once weighed, the samples were transferred to 250 mL wide mouth-Erlenmeyer flasks using 50 mL of filtered deionized (DI) water; then 20 mL of hydrogen peroxide (30%; VWR, Mississauga, ON,

Canada., cat. 89097-776) was added to the solution. The solution was allowed to sit overnight to promote the rehydration, and disaggregation of the dried biosolids sample (Lavoy and Crossman 2021). Iron (II) (MP Biomedicals, Ottawa, ON, Canada., cat. 158048) was then introduced to the sample in 5 × 2 mL aliquots at 10–15-minute intervals. This helped to maintain a stable reaction rate, while meeting the 2:1, hydrogen peroxide to iron (II) ratio, which is considered optimal for organic matter digestion (Lavoy and Crossman 2021). During the initial reaction, temperature was monitored to ensure the solution did not exceed 50˚C, since temperatures exceeding 70˚C have been shown to degrade microplastics (Munno et al. 2018). In the case of high temperatures, a cold-water bath was kept on hand to cool the digestate (Munno et al. 2018, Simon et al. 2018). The reaction was allowed to proceed for up to 24-hours before being passed through a 20 µm metal sieve. The portion retained on the sieve (residue) was transferred back to the flask using 50 mL of filtered DI water, and the liquid portion (filtrate) was discarded.

Enzyme digestions have been shown to be an effective method for organic matter removal, especially when applied to more complex organics, such as starches, fats, proteins, and cellulose (Wang et al. 2018, Lavoy and Crossman 2021). However, enzyme digestions are often considered costly, time consuming, and are not recommended as standalone digestion methods (Prata et al. 2019, Liu et al. 2022). Nonetheless, enzyme based septic tank cleaning products such as Bioclean® have been shown to remove 22% more organic matter compared to control treatments (Lavoy and Crossman 2021). Thus, Bioclean® was used due to its low cost, wide availability, and relatively fast reaction time (Lavoy and Crossman. 2021).

The Bioclean® solution was prepared by mixing 8 g of Bioclean® (Superior BioSolutions LLC, Grand Rapids, MI, USA., cat. SYNCHKG016874) for every 50 mL of water (Lavoy and Crossman 2021). After 30 minutes of stirring, the solution was sieved to 20 µm; the

solids were discarded, and the Bioclean® solution was retained (Lavoy and Crossman 2021). Then 50 mL of the Bioclean® solution was added to the Erlenmeyer flasks from the first Fenton's digestion (Flasks should contain: 50 ml Bioclean®, 50 ml Filtered DI, and solids >20 µm). The flasks were then incubated in an oven at 55°C for 48-hours (Lavoy and Crossman 2021). Afterwards the samples were sieved again to 20 µm, the liquid portion was discarded, and the solids were flushed back into the Erlenmeyer flask with 50 mL of filtered DI water. Then, using the methods previously described, the second Fenton's digestion was performed.

3.2.3.2 Mineral Separation

Following organic matter removal, a series of two water separations (1.0 g/cm^3) and one sodium iodide separation (1.6–1.8 g/cm^3) were used to liberate microplastics from the remaining mineral material (Lavoy and Crossman 2021, Wang et al. 2018). Typically, the density of plastic ranges from 0.8–1.6 g/cm^3, organic matter is between 1.0–1.4 g/cm^3, and mineral densities are ~2.65 g/cm^3 (Anger et al. 2018, Hurley et al. 2018, Prata et al. 2019). Sodium iodide separations have been shown to be capable of recovering both low and high-density polymers with >99% efficiency (Liu et al. 2022). However, this does not discount the importance of the water density separations that not only target low density polymers but are crucial to clean the sample and prevent the oxidation of the sodium iodide solution.

To conduct the initial water density separation, the flask containing the finished solution from the second Fenton's Digestion (~100 mL) was diluted using an additional ~150 mL of pre-filtered DI water. The solution was stirred on a magnetic stir plate for ~1 minute and was left to settle for a minimum of 6 hours (Lavoy and Crossman 2021). After settling, the liquid fraction of the sample was decanted and filtered though a 4.2 cm glass fibre filter paper rated for 1.1 µm particle retention (VWR, Mississauga, ON, Canada., cat. 28297-504), which was retained for

inspection. The flask was then re-diluted with ~250 mL of filtered DI water before stirring, settling, and filtering the sample in the same manner as the first separation (Lavoy and Crossman 2021).

Lastly, the settled fraction from the second water density separation was rinsed thoroughly into a density separation unit using the sodium iodide solution (prepared to a density between 1.6–1.8 g/cm^3 using pre-filtered DI water; Sigma Aldrich, Oakville, ON, Canada., cat. 746371). The density separation unit was constructed using a PVC pipe, a ball valve, and a ceramic tile (Appendix B3; Coppock et al. 2017, Lavoy and Crossman 2021, Wang et al. 2018). The density separation unit containing the sample and sodium iodide solution was covered, stirred for ~1 minute, then left to settle for 24 hours. Though shorter settling times have been suggested, the separation quality increases with time (Lavoy and Crossman 2021). Afterwards, the ball valve was turned to trap the settled portion in the bottom of the unit, while the top portion was filtered and rinsed through a 1.1 µm glass fibre filter paper. The filter paper was retained for visual inspection, while the remaining sodium iodide was filtered, and density adjusted prior to its reuse.

3.2.4 Microplastic Identification and Characterization

Once the samples were isolated on a filter paper, anthropogenic debris were manually counted using a stereomicroscope at 5–35× magnification (AmScope, Irvine, CA, USA., model. ZM-1 Series). This study quantified debris with a major axis between 20 µm and 5 mm. During counting, the debris were sorted into the following shape-based categories: fibres, films, fragments, beads, and foams. Characteristics key to the identification of anthropogenic debris include: an unnatural colour, material homogeneity, particle resiliency, reflective surfaces, and

limited fraying (Kosuth et al. 2018). Images of suspected microplastics were captured using a Teledyne Lumenera Infinity 2 camera (Teledyne Lumenera, Ottawa, ON, Canada., model. INFINITY2-1R). Further, hot needle tests were performed to establish the proportion of microplastics in the anthropogenic debris counts (Brander et al. 2020, Prata et al. 2019). Photographs of anthropogenic debris were deleted, and the image analysis software Infinity Analyse was used to measure the major and minor axis of microplastic particles.

A subset of microplastics were randomly selected for polymer identification using Raman Spectroscopy (Oxford Instruments, Ulm, Germany., model. WITec alpha300R confocal Raman Microscope). In the case of fibres, 31 plastics were selected from Plant 1, and 30 were selected from Plant 2. The sample size for fragments (P1 = 13, P2 = 9), foams (P1 = 3, P2 = 6), films (P1 = 2, P2 = 4), and beads (P1&2 = 2) was determined based on the relative proportions of each shape type. Raman scans were completed using lasers with wavelengths of 532 and 785 nm. All particles were first scanned using the 785 nm laser, and if a clean spectrum was not produced, the 532 nm laser was used (Lavoy and Crossman 2021). Several scanning variables can be altered in order to optimize spectral quality, such as the number of scans (accumulations), the length of each scan (integration time), the laser power/position, and the magnification level. Though these settings were adjusted on a case-by-case basis, scans were completed with a magnification between 20× and 100×, integration times between 0.05 and 50 seconds, the number of accumulations between 1 and 300, and the power level between 0.1 and 15 mW. Once produced, spectrum analysis and polymer identification were conducted through the open access database *OpenSpecy* (Cowger et al. 2021).

3.2.5 Data Analysis

To better understand the quantity of plastic in a sample, mass based microplastic concentrations were calculated (Kalcikova et al. 2017, Simon et al. 2018). To estimate microplastic mass, particle counts, average particle volumes, and average polymer densities were used (Simon et al. 2018). Microplastic volumes were calculated using the major and minor axis measurements, where fibres were calculated as cylinders, films were calculated as rectangular prisms, fragments and foams were calculated as ellipsoids, and beads were calculated as spheres (Appendix B4). Then, using the relative abundance of polymers and their known densities, a weighted average density was determined for each microplastic shape category. Since foams typically contain 95–98% air, the weighted average density of foams was adjusted to account for a void fraction of 96.5%. The mass of plastic (g) in each shape category was calculated (Appendix B5; Simon et al. 2018) as the product of density (g/cm^3), volume (cm^3), and microplastic count (n).

When comparing datasets, multiple statistical tests were used. All statistical tests were performed using Past v4.16c (Hammer et al. 2001). To evaluate the distribution of a dataset, the Shapiro-Wilk test for normality was used ($\alpha = 0.05$). The means of parametric datasets were compared using two-sample, equal-variance, t-tests at a significance level of $\alpha = 0.05$. In instances where non-normal distributions existed, the skew of the data was described visually using a violin plot (Hintze and Nelson 1998). When trying to determine whether a difference of medians exists between two datasets, the Mann-Whitney U test was used at a significance level of $\alpha = 0.05$. However, if the test compared medians of three or more datasets, the Kruskal-Wallis one-way analysis of variance was used ($\alpha = 0.05$). If a significant difference in medians existed, the Mann-Whitney pairwise post-hoc test was applied to rank the datasets ($\alpha = 0.05$).

Treatment efficiencies were calculated as the relative proportion (%) of plastic or biosolid removed from the tank compared to the total amount of plastic or biosolid present in the tank prior to treatment (Appendix B6). Pre-treatment microplastic and biosolid totals were based on the pre-treatment microplastic concentrations and moisture contents, as well as the total volume of biosolids added to each tank (20 L). The amount of material removed was based on the post-treatment microplastic concentrations and moisture contents, as well as the volume of biosolids removed from each tank. To account for the difference in volume extracted in the short and long treatments (0.75 and 1.75 L), extraction volumes were normalized to 1 L during calculation. Conversions between wet and dry concentrations were made using the moisture content of the associated sample. Preferred treatments were identified by determining the treatment parameters that maximised microplastic removal, while minimizing biosolids removal. Using the preferred treatment, theoretical microplastic diversion rates were calculated for each of the study plants using annual biosolids production data from 2021.

3.2.6 Quality Assurance and Quality Control

As in any study, controlling for contamination is critical in the analysis of microplastics. Contamination can be introduced at several points through sample preparation, and analysis. Analytical blanks (n = 3) were used to assess the degree to which contamination affected the results (Brander et al. 2020, Prata et al. 2019). One analytical blank was included for every ten samples analysed. As well, variability was monitored by including one set of duplicate samples for ever ten samples analysed. In addition, air blanks were used to monitor the rate of atmospheric microplastic deposition in the laboratory space (Coppock et al. 2017, Prata et al. 2019). Nonetheless, to reduce the impact of contamination from microplastics in the indoor atmosphere, all samples and beakers were covered during analysis, and any time spent uncovered

was recorded (Coppock et al. 2017, Prata et al. 2019). To assess data quality, the relative standard deviation (RSD = 44%), and method detection limit (MDL = 32.0 mp/g) were calculated using the blank data. While analysing samples, a series of best practices were employed. All solutions used in the analysis were filtered to remove microplastics prior to their use (Brander et al. 2020). All glassware was washed thoroughly with soap and water, and then rinsed 3× with filtered DI water (Brander et al. 2020). When interacting with samples, analysts wore laboratory coats and cotton clothing to avoid contamination from synthetic garments (Brander et al. 2020, Prata et al. 2019).

3.3 Results and Discussion

3.3.1 Microplastic Characterization Pre-Treatment

The microplastic count concentrations in biosolids collected pre-treatment from each plant (Table 2) were significantly different (two-sample, equal-variance, t-test, $p = 0.002$). The microplastic concentration in Plant 2 (563.7 ± 60.7 mp/g dw) was nearly double Plant 1 (284.2 ± 88.8 mp/g dw). Despite the difference in count concentration, the relative proportion of microplastic shapes was consistent between the two plants (Figure 7). In both cases, fibres were the most common shape identified, accounting for 68.6% and 64.4 % of the total number of microplastics in Plant 1 and 2 respectively. In a previous study, Li et al. (2018) also found fibres to be the most dominant microplastic shape in biosolids at 63%, despite their evaluation of slightly different shape categories. Fragments were the next most abundant microplastic shape at 28.2% and 26.2% for Plants 1 and 2, respectively. Foams (1.5%, 4.7%) and films (1.5%, 4.6%) had nearly identical counts, with a higher proportion of the total counts in Plant 2. The least common microplastic type in both plants were microbeads (0.2%, 0.1%). The low microbead proportions likely reflect the national ban on the import and sale of products containing

microbeads in July 2018 (Government of Canada, 2018). It should be noted that due to the low counts in this study, data on microbead size and polymer composition was pooled between plants and treatments to help increase the sample size.

Table 2: Mean of the microplastic count and mass concentrations (± one standard deviation) per gram dry weight (g dw) of biosolids and mean microplastic characteristics in biosolids collected prior to treatment from the two studied WWTPs (i.e., Plants 1 and 2). Microplastic count concentration (mp/g dw), size along the major and minor axis (µm), particle volume (cm^3), number of size observations (n), polymer density (g/cm^3), number of density observations (n), and mass concentration (µg/g dw) are described for fibres, films, fragments, beads, and foams.

Shape Category	Plant 1							
	Count	*Size*				*Density*		*Mass*
	Count Conc. (mp/g dw)	Major Axis(µm)	Minor Axis(µm)	Volume (cm^3)	Obs. (n)	Density (g/cm^3)	Obs. (n)	Mass Conc. (µg/g dw)
Fibres	194.8 ± 50.6	549.39	20.79	1.87 E-07	21	1.19	31	43.3 ± 11.3
Films	4.3 ± 2.8	393.82	218.43	1.79 E-06	10	1.29	2	9.9 ± 6.5
Fragments	80.2 ± 33.3	115.49	69.44	2.92 E-07	17	1.14	13	26.7 ± 11.1
*Beads**	0.6 ± 1.2	66.06*	64.46*	1.46 E-07*	11*	1.08*	2*	0.1 ± 0.2
Foams	4.3 ± 4.5	226.02	148.50	2.61 E-06	10	0.03	3	0.4 ± 0.4
Total	284.2 ± 88.8							80.3 ± 27.8
Shape Category	Plant 2							
	Count	*Size*				*Density*		*Mass*
	Count Conc. (mp/g dw)	Major Axis(µm)	Minor Axis(µm)	Volume (cm^3)	Obs. (n)	Density (g/cm^3)	Obs. (n)	Mass Conc. (µg/g - dw)
Fibres	362.8 ± 57.6	499.34	18.67	1.37 E-07	37	1.25	30	62.2 ± 9.9
Films	25.9 ± 25.8	389.54	191.27	1.39 E-06	10	1.24	4	44.5 ± 44.3
Fragments	147.7 ± 21.8	123.26	72.40	3.38 E-07	29	1.18	9	58.9 ± 8.7
*Beads**	0.7 ± 1.5	66.06*	64.46*	1.46 E-07*	11*	1.08*	2*	0.1 ± 0.2
Foams	26.5 ± 23.3	190.61	127.33	1.62 E-06	10	0.03	6	1.4 ± 1.2
Total	563.7 ± 60.7							167.1 ± 31.0

Note, cells marked with * indicate that the data were pooled between plants owing to low counts.

