Microplastics in Muskoka-Haliburton Headwater Lakes, Ontario, Canada

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Research Article

Keywords: Plastic pollution, microfibres, temporal variability, sediment, depth samples

Posted Date: January 27th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1181662/v1>

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Title

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Abstract

Microplastics (mp) are a growing environmental concern due to their ubiquity in terrestrial and aquatic environments. Nonetheless, there is limited knowledge on their abundance in lakes in rural areas. In this study, we surveyed 14 headwater lakes in Muskoka-Haliburton, Ontario, to assess the spatial and temporal variability of microplastics. The average microplastic concentration across the study lakes was 1.78 mp/L during May–June 2019, with limited spatial variability (coefficient of variation = 22%). Further, microplastic abundance was weakly correlated with lake area (r_s : 0.469), the number of shoreline residences (r_s : 0.399), and watershed area (r_s : 0.350), suggesting that diffusive inputs, such as atmospheric deposition, were the dominant source of microplastics to the study lakes. In contrast, microplastics showed a distinct temporal (seasonal) variability, as the average concentration in August 2019 (0.91 mp/L) was significantly lower ($p < 0.05$) compared with May and June 2019. While microplastic abundance was generally higher in the metalimnion (0.70 mp/L) and epilimnion (0.67 mp/L), there was no significant difference by stratified layer. The annual percent removal of microplastics in lake sediment was estimated to be 14%, suggesting that for most of the study lakes, sediment burial was not a dominant sink for microplastics. Effective management of microplastic pollution requires an understanding of the interlinkages between microplastics in the atmosphere, lake water, and sediment. In rural areas, microplastic abundance appears to be dominated by atmospheric inputs, suggesting limited need for spatial monitoring. Temporal monitoring however is required to understand seasonal changes and long-term trends in microplastic abundance and delivery.

Keywords

Plastic pollution, microfibrils, temporal variability, sediment, depth samples

Acknowledgements

The authors would like to thank members of the Ministry of the Environment, Conservation and Parks, especially Ron Ingram, and summer co-op students at the Dorset Environmental Science Centre, for routinely collecting lakes samples for analysis as well as Tim Field for providing additional background information on the study sites. The authors would also like to thank Jill Crossman for facilitating access to micro-Raman spectroscopy in the Great Lakes Institute for Environmental Research at the University of Windsor.

1. Introduction

The mass production of plastic materials coupled with the mismanagement of waste has led to an international plastic crisis (Cole et al. 2011; Li et al. 2018). Consequently, large quantities of plastic waste exist in the environment with microplastics becoming ubiquitous and pervasive. Microplastics, particles less than 5 mm in size, are either manufactured to be microscopic in size (primary microplastics) or arise through the fragmentation of larger plastic pieces (secondary microplastics) via biological degradation, mechanical transformation, and photodegradation (Cole et al. 2011; Lebreton et al. 2018; Sa et al. 2018). Microplastics can originate from a variety of sources such as textiles, cleaning products, tire wear particles, cosmetic and personal care products and can enter the environment through a number of different pathways including effluent from wastewater treatment plants, atmospheric deposition, road wear suspension, and storm-water run-off (ECCC 2020; Kallenbach et al. 2021; Triebkorn et al. 2019; Lenaker et al. 2019).

Microplastics are of widespread concern due to the potential risks they pose to organisms (Wright et al. 2013; Sussarellu et al. 2016). Microplastics have a tendency to be ingested by all levels of the food web, with studies reporting on microplastic ingestion in invertebrates, fish, turtles, birds, and mammals (Li et al. 2018; Kallenbach et al. 2021; Duncan et al. 2018, Carlin et al. 2020; Di Renzo et al. 2021). Furthermore, microplastics contain chemical additives (e.g., antimicrobials, phthalates, flame retardants, dyes, etc.), many of which are toxic, and they can potentially adsorb and accumulate toxic compounds, such as trace and heavy metals, pathogens, pharmaceuticals, and persistent organic pollutants (Hirai et al. 2011; Mato et al. 2011; Rochman et al. 2014; Remy et al. 2015). The ingestion of microplastics and their associated chemicals can result in the uptake and accumulation of toxic compounds, clogging or obstruction of feeding appendages and/or the digestive system, oxidative stress, reproductive disruption, carcinogenesis, neurotoxicity and in some cases death (Kallenbach et al. 2021; Gomes et al. 2021; Li et al. 2018; Baldwin et al. 2016; Sussarellu et al. 2016).