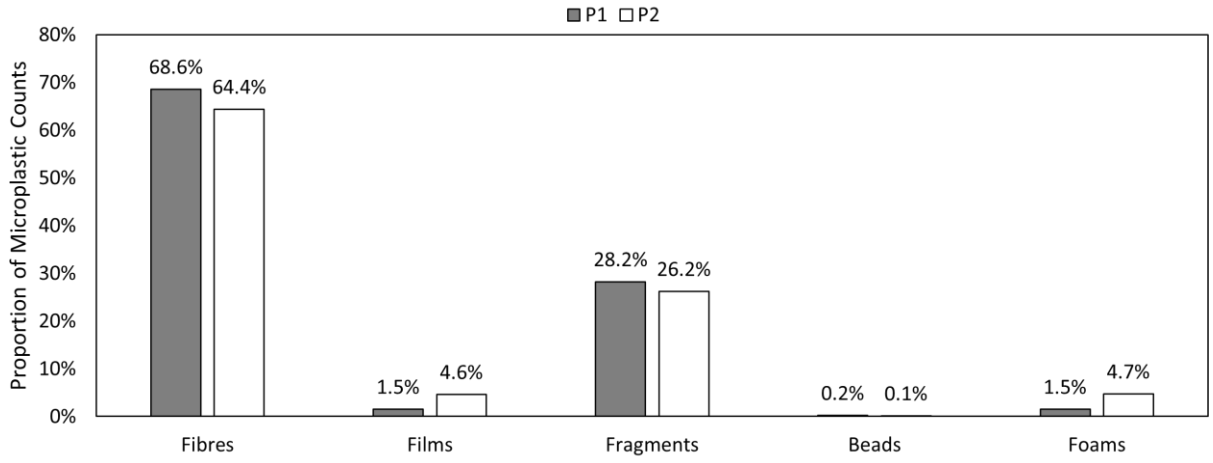

Figure 7: The mean proportion (%) of microplastic shapes identified within the biosolids collected before treatment from each of the studied WWTPs. P1 indicates Plant 1, and P2 indicates Plant 2.

The microplastic lengths for both the major and minor axes of all shape types (Figure 8) were found to have non-normal distributions (Shapiro-Wilk $p < 0.05$). The data were positively skewed, meaning most observations were from small particles (Figure 8), an observation which is consistent with previous studies (Coalition Clean Baltic 2017, Hengstmann et al. 2019, Kalcikova et al 2017). There was no significant difference in the size of the major and minor axis of each shape category between plants (Mann-Whitney U $p > 0.05$); as such, the data were pooled to increase sample size (Figure 8). However, there were significant size differences found between shape categories (Kruskal-Wallis $p < 0.001$). For the major axis, the categories were ranked from largest to smallest as follows A: fibres, AB: films, B: foams, and C: fragments and beads (Figure 8; Mann-Whitney $p < 0.05$). For the minor axis, the categories were ranked from largest to smallest as follows A: films, AB: foams, BC: beads, C: fragments, and D: fibres (Figure 8; Mann-Whitney $p < 0.05$). While, fibres were found to have the greatest major axis, they had the smallest minor axis, which has direct implications for their volume.

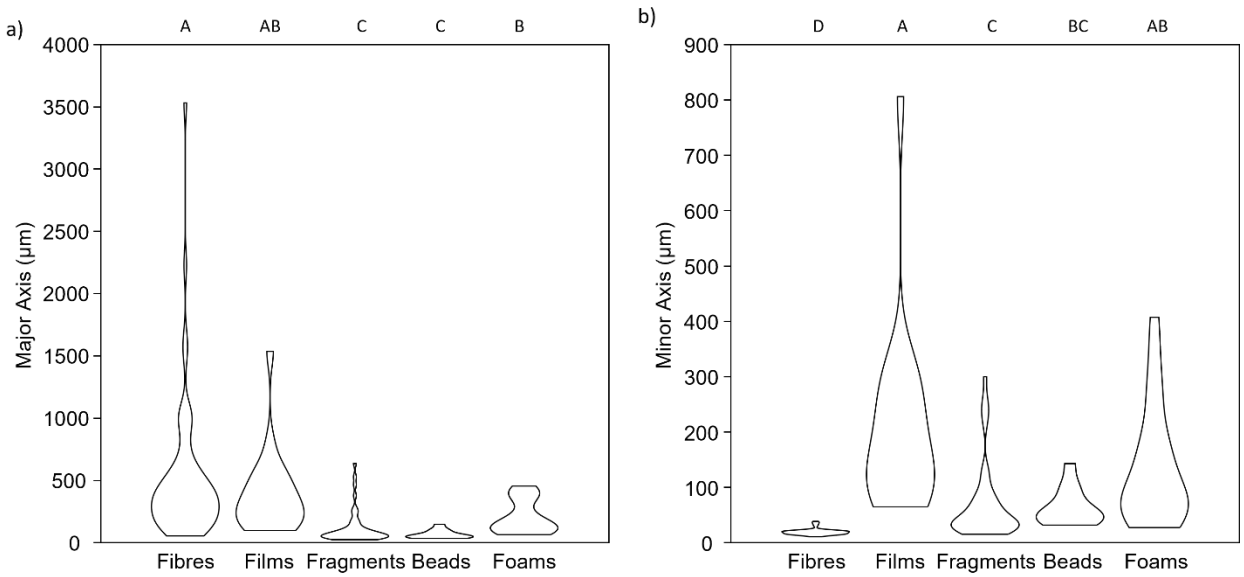

Figure 8: Violin plot showing the length of the major (a) and minor (b) axis of each microplastic shape category across the two study plants (pooled data). Note the difference in the scale of the y-axis between the two plots. The thickness of each violin represents the distribution of size observations, where thick regions have a greater number of observations than thin regions. The letters in the plot indicate the rank of each shape category from largest to smallest (A–D).

The volumes of individual microplastics were also positively skewed (Shapiro-Wilk $p < 0.05$); that is, most observations come from less voluminous microplastics. Further, there was no significant difference in the average volume of the microplastics observed in biosolids collected from the two plants (Mann-Whitney U $p > 0.05$); that is, the fibres in biosolids from one plant were not more voluminous than the fibres from the other. This suggests that microplastic size in biosolids was not influenced by the wastewater treatment source. The data were then pooled between plants to increase sample size (Figure 9). Significant differences were found in volume between the microplastic shape categories (Kruskal-Wallis $p < 0.001$). The shape categories were ranked from most to least voluminous as follows A: films and foams, B: fibres, fragments and beads (Figure 9; Mann-Whitney $p < 0.05$). This highlights the importance of considering microplastic volume when inferring the relative burden of each microplastic particle. Generally,

an individual foam or film particle will occupy a greater volume than an individual fibre, fragment, or bead (Figure 9).

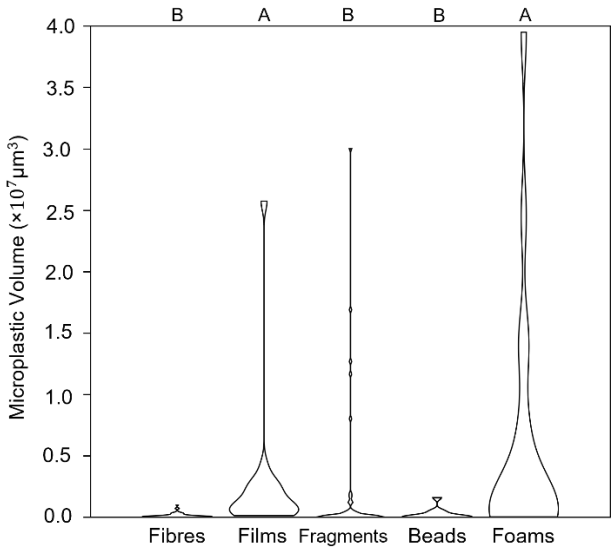

Figure 9: Violin plot showing the volumes of each microplastic shape category across the two study plants. The thickness of each violin represents the distribution of size observations, where thick regions have a greater number of observations than thin regions. The letters on the plot indicate the rank of each shape category from largest to smallest (A–B).

The polymeric composition of fibres by count was almost identical in the biosolids from the two plants (Figure 10). Polyester (or polyethylene terephthalate [PET]) was identified as the dominant polymer, at 52% in Plant 1 and 60% in Plant 2. Similarly, Zhang et al. (2019) found that PET represented 65% of fibres in China. The next most common polymer was Polypropylene [PP] (P1 = 23%, P2 = 20%), followed by Polyamide [PA] (P1 = 10%, P2 = 10%). Though other trace polymers (polymer representing less than 3.23%) were detected, they were grouped as "other" due to their low prevalence. The "other" category represented 16% of the fibres in Plant 1, and 10% of the fibres in Plant 2. Using the known density of each polymer type (Appendix B7), a weighted average fibre density was determined for each plant. The average fibre density was 1.19 g/cm^3 and 1.25 g/cm^3 in Plant 1 and Plant 2, respectively. The difference in density was explained by the slightly larger proportion of PP and PA found in Plant 1 (Figure

10), which have lower densities compared with PET. The other microplastic shape categories did not show the same similarities in polymer type in the two plants, which may be due to the lower sample size analysed for polymer composition. The diversity of polymers observed differed between shape categories. For example, in foams, most observations came from LDPE. In contrast, fragments exhibit a much higher degree of polymer diversity. This is because foams are more specific in their source definition, whereas fragments are much more general, where a broader range of particles may fit.

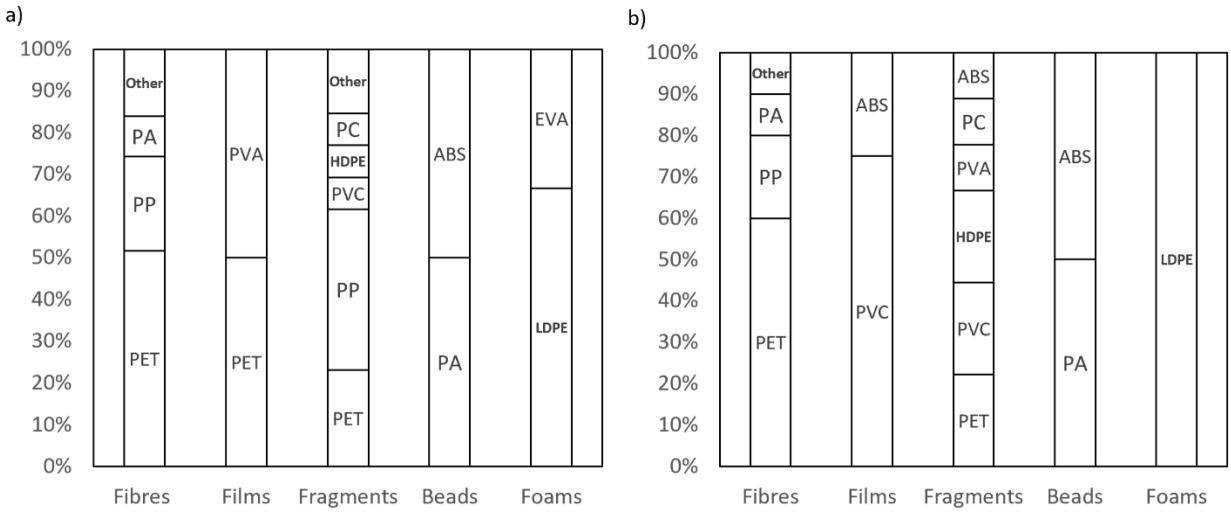

Figure 10: The mean proportion (%) of plastic polymers that were identified in a) Plant 1, and b) Plant 2 before settling treatments were applied. The data are ordered by microplastic shape category.

Consistent with the count concentration, the mass concentration of microplastics was significantly higher in biosolids from Plant 2 at 167.1 ± 31.0 µg/g, compared with biosolids from Plant 1 at 80.3 ± 27.8 µg/g (t-test, $p < 0.05$). There was a slight shift in the relative abundance of each shape category due to the conversion from count to mass-based concentrations (Table 2). Fibres were still the most prominent shape, however, the mass proportion of fibres (P1 = 53.9%, P2 = 37.2%) as shown in Figure 11, was reduced compared to it's count proportion (P1 = 68.6%, P2 = 64.4%) as shown in Figure 7. This was attributed to the relatively small volume of plastic

contributed by individual fibres (Figure 9). Typically, more voluminous microplastics account for a greater proportion of the total microplastic mass, but this is not always the case. Films serve as an example where high relative microplastic volumes (Figure 9) contributed to an increase in the proportional representation of their shape category (Figures 7 and 11). In contrast, the relative proportion of foams decreased from counts to masses (Figures 7 and 11) despite their high relative microplastic volume (Figure 9). Instead, low density (Table 2) caused the reduction in the relative abundance of foams by mass.

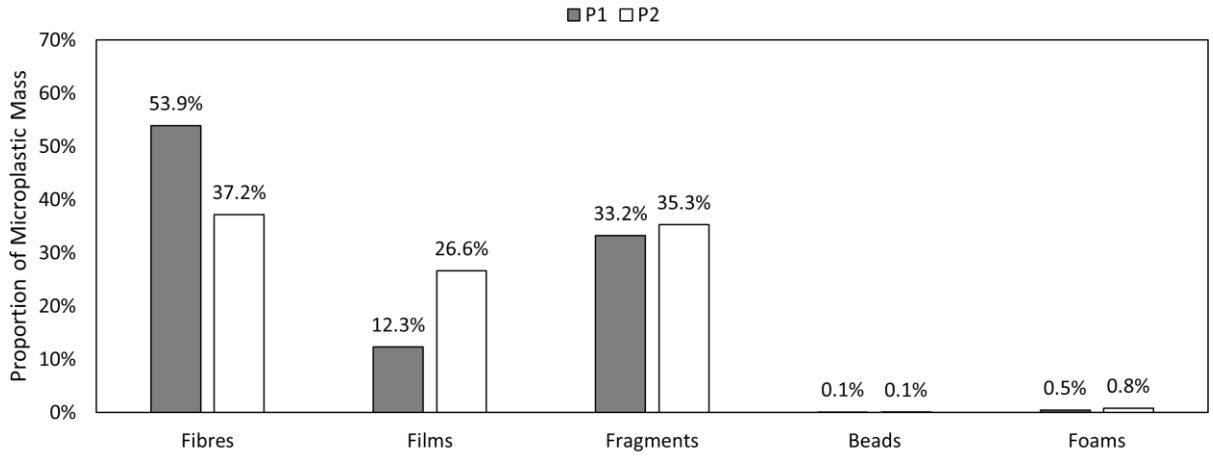

Figure 11: The mean proportion (%) that each shape category contributes to the total mass of plastic identified within the biosolids sample (P1 indicates Plant 1, and P2 indicates Plant 2).

Although the study plants had different microplastic concentrations, there were no differences in their characteristics (size, polymer type, and relative shape abundance). The microplastic count concentrations found in this study (Table 2) were very similar to values previously reported on microplastic concentrations in dry biosolids collected from 11 WWTPs in Ontario, Canada, where counts ranged between 188.2 and 512.0 mp/g dw, with an average of 372.3 (±27.5) mp/g dw (Letwin et al. 2023). In contrast, the mass concentrations in this study tended to be lower relative to other studies from the literature. For instance, Rasmussen et al. (2021) reported a microplastic mass concentration in biosolids of 569.7 µg/g dw. This difference

between count and mass concentrations is best exemplified by Lofty et al. (2022), who reported a biosolids count concentration of 24.7 mp/g and a mass concentration of 10,000 µg/g. This inconsistency was explained in part because these authors only included larger particles with greater masses (1–5 mm). By only including large microplastics, the resulting count concentrations would be reduced, while disproportionally increasing the mass concentration (Rasmussen et al. 2021). It should be noted that differences in methodologies commonly underly inconsistencies between reported values.

3.3.2 Comparison of Microplastics Pre- and Post-Treatment

It is important to understand whether the treatment procedure influenced the types of microplastics recovered prior to assessing the effectiveness of each type of treatment. In general, average treatment microplastic concentration in the upper horizon of the settling tanks was high compared with the pre-treatment concentration (Tables 2 and 3). In Plant 1, the average of all treatments compared with pre-treatment increased the count-based concentration from 284.2 ± 88.8 mp/g to 618.1 ± 348.5 mp/g; and mass-based concentrations from 80.3 ± 27.8 µg/g to 153.2 ± 80.2 µg/g. In Plant 2, on average the count-based concentration increased from 563.7 ± 60.7 mp/g to 3383.8 ± 1833.2 mp/g; and the mass-based concentration increased from 167.1 ± 31.0 µg/g to 573.6 ± 297.9 µg/g. Treatment increased the concentration of all shape categories except for films (Tables 2 and 3). This clearly shows that settling influences the concentration of microplastics observed in biosolids, consistent with Crossman et al. (2020). Between plants, similar trends were observed in the proportion of shapes that made up the microplastic counts and masses (Figure 12). Treatment appeared to increase the proportion of fibres and foams, decrease the proportion of films and fragments, and had no effect on the proportion of beads. The proportional increase in foams and decrease in fragments can be explained under the premise

that, in settling treatments, low-density plastics like foams are more easily suspended than dense granules like fragments (Bilgin et al. 2020, Kwon et al. 2022). Further, it should be noted that these are proportional changes, as the absolute concentrations of all shape categories except films were found to increase with treatment (Tables 2 and 3).