Studies on microplastics have primarily concentrated on marine environments, where plastic litter conspicuously accumulates (Li et al. 2018). Freshwater environments have largely been overlooked as sinks for microplastics as they were only thought to be conduits for plastics to the ocean (Hoellein and Rochman 2021). As such, very few studies have been conducted on microplastics in freshwater environments. Those that have examined freshwater rivers and lakes (e.g., Felismino et al. 2021; Lenaker et al. 2019; Anderson et al. 2017; Grbić et al. 2020) have primarily focused downstream of wastewater treatment plants or urban environments, with few studies on microplastics in rural areas distant from major anthropogenic point sources. Furthermore, very few studies have examined the spatial and temporal variability of microplastics. The objective of this study was to quantify the abundance of microplastics in headwater lakes in Muskoka-Haliburton. During summer 2019, a regional assessment of microplastics was conducted in 14 headwater lakes with water samples being collected on multiple occasions. At a subset of the study sites, lake sediment samples and water samples from each stratified layer were also collected. We specifically wanted to assess the spatial and temporal variability in microplastic abundance in small, rural lakes to understand their role in the microplastic cycle.

2. Materials and Method

2.1. Study Area

The Muskoka-Haliburton region is comprised of two adjacent municipalities in south-central Ontario, Canada, the District Municipality of Muskoka, more commonly referred to as 'Muskoka', and Haliburton

County. The region, which is located approximately 2.5 hours (250 km) north of Toronto, is commonly referred to as cottage country as it sees more than 2.1 million tourists and visitors each year during the summer period. The Muskoka-Haliburton region spans 8,041 km², has a year-round population of 78,661 and contains more than 1,200 lakes. The region has a humid continental climate with a 30-year annual precipitation average of 1,008 mm, of which 30% falls as snow (Yao et al. 2009).

2.2. Study Sites

The study focused on 14 small rural lakes in Muskoka-Haliburton (Figure 1; Table 1) that are part of a long-term monitoring network operated by the Ministry of the Environment, Conservation and Parks' Dorset Environmental Science Centre to assess the impacts of multiple stressors (e.g., cottage development and long-range atmospheric transport of pollutants) on aquatic ecosystems (Dillon and Molot 2005; Girard et al. 2007). All lake catchments are primarily forested. Agricultural activity is non-existent except in the vicinity of Three Mile Lake, and there is some residential development on most of the study lakes (Hall and Smol 1996; Table 1). The residences that are present are located on the shores of the lakes rather than inland and are serviced by septic systems (Hall and Smol 1996). There are several rural municipalities in the study region with seasonal recreation being the dominant form of human activity; however, the study lakes are more than 200 km from large urban or industrial centers (Hall and Smol 1996; Scheider et al. 1979). The lakes range from mesotrophic to oligotrophic and are single lake basins, with the exception of Red Chalk Lake and Three Mile Lake, which each have two basins (Dillon and Molot 2005; Girard et al. 2007). Samples from these lakes were collected from the main and east basin for Red Chalk Lake and from Hammell's Bay for Three Mile Lake. All lakes are headwater lakes with the exception of Red Chalk Lake which receives discharge from Blue Chalk Lake.

2.3. Lake Water Sampling

Lake water samples (1.0–1.5 L) were collected by the Ministry of the Environment, Conservation and Parks between May and October 2019, from the deepest point of each of the study lakes, 1-metre below the surface with replicate samples (n=3) at 5 lakes (Table 1). At a subset of lakes (n=10), 1 L samples were also collected from the epilimnion, metalimnion and hypolimnion during August 2020 (Table 1). These samples were collected in a volume-weighted fashion from discreet depths (1 m, 3 m, 5 m, etc., throughout the epi-, meta-, or hypolimnion), with thermal layers determined from a temperature profile. All samples were collected using a peristaltic pump with Tygon tubing and stored in a dark refrigerator until processed.