Table 3: Mean microplastic concentrations and characteristics observed within biosolids from the two studied WWTPs (i.e., Plants 1 and 2) after conducting the treatments. Microplastic count concentration (mp/g dw), size along the major and minor axis (µm), particle volume (cm^3), number of size observations (n), polymer density (g/cm^3), number of density observations (n), and mass concentration (µg/g dw) are described for fibres, films, fragments, beads, and foams.

Shape Category	Plant 1							
	Count	*Size*				*Density*		*Mass*
	Count Conc. (mp/g dw)	Major Axis (µm)	Minor Axis (µm)	Volume (cm^3)	Obs. (n)	Density (g/cm^3)	Obs. (n)	Mass Conc. (µg/g dw)
Fibres	452.6 ± 267.7	487.55	20.00	1.53 E-07	121	1.23	84	85.6 ± 50.6
Films	3.8 ± 2.5	386.52	205.94	1.59 E-06	10	1.17	5	7.1 ± 4.7
Fragments	148.1 ± 74.3	128.43	71.32	3.42 E-07	45	1.18	11	59.9 ± 30.0
*Beads**	1.3 ± 2.2	66.06*	64.46*	1.46 E-07*	11*	1.08*	2*	0.1 ± 0.2
Foams	12.2 ± 13.1	178.49	110.71	1.15 E-06	10	0.04	7	0.5 ± 0.5
Total	618.1 ± 348.5							153.2 ± 80.2
Shape Category	Plant 2							
	Count	*Size*				*Density*		*Mass*
	Count Conc. (mp/g dw)	Major Axis (µm)	Minor Axis (µm)	Volume (cm^3)	Obs. (n)	Density (g/cm^3)	Obs. (n)	Mass Conc. (µg/g dw)
Fibres	2436.9 ± 1342.2	523.13	18.88	1.47 E-07	512	1.26	72	449.1 ± 247.4
Films	10.2 ± 3.2	381.73	260.15	1.88 E-06	10	1.26	3	24.1 ± 7.6
Fragments	360.0 ± 173.6	98.49	60.55	1.89 E-07	94	1.18	26	80.1 ± 38.6
*Beads**	5.9 ± 4.8	66.06*	64.46*	1.46 E-07*	11*	1.08*	2*	1.0 ± 0.8
Foams	570.8 ± 472.0	167.03	106.71	9.96 E-07	128	0.03	14	19.2 ± 15.9
Total	3383.8 ± 1833.2							573.6 ± 297.9

Note, cells marked with * indicate that the data were pooled between plants owing to low counts.

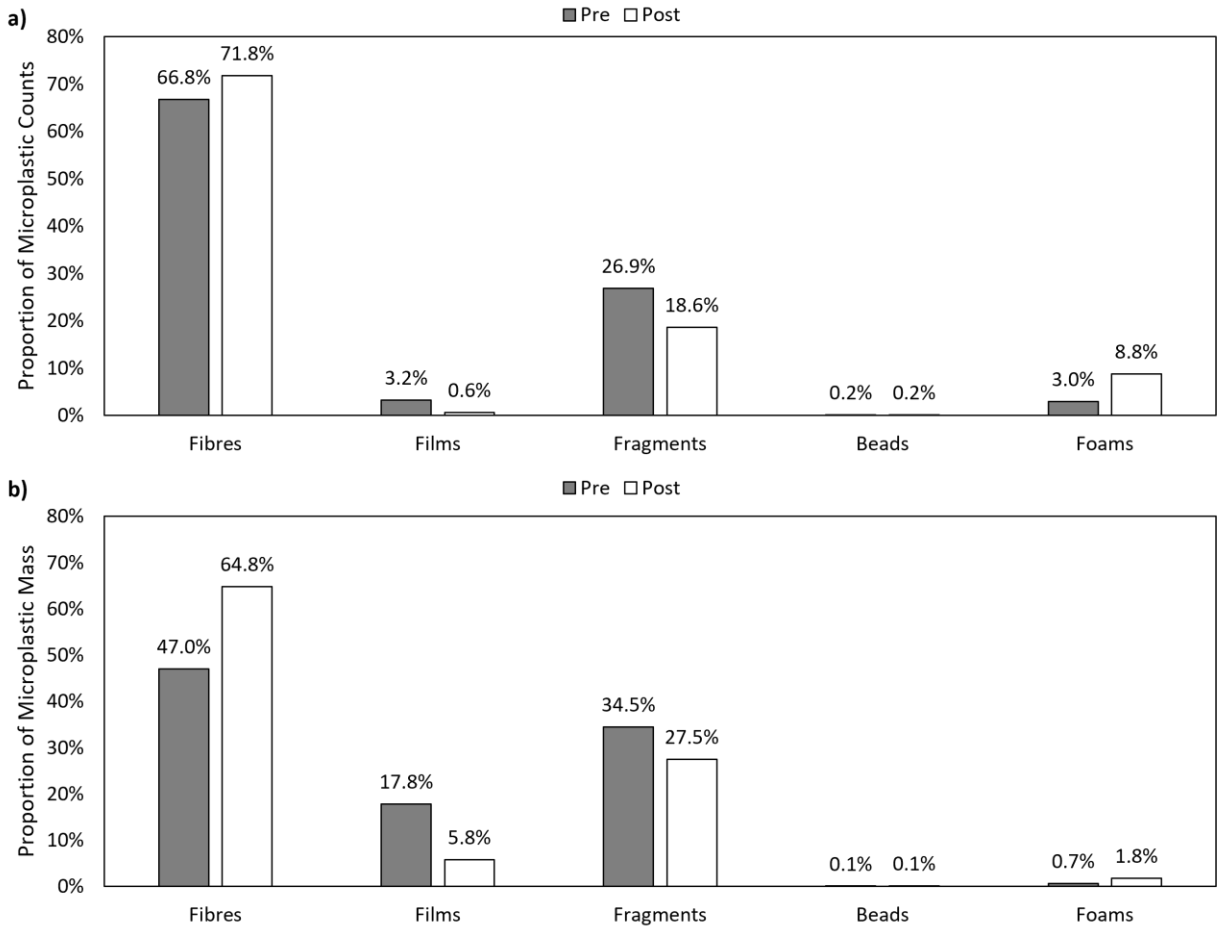

Figure 12: The effect of treatment on the mean proportion (%) that each shape category contributes to the total (a) count, and (b) mass of microplastics. Pre and Post are used to indicate the condition before and after conducting the settling treatment.

Treatment did not have a substantial influence on the size of the major and minor axes of microplastics. Other than slight differences in the distribution range and shape, there were no significant differences in the medians of either the major or the minor axes of any of the microplastic shape categories (Appendix B8; Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p < 0.05$). Additionally, there were no significant differences in the volume of the microplastics between the pre- and post-treatment samples for all shape categories (Appendix B9, Shapiro-Wilk $p < 0.05$, Mann-Whitney U $p < 0.05$). The pre-treatment samples were ranked A: foams and films, B: fragments, beads, and fibres (Figure 3). Whereas in the post-treatment samples, the ranking was

A: foams, B: films, C: fragments, beads, and fibres (Mann-Whitney $p < 0.05$). Apart from the separation of foams and films in the rankings, and a slight difference in data range, there was no difference in the volume of microplastics before and after conducting treatments.

The polymeric composition of fibres pre- and post-treatment was nearly identical (Appendix B10). The three main fibre polymers were again PET, PP, and PA, with the remaining trace polymers categorized as "other". The proportion of PET increased from 56% to 60%, PP decreased from 21% to 15%, PA increased from 10% to 18%, and other decreased from 13% to 7% for the post treatment fibres. However, there was no difference in the count-weighted densities of the fibres in each treatment phase (t-test $p > 0.05$). This was also observed in all other shape categories, where treatment did not have a significant influence on the count-weighted densities of the polymers that were recovered (t-test $p > 0.05$). Though there was no apparent pattern in the polymers identified pre- and post-treatment, there was a much greater polymer diversity observed post-treatment (Appendix B10). This increase in polymer diversity was attributed to the greater sample size for the polymeric analysis post-treatment (Table 3) as compared to pre-treatment (Table 2). Since it is unlikely that the plant or treatment phase influenced the density of the observed polymers, all polymer observations for each shape category were pooled (Appendix B11). Microplastic shape was found to influence polymer density (Kruskal-Wallis $p < 0.001$); where the order of microplastic shapes from most to least dense was as follows, A: fragments, AB: films, B: fibres, and C: foams (Mann-Whitney $p < 0.05$). Beads were not included in this analysis due to their low sample size.

3.3.3 Assessment of Treatment Efficiencies

In the upper horizon of the biosolids settling tanks, microplastic count concentrations increased in 15 of the 16 trials, and microplastic mass concentrations increased in 14 of the 16

trials (Table 4). Overall, this indicates that settling treatments are capable of concentrating microplastics in the upper horizon of the settling tank. Of the treatment variables employed, neither the stirring methods, nor settling times significantly influenced the ability of the treatments to concentrate microplastics in the upper layer of the settling tank (t-test $p > 0.05$). However, scooping (surface skim) was found to yield a significantly greater microplastic concentration potential compared to syphoning (t-test, $p < 0.05$). As well, using the re-wetted cake from plant 2 generated a significantly greater microplastic concentration compared to using the un-altered liquid biosolid from plant 1 (t-test $p < 0.05$). This observation may be attributed to a change in the biosolids matrix during the drying and re-hydration process. The unaltered biosolids had a very homogenous composition, where despite thorough mixing, the rehydrated cake maintained more of a suspended solids like composition. This suspended solids like composition may have aided in the separation of microplastics from biosolids, while simultaneously improving the ability of the biosolids to settle.

Additionally, moisture contents of the extracted biosolids were found to increase in 11 of the 16 trials. As the inverse measure of total solids, the moisture content essentially indicates a biosolids concentration. As such, settling treatments not only show promise of increasing microplastic concentrations, but also show potential to reduce biosolids concentrations, thus minimising the loss of biosolids during treatment. Of the treatment variables employed, neither the harvest, nor stirring methods were found to significantly influence the moisture content of the biosolids removed (t-test $p > 0.05$). However, the moisture content in trails using re-hydrated cake were significantly greater than in trails using the unaltered biosolids (t-test $p < 0.05$). This proportional reduction of biosolids in the upper horizon may be attributed to the rehydrated cake's improved settling ability, given its suspended solid like composition. Additionally, trails

that used long settling times had significantly higher moisture contents than trails that used short settling times (t-test $p < 0.05$). This could be attributed to a sort of dilution effect during sampling. Where long settling treatments extracted a larger sample volume (1750 mL) over a longer time (21 days) than short settling treatments (750 mL over 9 days). During sampling, surface level solids were extracted at a higher rate in beginning of the treatment cycle, as opposed to at the end of the treatment cycle. Due to this effect, long sampling periods experienced a dilution effect, thus increasing the moisture content, and reducing the proportional removal of biosolids.

Table 4: Moisture content (%), mean dry microplastic count concentration (mp/g), and dry mass concentration (µg/g) from each settling treatment for biosolids collected from plant 1 and plant 2. Treatment names were selected based on the treatment variables employed, e.g., stir vs. un-stir, short vs. long (9 vs. 21 days), and scoop vs. syphon. The values are presented as dry concentrations in terms of both microplastic mass and count. Initial concentration prior to treatment is included as a reference.

Treatment	**Moisture Content (%)**		**Dry Count Conc. (mp/g)**		**Dry Mass Conc. (µg/g)**	
	Plant 1	Plant 2	Plant 1	Plant 2	Plant 1	Plant 2
Un-stir Short Syphon	97.97	99.02	301.5	763.6	86.0	132.5
Stir Short Syphon	97.50	99.86	321.7	4358.1	85.3	652.4
Un-stir Short Scoop	94.42	99.02	278.1	3099.8	78.1	513.3
Stir Short Scoop	97.38	99.73	335.4	5485.0	83.9	863.3
Un-stir Long Syphon	99.08	99.61	1028.9	1002.1	253.3	210.4
Stir Long Syphon	98.76	99.73	716.5	2535.2	163.3	471.8
Un-stir Long Scoop	97.41	99.61	1091.6	4644.3	260.4	796.0
Stir Long Scoop	98.37	99.44	871.0	5182.0	215.6	949.3
Initial	97.97	98.22	284.2	563.7	80.3	167.1

Treatment efficiencies were estimated by comparing the total amount of plastic and biosolid that was removed during each treatment, to the total amount in the tank prior to treatment. Across all treatments, between 1.95% and 24.50% (RSD = 64.5%) of total microplastic counts were removed, between 1.38% and 20.68% (RSD = 72.1%) of total plastic mass was removed, and between 0.39% and 13.74% (RSD = 93.6%) of biosolid mass was

removed. When assessing the effectiveness of each treatment variable (e.g., Stirring: Stirred vs. Un-Stirred), eight observations were grouped for each variable, and the average values from each group were compared (Figures 13 and 14). However, given the nature of the experimental design, there was a high degree of variability in treatment efficiencies between observations that belonged to the same group (e.g., Stirred). This is because of the influence of several non-target variables (e.g., harvest method, settling time, and biosolid type). The variability associated with the influence of these non-target variables made it difficult to draw statistically significant conclusions about the data. Fortunately, the non-target variables are arranged in way which should influence each group (e.g., Stirred vs. Un-Stirred) in the same way.

Preferred treatments were identified based on their ability to maximize microplastic liberation while minimizing biosolids removal. In terms of the harvest method, scooping generated data showing significantly greater microplastic removal (masses and counts; t-test, $p < 0.05$), no significant difference in biosolids removal (t-test, $p > 0.05$), and no significant difference in the overall plastic to biosolid removal ratio (t-test, $p > 0.05$). Ultimately, the scooped trials were favored, despite limited statistical basis. In terms of stirring, there were no significant differences in microplastic or biosolid removal, or the overall plastic to biosolid removal ratios (t-test, $p > 0.05$). Despite not finding a statistically significant difference, un-stirred tests appear to remove greater amounts of microplastics and biosolids as compared to stirred tests; however, stirred tests appear to generate better plastic to biosolid removal ratios as compared to un-stirred tests. Due to this, stirred tests were preferred under the premise that treatments should maximise plastic removal while minimizing biosolids removal. In terms of settling times, there were no significant differences in microplastic or biosolid removal, or the overall plastic to biosolid removal ratios (t-test, $p > 0.05$). Despite not finding a statistically

significant difference, long settling times appeared to remove greater amounts of microplastics and lower amounts of biosolids as compared to the short settling times; overall, the long settling times appeared to generate better plastic to biosolid removal ratios as compared to short settling times. As such, long settling times were preferred to short settling times. Finally, in terms of biosolid source, re-wetted cake was found to generate significantly lower microplastic and biosolids removal as compared to unaltered liquid biosolids (t-test, $p < 0.05$). Additionally, re-wetted cake generated a significantly greater plastic to biosolids removal ratio as compared to the un-altered liquid biosolid (t-test, $p < 0.05$). As such, the use a re-wetted cake was favored to the use of un-altered liquid biosolid. Overall, the favored results for each of the four variables are as follows, harvest method: scoop, stirring: stirred, settling time: long, biosolid type: re-wetted cake.

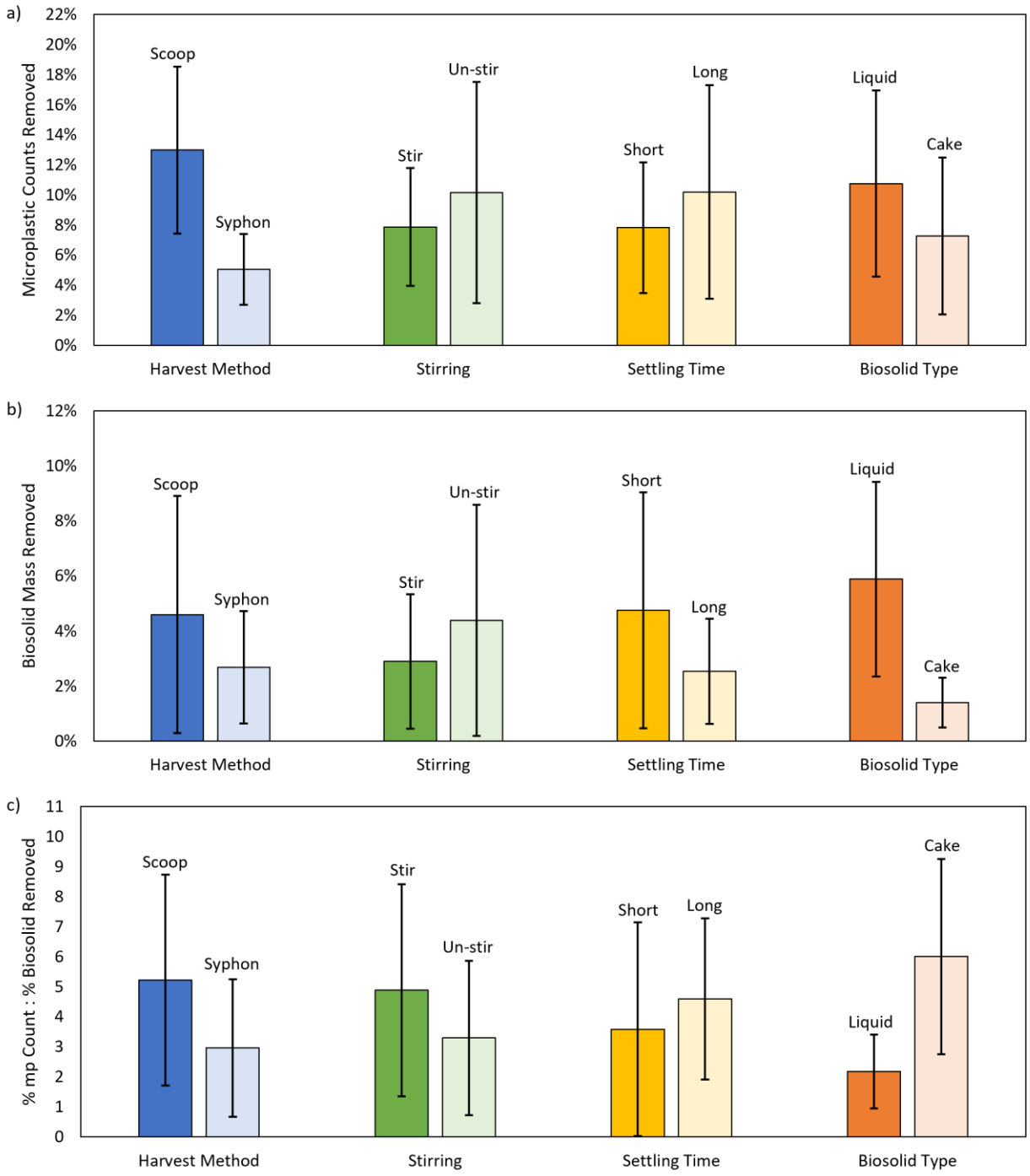

Figure 13: A comparison of each treatment variable's (a) percent microplastic count removal, (b) percent solids removal, and (c) ratios of the microplastic count and biosolids removal. Percentages were obtained by comparing the amount of plastic and solids that were removed during treatment, to the total amount of plastic and solids in the tank prior to treatment. All error bars indicate ± one standard deviation.

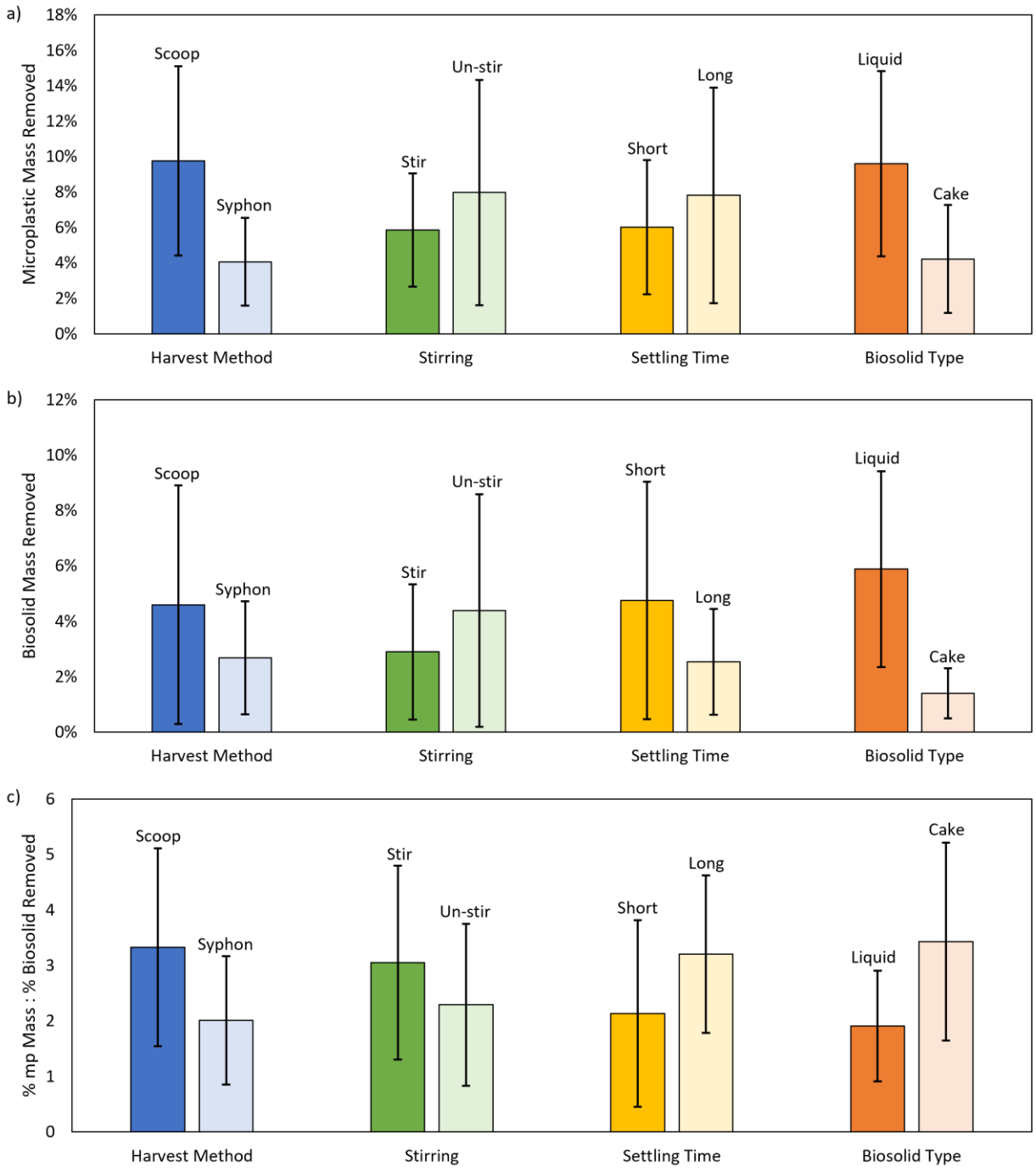

Figure 14: A comparison of each treatment variable's (a) percent microplastic mass removal, (b) percent solids removal, and (c) ratios of the microplastic mass and biosolids removal. Percentages were obtained by comparing the amount of plastic and solids that were removed during treatment, to the total amount of plastic and solids in the tank prior to treatment. All error bars indicate ± one standard deviation.

3.3.4 Microplastic Diversion Potential

Annual microplastic diversion potentials were estimated using the preferred treatment parameters which were identified in the previous section (Table 5). First, the total annual microplastic and biosolids exports were estimated using microplastic concentrations, moisture contents, and the total volume of biosolids produced by each plant over the course of one year. In general, the number of microplastics retained in the biosolid annually (P1 = 4.49 ± 1.40 E+11 mp/year, P2 = 1.06 ± 0.11 E+12 mp/year) was comparable to previous studies. In their study, Gies et al. (2018), reported approximately 1.64 E+12 microplastics retained in biosolid annually. Further, Carr et al. (2016) reported 1.09 E+09 mp/day retained in biosolids, which equates to 3.98 E+11 mp/year. In terms of mass however, the values differed substantially. One study reported the accumulation of 27.3 kg of microplastic in biosolid each day, which equates to 9964.5 kg/year (Rasmussen et al. 2021). These values are related to the reported microplastic mass concentration (569.7 μg/g dw), and rate of biosolid production (7.97 tonnes biosolid/hr dw; Rasmussen et al. 2021). This study reported a lower rate of biosolid production, and lower microplastic mass concentrations, which resulted in the lower microplastic mass exports (Table 5; P1 = 127 ± 43 kg/year, P2 = 313 ± 58 kg/year).

Under the recommended treatment (Stir, Scoop, Long), Plant 1 had the capacity to divert 10.8% of microplastic mass, and 12.3% of counts, while removing 4.0% of the dry solids mass (Table 5). Whereas Plant 2 had the capacity to divert 8.9% of microplastic mass, and 14.5% of counts, while only removing 1.6% of the dry solids mass (Table 5). Though these treatment efficiencies may sound promising, they are not yet indicative of a real-world solution. Microplastics accounted for just 0.01% and 0.02% of the dry biosolid mass in Plants 1 and 2, respectively. Despite being able to remove proportionally more microplastics than biosolid, the

current treatments need further refinement. Given that microplastics represented a low proportion of the total biosolid mass, 63.5 tonnes of biosolid would need to be removed to divert 13.7 ± 4.7 kg of microplastics from Plant 1, and 29.5 tonnes of biosolid would need to be removed to divert 28.0 ± 5.2 kg of plastic from Plant 2 (Table 5).

Table 5: Comparison of the two study plants, and their potential for plastic diversion if biosolid settling were implemented. Diversion was estimated using the values from the stirred, and scooped treatments with long settling times (Table 4).

Plant Metrics	**Plant 1**	**Plant 2**
Service Population	123,503	84,000
Average Daily Flow – 2021 (m^3/day)	36,477	38,645
Dry Mass Biosolid Exported per Year – 2021 (tonnes/year)	1580	1873
Recommended Treatment: Mass of Biosolid Removed (tonnes/year)	63.5	29.5
Microplastic Count Concentration (mp/g dw)	284.2 ± 88.8	563.7 ± 60.7
Number of Microplastics in Biosolid per Year (mp/year)	4.49 ± 1.40 E+11	1.06 ± 0.11 E+12
Recommended Treatment: Number of Microplastics Diverted (mp/year)	5.53 ± 1.73 E+10	1.53 ± 0.16 E+11
Microplastic Mass Concentration (µg/g dw)	80.3 ± 27.8	167.1 ± 31.0
Mass of Microplastics in Biosolid per Year (kg/year)	127	313
Recommended Treatment: Mass of Microplastics Diverted (kg/year)	13.7 ± 4.7	28.0 ± 5.2

Should the need arise, settling treatments to remove microplastics from biosolids could be implemented with relative ease. The process would require an additional settling basin where liquid biosolids could be retained and allowed to settle, as well as infrastructure to support the stirring and harvesting of biosolids. The treatments could be implemented onsite at a WWTP, or prior to treatment at a secondary biosolids refinement facility. Overall, this series of settling treatments demonstrated the ability to concentrate microplastics in the surface horizon; however, the process must be refined to further increase treatment efficiencies. Continued investigation of settling times, stirring regiments, and harvesting protocols are also required to develop a practical approach to reduce microplastic loads in biosolids. In addition, the incorporation of

chemical treatments such as surfactants may aid in the refinement of microplastic liberation potentials.

Alternative treatment solutions to reduce microplastic loads in wastewater have been proposed, but few for biosolids in particular. For example, thermal treatments have been suggested to reduce the abundance of microplastics. It is common for biosolids to be incinerated when they are not land applied. Though this process drastically reduces microplastic loads, the nutrient resource in biosolids is also lost. In contrast, thermal regeneration techniques using steam have been shown to degrade microplastics without the loss of biosolids (Kim et al. 2021). Though these treatments may produce results quickly, melting, and vaporizing microplastics can release harmful chemicals to the environment (Kim et al. 2021). As an alternative, microbial colonies, though slower acting, have been shown to degrade plastics with minimal environmental consequence (Misraa et al. 2020, Yuan et al. 2020). By using plastics as a carbon source, select bacteria such as *Ideonella sakaiensis*, turn plastic into carbon dioxide, water, and biomass (Misraa et al. 2020, Yuan et al. 2020). Though promising in theory, the practicality of microbial approaches has not been tested for treating microplastics in WWTPs. Nonetheless, this study demonstrates that biosolid settling treatments have the potential to divert upwards of 10% of microplastics from the terrestrial environment. Unlike other methods, settling is unique in the sense that it can be implemented with minimal modification to existing infrastructure, while simultaneously having low energy costs. As such, settling treatments can be applied with relative ease, thus making them a viable option for microplastics treatment that may be implemented depending on legislative need.

3.4 Conclusions

Given the ubiquitous presence of microplastics in the environment, it is important to develop strategies that move us closer towards zero plastic pollution. It is well established, that the land application of biosolids is a major pathway by which contaminants, including microplastics enter the environment. This study demonstrated that between 2.0 and 24.5% of microplastic counts or 1.4 and 20.7% of microplastic mass could be diverted from land application by settling treatments, while only removing between 0.4 and 13.8% of dry biosolid mass. Though these values are not yet indicative of efficient microplastic liberation, it suggests that settling has the potential to concentrate microplastics in the upper horizon of a settling tank. Of the treatment variables analysed, stirred treatments were preferred to unstirred treatments, scooped (surface skim) treatments were preferred to syphoned treatments, and long settling times (21 days) were preferred to short settling times (9 days). Further, it should be noted that microplastic separation efficiency was higher in treatments using re-wetted cake compared with un-altered liquid biosolid. Future research will require method refinement to produce treatments that liberate microplastics from biosolids with greater efficiency. Similarly, the fate of microplastics after they have been separated from biosolids should be considered. Since the recycling of microplastics is not feasible, and landfilling/incineration is less than ideal.

Chapter 4: Conclusion

4.1 Summary and General Conclusions

This thesis had two objectives: (a) to quantify and characterize annual microplastic export in the biosolid and final effluent streams of a Canadian wastewater treatment plant, and (b) to investigate the feasibility of using a settling-based approach to liberate microplastics from biosolids prior to land application. The first objective was addressed in Chapter 2, while the second objective was addressed in Chapter 3. Chapter 2 was inspired by a need to understand the seasonal dynamics of microplastic emissions; the study carefully examined microplastic concentrations and characteristics in a wastewater treatment plant between October 2020 and September 2021. It estimated the annual microplastic exports from a wastewater treatment plant by considering monthly variations in microplastic concentrations, wastewater flows, and biosolid production. As identified by the literature, and supported by the results of Chapter 2, the majority of microplastics processed by wastewater treatment plants are retained in the biosolids. These biosolids may then be land applied as an agricultural amendment, which is a major source of microplastics to the terrestrial environment. Therein lies the justification for the second thesis objective. While wastewater treatment is considered relatively effective at preventing the export of microplastics to the aquatic environment, these microplastics are merely being diverted to the terrestrial environment. As such, Crossman et al. (2020) identified the need to reduce microplastic loads in the biosolids of wastewater treatments prior to their use in agriculture. Further, Crossman et al. (2020) observed lower microplastic concentrations in thickened biosolids that underwent a settling phase at a secondary biosolids refinement facility compared with unaltered biosolids. Ultimately, Dr. Crossman's work at the University of Windsor served as

inspiration for Chapter 3, and she played an integral role in the conception and development of settling strategies to reduce microplastic loads in biosolids.

In chapter 2, weekly samples of biosolids and final effluents were collected from a conventional style wastewater treatment plant in Southern Ontario that services a population of approximately 84,000 people. These samples were then composited to make monthly samples and were analysed for microplastics. Count, shape, size, and polymer measurements were taken, and used to produce data on microplastic volumes, densities, and masses. These microplastic attributes were then compared between the biosolids and final effluents to determine whether there was any indication of microplastic selectiveness during the wastewater treatment process. The results suggested that granular microplastic shapes with centralized masses (e.g., fragments, beads, and foams) were preferentially retained in the biosolids, whereas linear and flat microplastics with a more dispersed mass (e.g., fibres and films) were more likely to avoid being retained in the biosolids, thus ending up in the final effluents. Settling tendencies are believed to play a key role in determining the rate at which microplastics were retained in the biosolids. Average annual microplastic concentrations in the final effluent were 3.7 ± 2.1 mp/L (RSD = 58%) by count, and 2.9 ± 2.0 µg/L (RSD = 70%) by mass. While the average annual microplastic concentration in the biosolids was 169.1 ± 25.7 mp/g dw (RSD = 15%) by count, and 143.3 ± 31.5 µg/g dw (RSD = 22%) by mass. The microplastic concentrations obtained for biosolids tended to be similar to previous microplastic concentration values reported in the literature, whereas for the final effluent, the count concentrations were low compared to the literature, while the mass concentrations were quite similar to values reported in the literature. Overall, microplastic concentrations were more variable in the final effluent compared to the biosolids. Annual microplastic exports from the final effluent were estimated to be 50.8 ± 14.8 billion

particles (14.3% of total mp counts), or 39.8 ± 18.3 kg (13.4% of total mp mass). On the other hand, annual microplastic exports from the biosolids accounted for 303.3 ± 19.8 billion particles (85.7% of total mp counts), or 256.9 ± 34.9 kg (86.6% of total mp mass). This means that over the course of the year, the plant was found to export a total of 354.1 ± 24.7 billion microplastic particles, which equated to a mass of 296.7 ± 39.4 kg. The total microplastic exports (biosolid and final effluent) as well as the microplastic exports in the biosolids did not show evidence of seasonal trends, whereas the microplastic exports in the final effluents were lowest in the fall/winter, and highest in the spring/summer. This observation may be explained in part by considering seasonality in relation to plant flows. In periods of increased plant flows, the plant experienced decreased microplastic treatment capacity ($r = –0.62$). Meaning, that as the plant flow increased, the WWTPs ability to remove microplastics decreased. High wastewater flows were thought to reduce the retention time of wastewater, which reduced treatment efficiency, thus resulting in a higher proportion of microplastics in the final effluent. Further, both precipitation and river flow were positively correlated with plant flows, and negatively correlated with microplastic treatment efficiencies.

In chapter 3, biosolid samples were collected from two wastewater treatment plants in south-central Ontario and were subjected to settling treatments under the influence of four key variables (biosolid type: un-altered liquid biosolid vs. re-wetted cake, settling time: long vs. short, stirring: stirred vs. un-stirred, and harvest method: scoop vs. syphon) to assess the efficiency of the treatments to liberate microplastics from biosolids. Microplastic attributes were compared between the biosolids of each plant before treatment, as well as between the biosolids before and after conducting the treatments. Beyond differences in microplastic concentration, there were no significant differences in the morphologies of individual microplastics that came

from either wastewater treatment plant. This suggests that microplastic profiles in biosolids from wastewater treatment plants within the same geographic region are likely to share very similar characteristics. Similarly, there were no significant differences in the morphologies of individual microplastics before and after conducting the settling treatments. This suggests that microplastic morphology influences microplastic settling tendencies to a lesser degree in biosolids settling, as compared to in the conventional wastewater treatment process. This may be an effect of settling in a semi-solid compared with settling in a liquid. Additionally, in biosolids, there is a greater chance of microplastics binding to organic matter, therefore nullifying the effect of microplastic morphology on settling. Treatment effectiveness was assessed on the ability of treatments to maximise the liberation of microplastics, while minimizing the removal of biosolids. Under this guideline, the ratio of plastic to biosolids removal was essential to determine treatment effectiveness. Across all treatments, between 1.95% and 24.50% (Average = 9.01%, RSD = 64.5%) of total microplastic counts were removed, between 1.38% and 20.68% (Average = 6.91%, RSD = 72.1%) of total plastic mass was removed, and between 0.39% and 13.74% (Average = 3.64%, RSD = 93.6%) of biosolid mass was removed. As a result, the ratio of microplastic count to biosolid removal ranged between 1.0 and 9.7 (Average = 4.1, RSD = 75.6%), while the microplastic mass to biosolid removal ranged between 0.8 and 5.7 (Average = 2.7, RSD = 60.0%). By comparing the microplastic to biosolid removal ratios, treatments were more effective in terms of microplastic count than they were in terms of microplastic mass. However, the treatment effectiveness was less variable in terms of microplastic mass. Ultimately, stirring was deemed superior to not stirring, scooping was deemed superior to syphoning, and long settling times were deemed superior to short settling times. Under the preferred treatment, Plant 1 had the capacity to divert 10.8% of microplastic mass, and 12.3% of counts, while

removing 4.0% of the dry solids mass (Table 5). Whereas Plant 2 had the capacity to divert 8.9% of microplastic mass, and 14.5% of counts, while only removing 1.6% of the dry solids mass (Table 5). Overall, the results of Chapter 3 demonstrate settling treatments to be a promising method of reducing microplastic loads in biosolids, however further method refinement is needed to produce more insightful data.

4.2 Study Limitations and Recommendations

Though the information presented in Chapter 2 is novel in it's year long assessment of trends in microplastic treatment, it is still limited in the sense that all temporal/seasonal conclusions were based only on one year's worth of data, at one wastewater treatment plant. Additionally, with the apparent influence of plant flows on microplastic treatment efficiencies, the study would benefit from a more in-depth evaluation of plant flows. Variables such as hydraulic retention time, and an evaluation of storm overflows would certainly strengthen our understanding of the interactions between plant flows and microplastic behavior, however, this data was not available at the time of the study. Overall, there is value in continued long-term monitoring of microplastic concentrations at wastewater treatment plants both regionally and globally. Observing a continued cyclic nature would contribute far more confidence in any conclusions made about temporal/seasonal trends in microplastic pollution. Additionally, it would allow researchers to delve deeper into any interactions between microplastics, and plant parameters (e.g., water quality, or plant flows/retention times), or other environmental parameters (e.g., climate). Whether the research is completed by wastewater treatment plant personnel, universities, a governing body, or a private institution, there should be resources allocated to the long-term monitoring of microplastics at wastewater treatment plants.

The greatest limitation of Chapter 3 is in its experimental design, and the influence of uncontrolled co-variables. By evaluating the effect of four variables at once, the experimental and analytical intensity were greatly reduced, however, the insight gained from the results was reduced as well. When attempting to determine the effect of one variable (e.g., harvest method), the influence of uncontrolled co-variables (e.g., settling time, stirring, and biosolid type) increased result variability, thus making it difficult to determine the effect of an individual variable (e.g., harvest method: scoop vs syphon). A major factor which influenced the experimental design was the scale at which the study was conducted. By conducting the treatments in 20 L settling basins, the resources required to conduct each experiment were overwhelming. To remedy both issues at once, future studies should focus less on the scale of the experiment, perhaps by first conducting bench top jar tests, and focus more on the isolation of control variables (individual experiments for control variables, which employ a greater degree of replication). By doing so, experiments could be conducted both with greater ease, while also producing greater insights on the effect of treatment variables on treatment efficiencies.

4.3 Significance of the Study

These studies further microplastic research as a whole by communicating microplastic burden in terms of both microplastic counts and masses. Far too few studies publish data using both count and mass values, despite these values being fundamentally related to each other. When either value is used in isolation, the data only provides a partial understanding of microplastic burden. Using microplastic counts to communicate the quantity of microplastics in a given medium is the foundation of microplastics analysis, however, it is limited by the fact that not all microplastics are equal. For example, a spherical microplastic with a diameter of 1 mm would have a volume that is one billion times greater than a sphere with a volume of 1 μm, yet

both particles would be considered one microplastic. Additionally, microplastics may fragment, which creates more microplastics; a change which would inflate microplastic counts despite there being no effect on microplastic mass. As such, microplastic counts are not suitable as a standalone method of communicating microplastic quantities. On the other hand, when microplastic mass data is used alone, it gives little to no indication of the number or size of the microplastics present in a given medium. These more nuanced microplastic characteristics have important implications for human and environmental health; and should therefore be communicated. By thoroughly communicating microplastic counts, sizes, polymer compositions, and masses, this study provides a comprehensive understanding of microplastic burden.

Chapter 2 provided valuable insight to annual variations in microplastic exports and treatment efficiencies at a wastewater treatment plant. It is the first study conducted to evaluate microplastic burden in the biosolids and final effluent of a wastewater treatment plant using monthly composite samples for a whole year. By doing so, this study provides researchers, policy makers, and industry professionals with a deeper understanding of the temporal variation of microplastic burden in a conventional wastewater treatment setting. This information is valuable because it provides insight as to how microplastics behave in wastewater treatment, and the variables which influence their behavior. This information has the potential to enable industry professionals to make process adjustments to optimize microplastic treatment within wastewater treatment plants, or to give policy makers the tools they need to build effective and realistic legislation surrounding microplastics in wastewater treatment plants. Continued research has the potential to reveal predictor variables which may be related to or serve as an indication of microplastic burden or treatment efficiencies in wastewater treatment plants. Such information would be incredibly useful for industry professionals.

Chapter 3 is the first study of its kind; it lays the foundation for the development of a method to reduce microplastic loads in biosolids. As an initial investigation into settling based biosolids treatments, the methods require further refinement, however, the results suggest that settling based treatments are a potentially viable method to reduce microplastic loads in biosolids. Results of this, and future research on the topic should be of particular interest to researchers, policy makers, or industry professionals who seek methods of reducing environmental inputs of microplastics, particularly through the lens of wastewater treatment and agriculture. If legislation around microplastic emissions from wastewater treatment plants were ever to be adopted, having a viable, low-energy method of reducing microplastic loads in biosolids will be crucial, and the results of this study indicate that settling based treatments may be a viable solution to this problem.

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Appendix A

Appendix A1 – *Sample List*

Table A1: A list of all samples collected during the October 2020 to September 2021 study period.

Month/Year	Biosolid	Final Effluent	Month/Year continued	Biosolid continued	Final Effluent continued
Oct-20	2020-10-01	2020-10-01	Apr-21	2021-04-06	2021-04-06
	2020-10-06	2020-10-06		2021-04-15	2021-04-14
	2020-10-14	2020-10-14		2021-04-20	2021-04-20
	2020-10-20	2020-10-20		2021-04-27	2021-04-27
	2020-10-27	2020-10-27	May-21	2021-05-03	2021-05-04
Nov-20	2020-11-09	2020-11-09		2021-05-11	2021-05-11
	2020-11-13	2020-11-13		2021-05-17	2021-05-18
	2020-11-18	2020-11-18		2021-05-26	2021-05-26
	2020-11-25	2020-11-25	Jun-21	2021-06-01	2021-06-01
Dec-20	2020-12-14	2020-12-14		2021-06-08	n/a
	n/a	2020-12-18		2021-06-16	2021-06-16
	2020-12-21	2020-12-21		2021-06-22	2021-06-22
	2020-12-23	2020-12-23		2021-06-29	2021-06-29
	2020-12-29	2020-12-29	Jul-21	2021-07-06	2021-07-06
Jan-21	2021-01-06	2021-01-06		2021-07-13	2021-07-13
	2021-01-13	2021-01-13		2021-07-20	2021-07-20
	2021-01-18	2021-01-18		2021-07-27	2021-07-28
	2021-01-25	2021-01-26	Aug-21	2021-08-08	2021-08-09
Feb-21	2021-02-03	2021-02-03		2021-08-10	2021-08-11
	2021-02-09	2021-02-09		2021-08-17	2021-08-18
	2021-02-16	2021-02-16		2021-08-24	2021-08-25
	2021-02-22	2021-02-22	Sep-21	2021-08-31	2021-09-01
Mar-21	n/a	2021-03-02		2021-09-07	2021-09-08
	2021-03-09	2021-03-09		2021-09-14	2021-09-15
	2021-03-16	2021-03-16		2021-09-21	2021-09-22
	2021-03-24	2021-03-24			
	2021-03-29	2021-03-30	Total Count	50	51

Appendix A2 – *Density Separator*

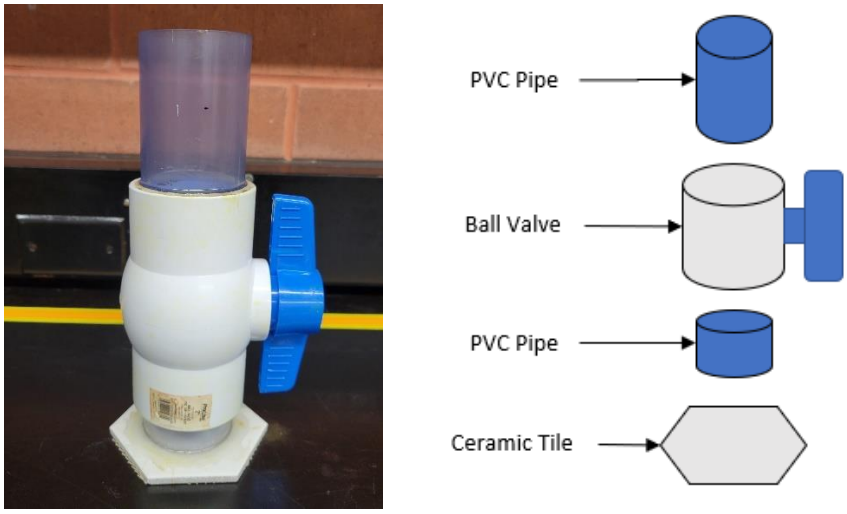

Figure A2: An image of a density separation unit, and the component pieces used in the study. Adapted from Coppock et al. 2017. Designed by Lavoy et al. 2021.

Appendix A3 – *Volume Calculations*

Table A3: The equations and variables used to estimate microplastic volumes.

Microplastic Shape	**Volume Formula**	**Variable Definition**
Fibre – Cylinder	$V = \pi r^2 h$	r = Minor Axis / 2 h = Major Axis
Film – Rectangular Prism	V = l * w * h	l = Major Axis w = Minor Axis h = Minor Axis (fibres)
Fragment – Ellipsoid	$V = 4/3\ \pi$ abc	a = Major Axis / 2 b = Minor Axis / 2 c = Minor Axis / 2
Bead – Sphere	$V=4/3\ \pi r^3$	r = Average of Major and Minor Axis / 2
Foam – Ellipsoid	$V = 4/3\ \pi$ abc	a = Major Axis / 2 b = Minor Axis / 2 c = Minor Axis / 2

Appendix A4 – *Mass Calculations*
The equations and variables used to calculate microplastic mass.

$$Mass\ of\ Plastic = Average\ Plastic\ Density \times Average\ Particle\ Volume \times Particle\ Count \times Void\ Fraction^{*}$$

Where:
Mass of Plastic (g)
Average Plastic Density (g/cm³)
Average Particle Volume (cm³)
Particle Count (n)
Void Fraction Only applied to foams (3.5%)*

Appendix A5 – *Flux Calculations*
The equations and variables used to calculate microplastic flux.

$$Mass\ of Microplastics = Microplastic\ Concentration \times Quantity\ of\ Media\ Processed$$

Where:
Mass of Microplastics (µg/month)
Microplastic Concentration (FE: µg/m³, BS: µg/kg)
Quantity of Media Processed (FE: m³/month, BS: kg/month)

$$Number\ of\ Microplastics = Microplastic\ Concentration \times Quantity\ of\ Media\ Processed$$

Where:
Number of Microplastics (mp/month)
Microplastic Concentration (FE: mp/m³, BS: mp/kg)
Quantity of Media Processed (FE: m³/month, BS: kg/month)

Appendix A6 – *Treatment Efficiency Calculations*
The equations and variables used to calculate microplastic treatment efficiencies.

$$Treatment\ Efficiency = \frac{Microplastics\ in\ Biosolids}{Total\ Microplastics} \times 100\%$$

Where:
Treatment Efficiency (%)
Microplastics in Biosolids (µg/month or mp/month)
Total Microplastics (µg/month or mp/month)
**Note: Total Microplastics = Microplastics in Biosolids + Microplastics in Final Effluent*

Appendix A7 – *Microplastic Sizes*

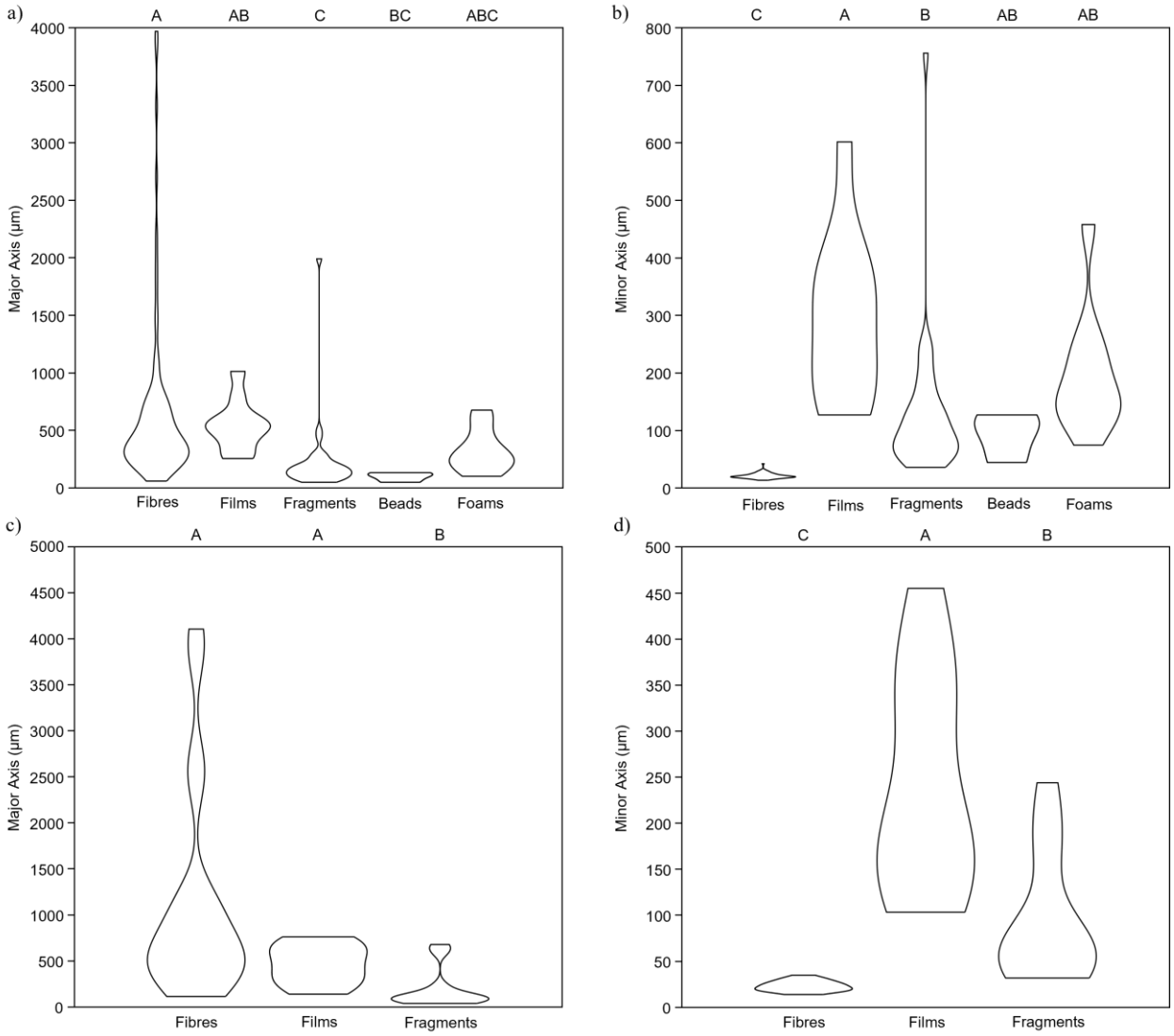

Figure A7: The distribution of microplastic sizes observed in the study (all lengths are un µm). Figures *a* and *b* show the lengths of major and minor axis observed in the biosolid, whereas *c* and *d* show the major and minor axis observed in the final effluent. Microplastic shapes were ranked largest to smallest (A–C) using a combination of the Shapiro-Wilk test for normality, the Kruskal-Wallis one-way analysis of variance, and the Mann-Whitney pairwise post-hoc test. All tests were conducted at a significance level where $\alpha = 0.05$.

Appendix A8 – *Microplastic Volumes*

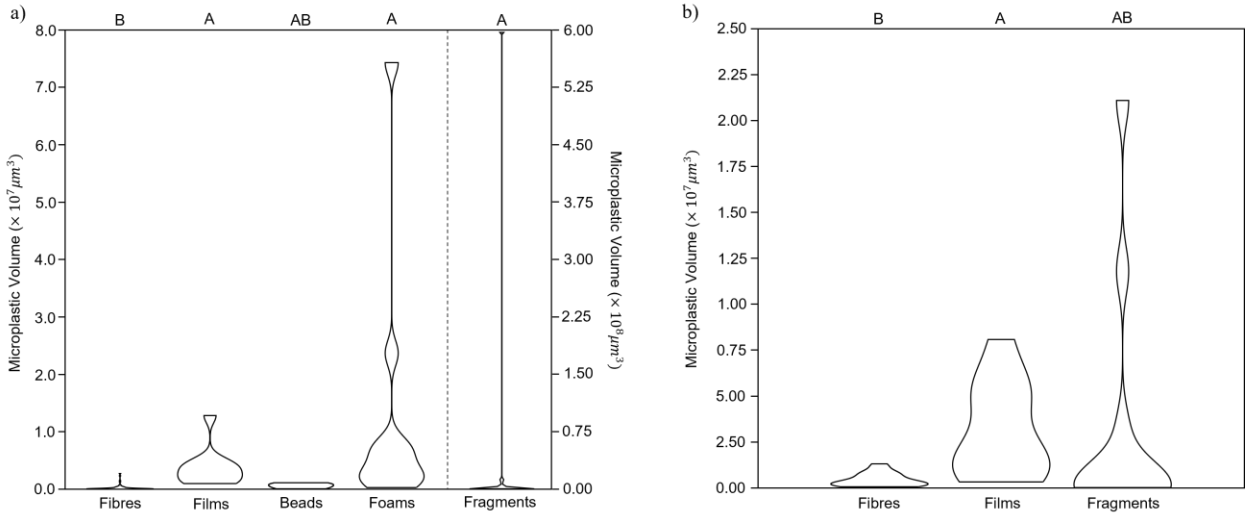

Figure A8: The distribution of microplastic volumes (presented in $\mu m^3 \times 10^7$ except for fragments in biosolids, which are presented in $\mu m^3 \times 10^8$) observed in (a) the biosolid, and (b) the final effluent. Microplastic shapes were ranked largest to smallest (A–B) using a combination of the Shapiro-Wilk test for normality, the Kruskal-Wallis one-way analysis of variance, and the Mann-Whitney pairwise post-hoc test. All tests were conducted at a significance level where $\alpha = 0.05$.

Appendix A9 – *Polymer Acronyms and Densities*

Table A9: All polymer types detected in this study, as well as their popular acronyms, and commonly accepted density values. Values obtained through (Driedger et al. 2015, Environment and Climate Change Canada, 2020, Liu et al. 2022).

Polymer	**Acronym**	**Density (g/cm^3)**
Polyester/Polyethylene Terephthalate	PET	1.38
Polypropylene	PP	0.905
Nylon/Polyamide	PA	1.14
Low Density Polyethylene	LDPE	0.92
High Density Polyethylene	HDPE	0.95
Polyvinyl Alcohol	PVA	1.19
Polyurethane	PU	1.14
Polyvinyl Chloride	PVC	1.38
Polydimethylsiloxane	PDMS	0.97
Polyacrylamide	PAM	1.20
Acrylonitrile Butadiene Styrene	ABS	1.025
Polytetrafluoroethylene	PTFE	2.20
Silicone	Sil.	1.70
Polystyrene	PS	1.005
Nitrile Rubber	NBR	1.00
Polymethyl Styrene	PMS	0.91
Polyoxymethylene	POM	1.41
Polyphenylsulfone	PPSF	1.29

Appendix A10 – *Microplastic Polymers*

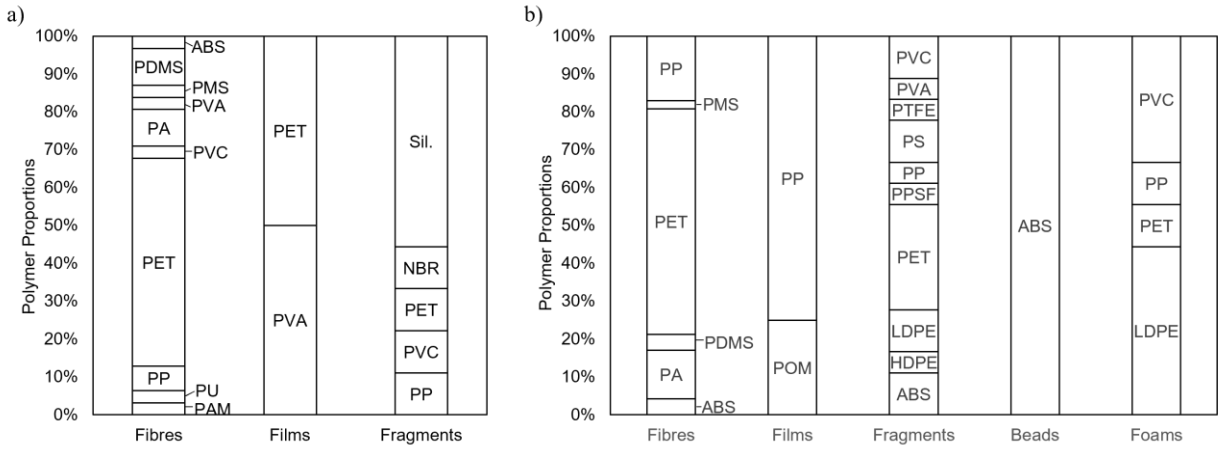

Figure A10: The relative proportion of plastic polymers identified in the (a) final effluent, and (b) biosolid. Polymer abundance is represented as a percentage for fibres, films, fragments, beads, and foams. Polymer acronyms are described in Appendix 9 (Table A-9).

Appendix A11 – *Microplastic Masses*

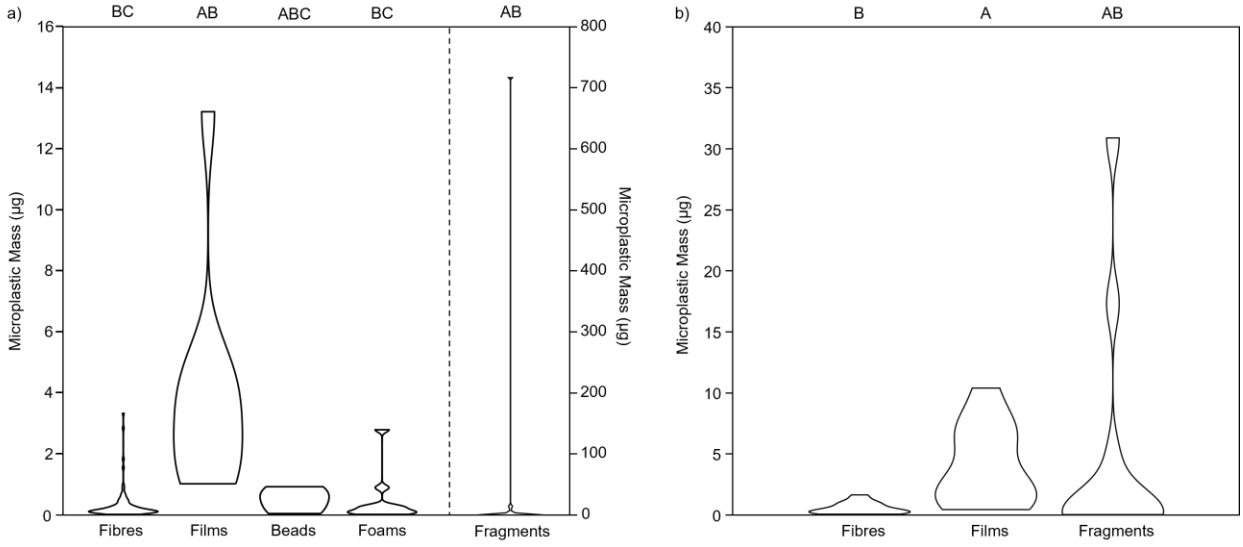

Figure A11: The distribution of microplastic masses (presented in µg) observed in (a) the biosolid, and (b) the final effluent. The difference in scale for fragments in (a) should be noted. Microplastic shapes were ranked largest to smallest (A–C) using a combination of the Shapiro-Wilk test for normality, the Kruskal-Wallis one-way analysis of variance, and the Mann-Whitney pairwise post-hoc test with Bonferroni Correction. All tests were conducted at a significance level where $\alpha = 0.05$.

Appendix A12 – *Annual Biosolid Data*

Table A12: Annual data for the biosolids from October 2020 to September 2021. The data include: microplastic concentrations, moisture contents, production rates (ww is wet weight), and total microplastic exports.

Month	Biosolid Count Concentration (mp/g dw)	Biosolid Mass Concentration (µg/g dw)	Moisture Content (%)	Biosolid Produced per Month (Tonnes [ww])	Number of Microplastics in Biosolid per Month	Mass of Microplastics (kg) in Biosolid per Month
October 2020.	206.3	173.0	74.62	527	2.76 E+10	23.1
November 2020.	168.2	144.7	74.85	506	2.14 E+10	18.4
December 2020.	183.8	140.4	75.76	620	2.76 E+10	21.1
January 2021.	186.9	150.2	75.61	587	2.68 E+10	21.5
February 2021.	212.1	217.6	75.47	570	2.97 E+10	30.4
March 2021.	147.7	121.3	74.63	602	2.26 E+10	18.5
April 2021.	172.2	140.4	75.15	610	2.61 E+10	21.3
May 2021.	148.2	123.0	74.27	546	2.08 E+10	17.3
June 2021.	171.6	158.4	73.34	606	2.77 E+10	25.6
July 2021.	161.4	130.7	72.24	581	2.60 E+10	21.1
August 2021.	147.0	132.5	72.47	675	2.73 E+10	24.6
September 2021.	123.6	87.8	72.67	583	1.97 E+10	14.0
Year Total	–	–	–	7013	3.03 E+11	256.9

Appendix A13 – *Annual Final Effluent Data*

Table A13: Annual data for the final effluent from October 2020 to September 2021. The data include: microplastic concentrations, plant flow, and total microplastic exports.

Month	Final Effluent Count Concentration (mp/L)	Final Effluent Mass Concentration (µg/L)	Total Monthly Flow (m^3)	Number of Microplastics in Final Effluent per Month	Mass of Microplastics (kg) in Final Effluent per Month
October 2020.	3.5	2.1	1,004,994	3.55 E+9	2.1
November 2020.	0.9	1.1	968,169	8.62 E+9	1.0
December 2020.	2.3	1.5	1,185,922	2.68 E+9	1.7
January 2021.	1.6	1.5	1,081,906	1.73 E+9	1.6
February 2021.	3.0	1.9	884,905	2.63 E+9	1.7
March 2021.	3.8	2.6	1,286,405	4.86 E+9	3.3
April 2021.	7.8	7.2	1,251,250	9.72 E+9	9.1
May 2021.	5.6	5.2	1,201,957	6.71 E+9	6.2
June 2021.	3.4	2.5	988,016	3.34 E+9	2.4
July 2021.	7.3	5.9	1,147,034	8.36 E+9	6.7
August 2021.	3.5	2.2	1,043,379	3.68 E+9	2.3
September 2021.	2.1	1.3	1,262,924	2.68 E+9	1.6
Year Total	–	–	13,306,861	5.08 E+10	39.8

Appendix A14 – *Wastewater Influent Burden*

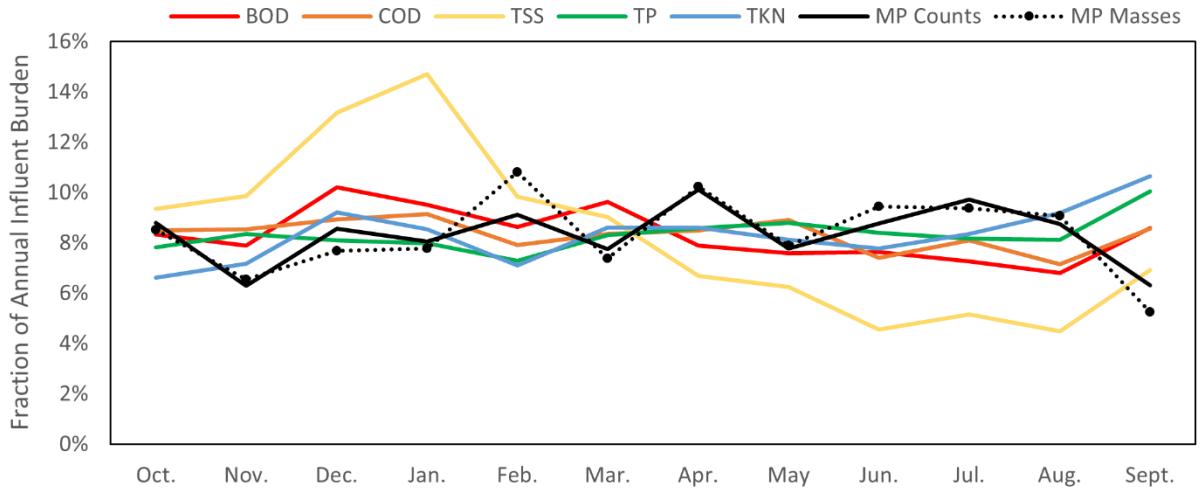

Figure A14: The degree that each month contributed to the total annual burden of each influent parameter. Values were obtained by using concentrations and flows to find the overall quantity of each parameter by month and year. Then the relative fraction that each month contributed to the annual total was calculated.

Appendix A15 – *Wastewater Effluent Burden*

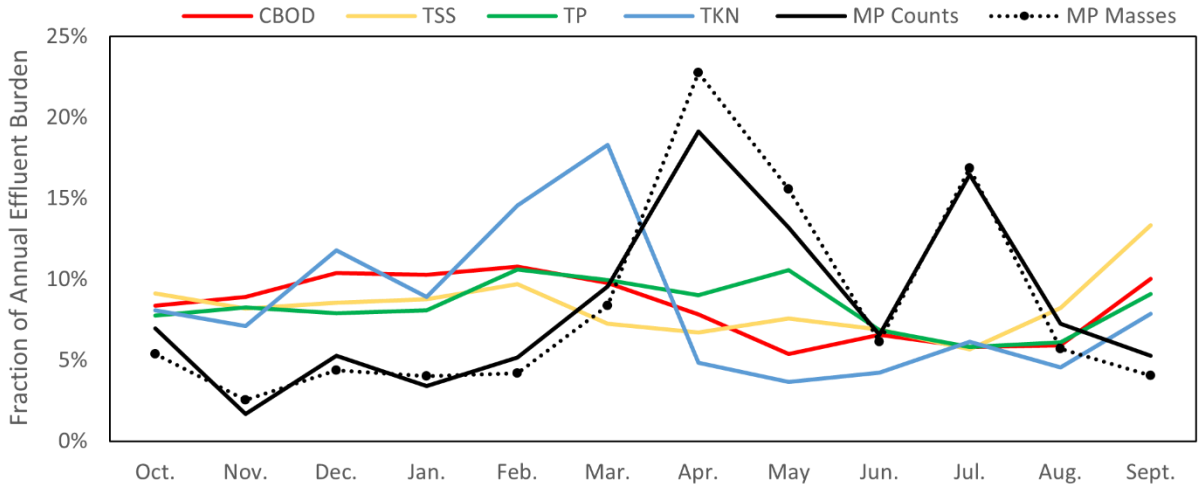

Figure A15: The degree that each month contributed to the total annual discharge of each effluent parameter. Values were obtained by using concentrations and flows to find the overall quantity of each parameter by month and year. Then the relative fraction that each month contributed to the annual total was calculated.

Appendix B

Appendix B1 – *Moisture Content*

The settling tendencies of four different moisture contents were observed: 90, 92, 96, and 99%. To achieve these levels, the liquid biosolids (~98%) from Plant 1 was diluted for the 99% trial, and de-watered for the 96, 92, and 90% trials. Biosolids de-watering was achieved by means of evaporation. The apparatus used (Figure B1a) was constructed using 150 L pots, 100V/2100W concrete mixers, and a 120V/1000W immersion heating coil. Temperature was monitored to ensure that a limit of 60ºC was not exceeded. The biosolids from Plant 2 were received as cake, and therefore required moisture additions. This was achieved by dilution and thorough mixing using the concrete mixers and cooking pots previously described. After adjusting the moisture content, 20L of each sample was transferred to a settling tank and left for 40 days to observe the natural settling patterns of each biosolid type at each moisture content. At the end of the experiment, the tanks were sampled by scooping distinct layers into a secondary vessel. Here the volume and mass of each layer was measured before mixing and retaining a 1 L sample for analysis.

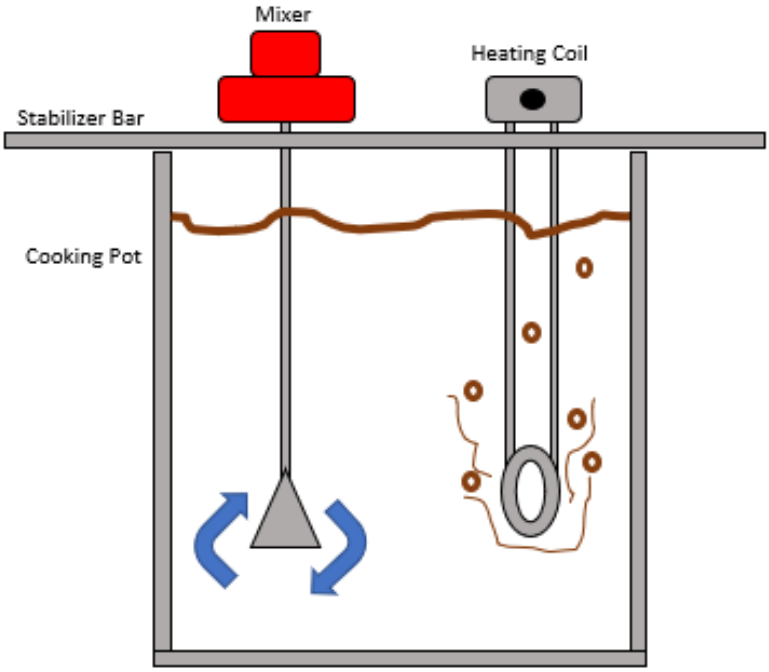

Figure B1a: A diagram of the evaporation system used to de-water biosolids.

Looking at the results of this test (Figure B1b), trials with greater moisture contents settled better. In the 99% and 96% trials, there was clear separation between water and biosolid, whereas no clear separation was found in the 92% and 90% trials. Despite being a more qualitative observation, the best results in Plant 1 were achieved at moisture contents with the least modification. The de-watering process was problematic and may have adversely affected biosolids properties. On the other hand, re-hydrating the biosolids was comparatively simple and

did not produce adverse effects. As such, it was decided that the biosolids from Plant 2 (~ 75%) should be re-hydrated to match that of Plant 1 (~ 98%). Additionally, it was found that through the settling process microplastics largely remained bound to biosolids as opposed to floating freely in the water columns (Figure A-2)

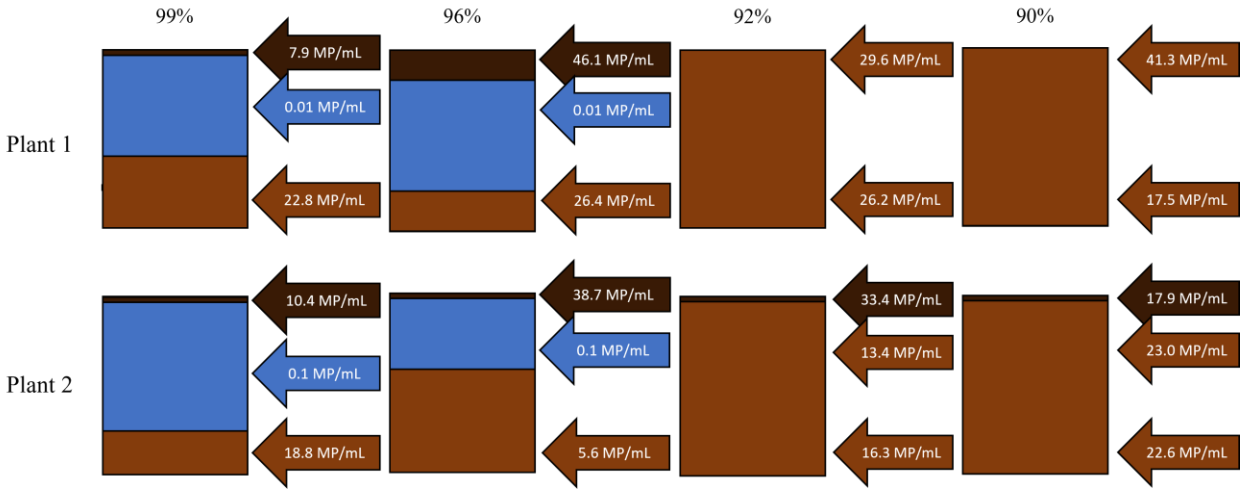

Figure B1b: The distribution of solids and microplastics after a 40-day settling period. Dark brown layers represent a dried "skin" layer, blue layers represent water, and light brown layers represent a biosolids slurry. Microplastic concentrations represented as mp/mL.

Appendix B2 – *Experimental Block Design*

Figure B2: The design used to evaluate what settling conditions enhance microplastic liberation. Two sets were completed, for each of Plant 1 (n = 8), and 2 (n = 8), making a total of 16 settling tanks.

Appendix B3 – *Density Separator*

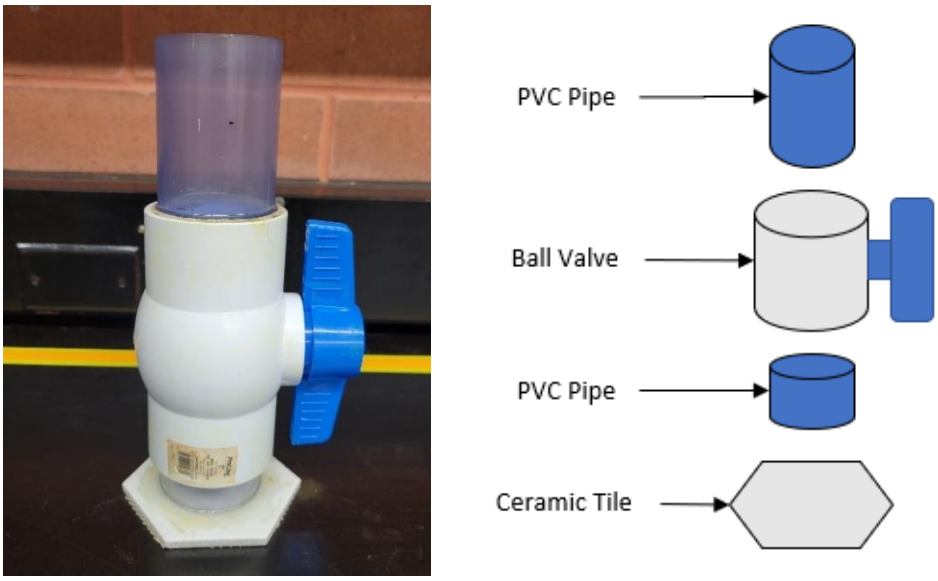

Figure B3: An image, and the component pieces of the density separation unit used in the study. Adapted from Coppock et al. 2017. Designed by Lavoy et al. 2021.

Appendix B4 – *Volume Calculation*

Table B4: Definitions of the equations and variables used to estimate microplastic volumes.

Microplastic Shape	*Volume Formula*	*Variable Definition*
Fibre – Cylinder	$V = \pi r^2 h$	r = Minor Axis / 2 h = Major Axis
Film – Rectangular Prism	V = l * w * h	l = Major Axis w = Minor Axis h = Minor Axis (fibres)
Fragment – Ellipsoid	$V = 4/3\ \pi$ abc	a = Major Axis / 2 b = Minor Axis / 2 c = Minor Axis / 2
Bead – Sphere	$V=4/3\ \pi r^3$	r = Average of Major and Minor Axis / 2
Foam – Ellipsoid	$V = 4/3\ \pi$ abc	a = Major Axis / 2 b = Minor Axis / 2 c = Minor Axis / 2

Appendix B5 – *Mass Calculation*

The equations and variables used to calculate microplastic mass.

$$Mass\ of\ Plastic = Average\ Plastic\ Density \times Average\ Particle\ Volume \times Particle\ Count \times Void\ Fraction^{*}$$

Where:
Mass of Plastic (g)
Average Plastic Density (g/cm^3)
Average Particle Volume (cm^3)
Particle Count (n)
Void Fraction* Only applied to foams (3.5%)

Appendix B6 – *Treatment Calculation*

The equations and variables used to calculate treatment efficiencies.

$$Percent\ MP\ Removal = MPs\ Removed / Total\ MPs$$

Where:

$$MPs\ Removed = Treatment\ MP\ Concentration * 1L$$
$$Total\ MPs = Initial\ MP\ Concentration * 20L$$

Appendix B7 – *Polymer Density*

Table B7: All polymer types detected in this study, as well as their popular acronyms, and commonly accepted density values. Values obtained through (Driedger et al. 2015, Environment and Climate Change Canada, 2020, Liu et al. 2022).

Polymer	**Acronym**	**Density (g/cm^3)**
Polyester/Polyethylene Terephthalate	PET	1.38
Polypropylene	PP	0.905
Nylon/Polyamide	PA	1.14
Low Density Polyethylene	LDPE	0.92
High Density Polyethylene	HDPE	0.95
Polyvinyl Alcohol	PVA	1.19
Polyvinyl Butyral	PVB	1.11
Polyvinyl Chloride	PVC	1.38
Polycarbonate	PC	1.20
Polyacrylonitrile	PAN	0.81
Acrylonitrile Butadiene Styrene	ABS	1.025
Polytetrefluoroethylene	PTFE	2.20
Polybutylene Terephthalate	PBT	1.35
Polystyrene	PS	1.005
Polyphenylene Sulphide	PPS	1.29
Polymethylstyrene	PMS	0.91
Ethylene Vinyl Acetate	EVA	0.948
Polymethylmethacrylate	PMMA	1.18

Appendix B8 – *Microplastic Sizes Pre/Post Treatment*

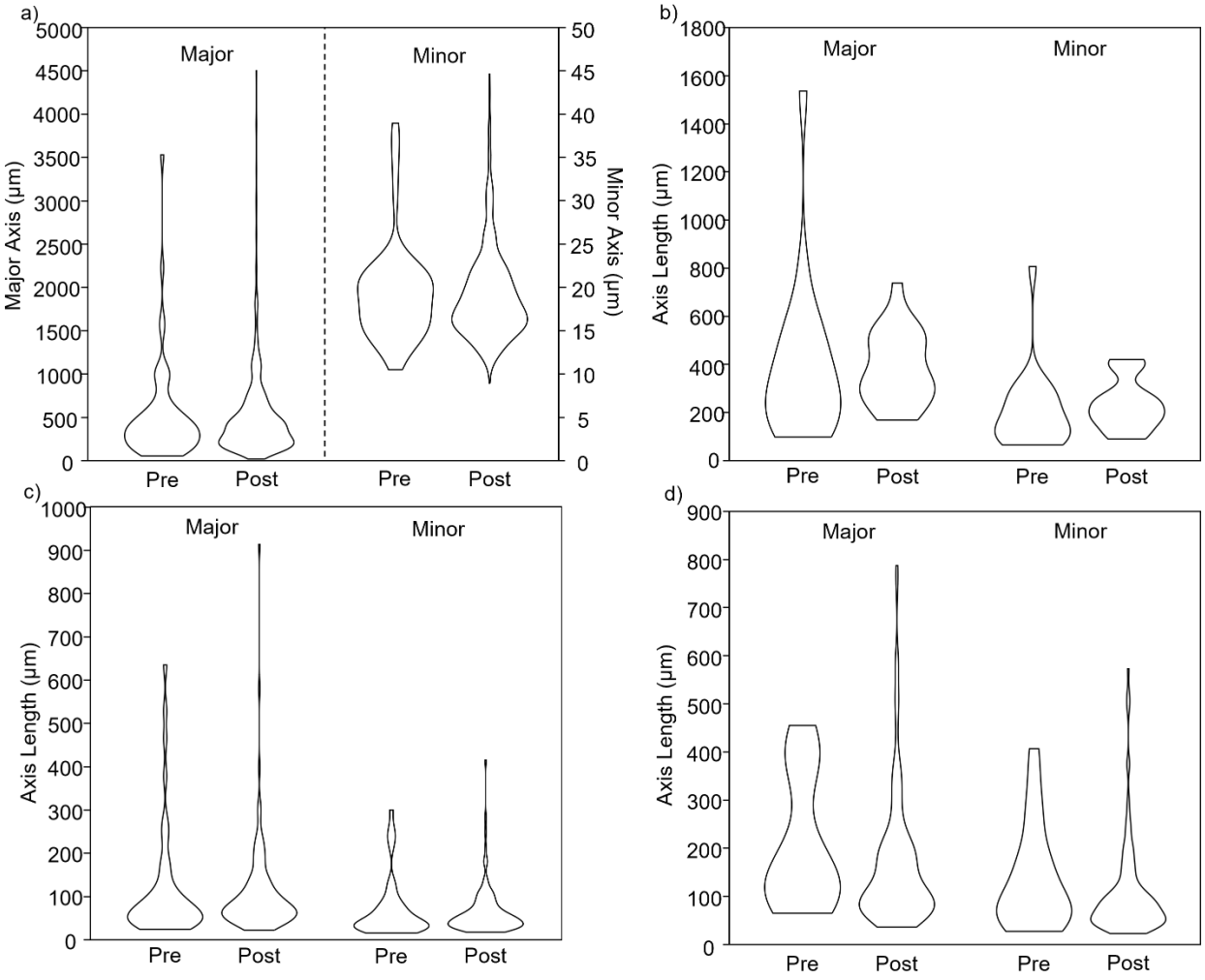

Figure B8: The size of the major and minor axis of each shape category, before and after treatment (data pooled across the two study plants). Fibres are presented in (a), films in (b), fragments in (c), and foams in (d). Note the secondary axis included in (a). Beads were not included as the pre- and post-treatment measurements were pooled due to their low sample size.

Appendix B9 – *Microplastic Volumes Pre/Post Treatment*

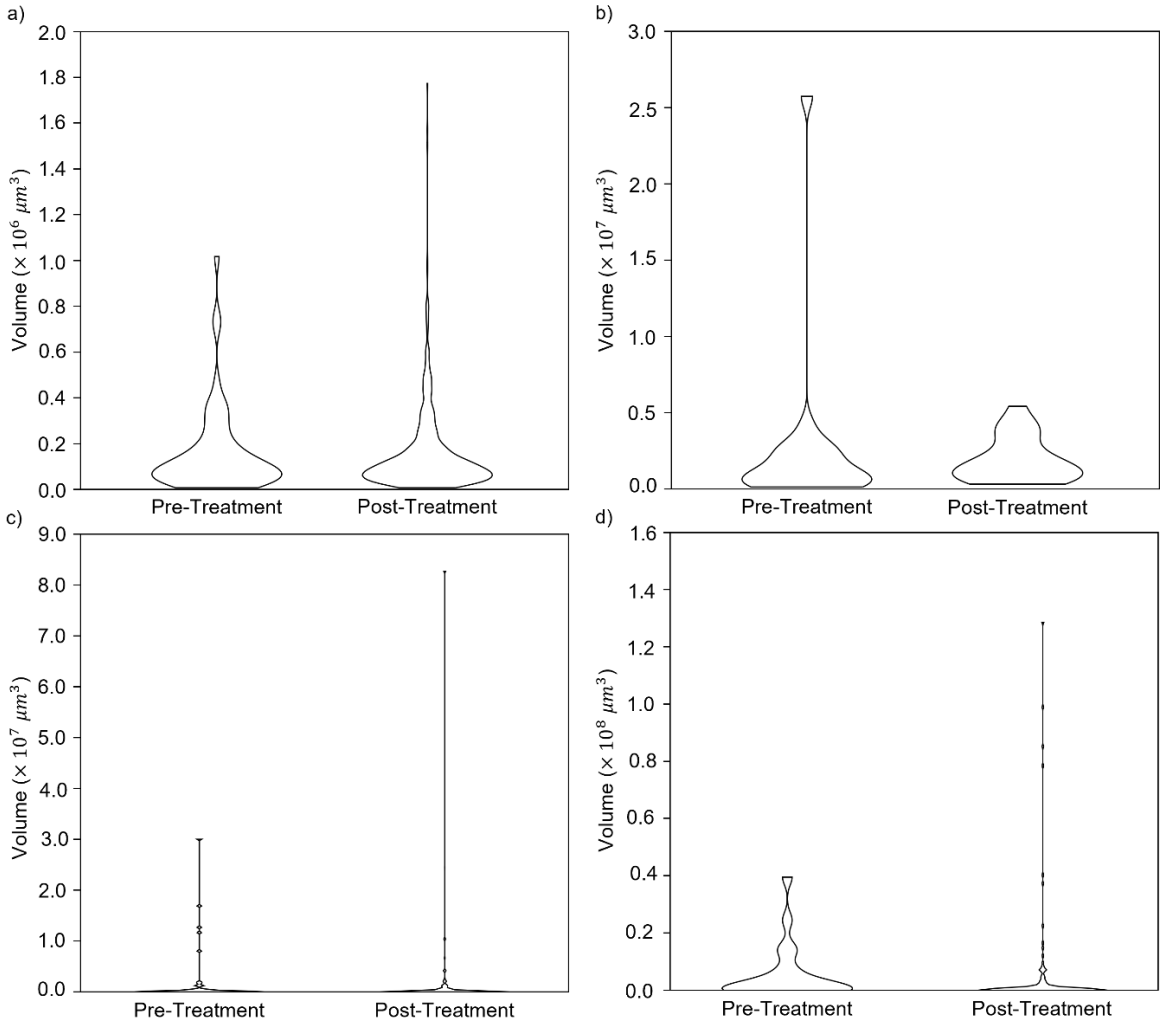

Figure B9: The volume per microplastic pre- and post-treatment (data pooled across the two study plants). For fibres (a), films (b), fragments (c), and foams (d). Note that beads are not included due to their pooled dataset before and after treatment.

Appendix B10 – *Polymer Proportions Pre/Post Treatment*

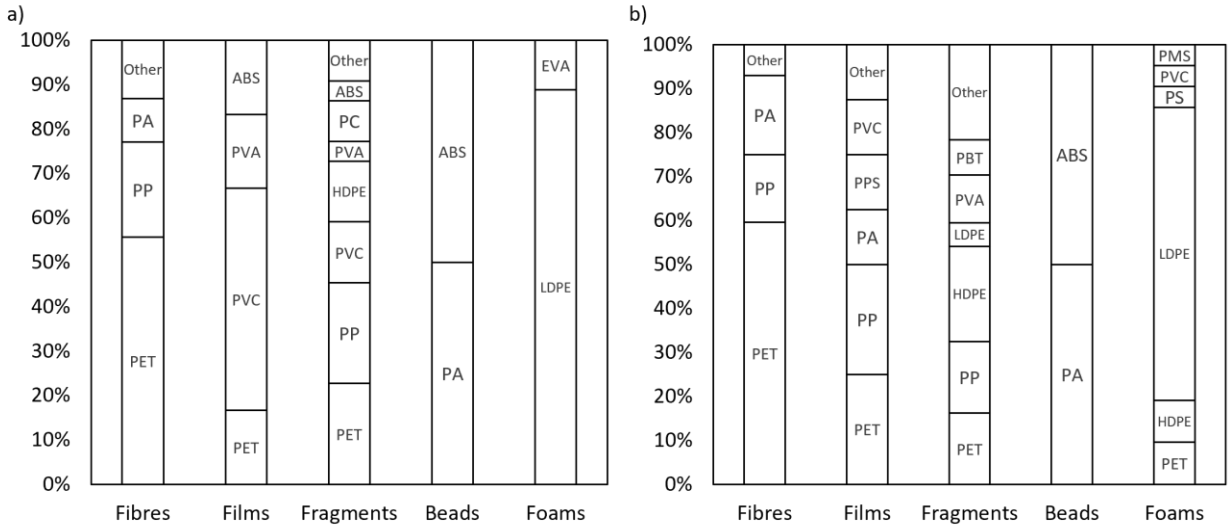

Figure B10: The proportion of plastic polymers detected in both plants (a) pre and (b) post-treatment. The data are ordered by microplastic shape category.

Appendix B11 – *Microplastic Shape Density Distributions*

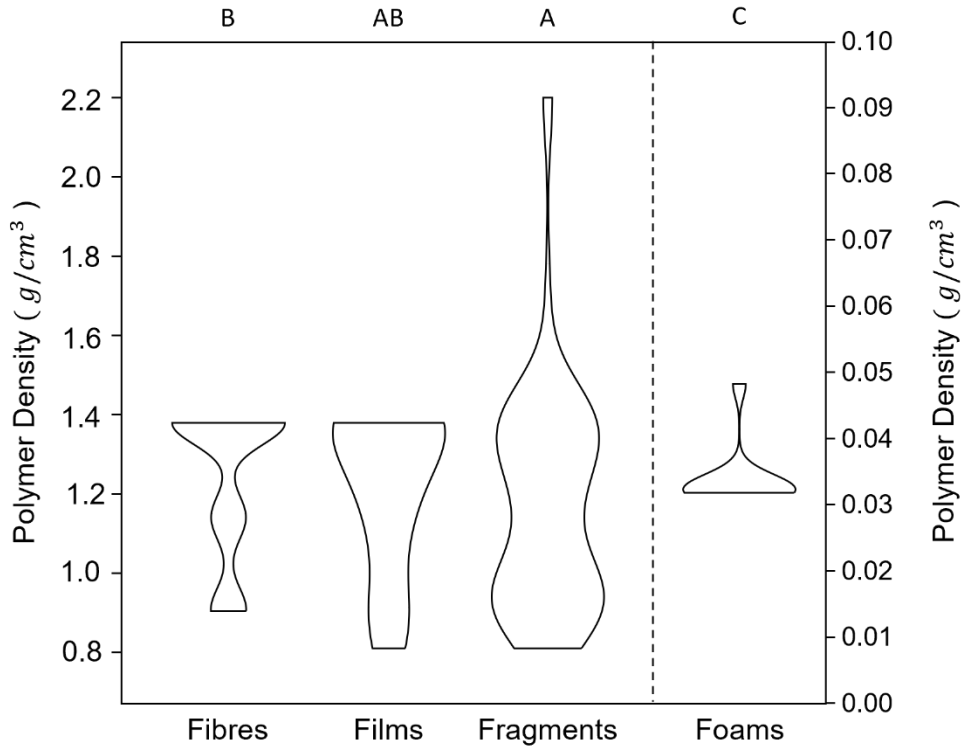

Figure B11: The distribution of polymer densities observed in each shape category.

Appendix B12 – *Post-Treatment Concentration Changes*

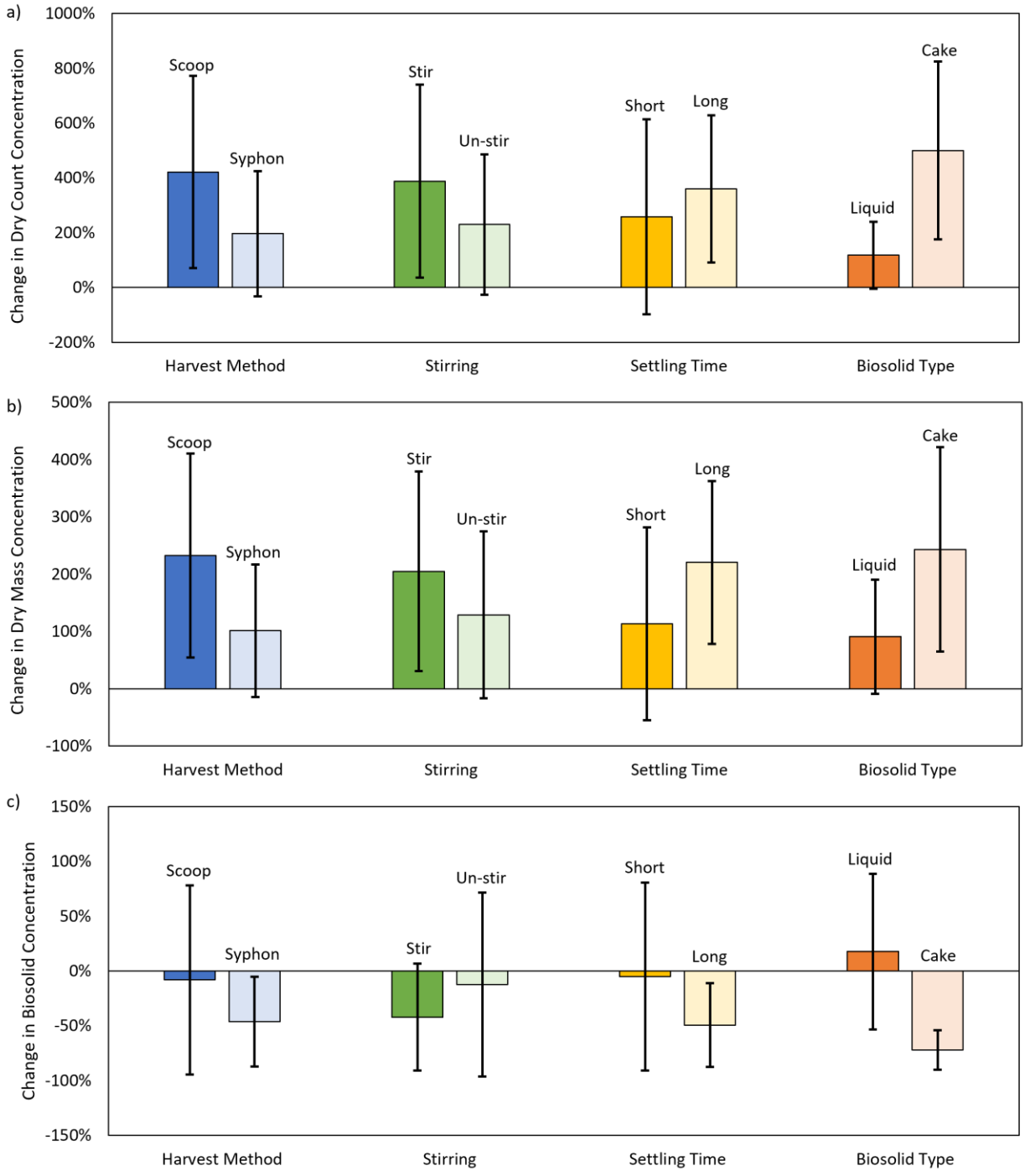

Figure B12: Each treatment variable's ability to alter the (a) microplastic count, (b) microplastic mass, and (c) biosolids mass. The variables examined include the harvest method: scoop (surface skim) vs. syphon, the stirring method: stir vs. un-stir, the settling time: short vs. long (9 vs. 21 days), and the biosolids type: liquid vs. cake. All error bars indicate ± one standard deviation.