2.4. Sediment Sampling

Sediment cores were collected from a subset of lakes (n=7) between June and September 2019 (Table 1) with duplicate sediment cores collected at 3 lakes. The sediment cores were collected from the deepest point of each lake using a Glew gravity corer attached to a clear Lucite™ core tube with an internal diameter of 7.62 cm. The sediment cores were extruded on shore using a Glew vertical extruder, an extruding tray and a 4-inch stainless steel paint scraper. Before extrusion, any water present on the surface of the sediment core was removed using a pipette and/or narrow plastic tubing and the first 3 cm of the sediment was extruded and placed into a sterile Whirl-Pak® bag. The middle section of the sediment core was discarded, and the 30–33 cm section of the sediment core was extruded and placed into a sterile Whirl-Pak® bag. In three of the lakes, the sediment cores were greater than 20 cm but less than 33 cm, so the last 3 cm of the sediment core was extruded. The 30–33 cm section, or the last 3 cm of the core, corresponds temporally to the pre-industrial era, prior to the invention of plastic; a number of lakes in the study area have been dated using Pb²¹⁰ and the pre-industrial era was determined to be at a depth of

between 15 and 20 cm in the sediment column (Mills et al. 2009). The stainless-steel paint scraper and extruding tray were rinsed in lake water, cleaned with Kimwipes™ and rinsed three times with filtered deionized (DI) water before sectioning the upper 3 cm and the 30–33 cm section (or deepest 3 cm) of each sediment core. Sediment samples were stored in a dark refrigerator until processed.

2.5. Microplastic Extraction: Lake Water

Samples were vacuum filtered through 1.6 µm Fisherbrand™ G6 glass-fibre filter papers and the filtrate was emptied into a graduated cylinder to determine the exact volume of the sample. Sample bottles were triple rinsed with either filtered B-pure™ water or filtered DI water and filtered through the same filter paper as the original sample. The filter papers were immediately transferred to petri dishes, sealed, and stored for analysis.

2.6. Microplastic Extraction: Sediment Samples

Sediment samples were oven dried at approximately 55°C for approximately 72 hours. Under a laminar flow fume hood, 3 g of dried sediment was digested from each sediment core following the wet peroxide oxidation method outlined in Roblin and Aherne (2020). The sample was sieved through 100 µm mesh and the material remaining on the mesh was density separated in zinc chloride (1.5 g/cm³), using an apparatus from Coppock et al. (2017). Any residual material still remaining on the mesh was rinsed with filtered B-pure™ and vacuum filtered onto a 1.6 µm Fisherbrand™ G6 glass-fibre filter paper. For the density separation procedure, a magnetic stir rod was added to the apparatus and the mixture was stirred at 650 RPM for five minutes, left to settle for five minutes, and pulsed three times to remove any trapped air bubbles. The mixture was left to sit for one more minute allowing the supernatant to become clear before the ball valve was closed. The top portion of the density separator was vacuum filtered through 1.6 µm Fisherbrand™ G6 glass-fibre filter papers and triple rinsed with filtered B-pure™ to remove any particles remaining. The bottom portion of the density separator was vacuum filtered through new glass-fibre filter papers and triple rinsed with filtered B-pure™ and the filter papers were individually sealed in sterile petri dishes for microscopic analysis.

2.7. Microplastic Identification

Using a Leica EZ44 stereomicroscope with an EZ4W0170 camera, the filter papers were visually analyzed at 32x magnification for the presence of anthropogenic particles following the visual identification criteria and protocol widely used by Roblin et al. (2020), Roblin and Aherne (2020) and Loppi et al. (2021). The five criteria used were: (i) unnaturally coloured (blue, red, purple, etc.) relative to the sample, (ii) no visible cellular structure or offshoots and appears homogenous in texture and material; (iii) not brittle and remains intact when poked, compressed or tugged with fine tweezers; (iv) shiny or glossy appearance; and (v) no similarities to natural fibres and has limited fraying. If two or more of the criteria were met, the particle was classified as an anthropogenic particle (i.e., synthetic but not necessarily plastic), the colour and proportion of criteria each particle met was recorded and each anthropogenic particle was photographed and measured using an image processing software (ImageJ; URL: imagej.nih.gov/ij). Particles were identified down to a size class of 50 µm but smaller particles were identified where possible. The size (length) of particles were manually estimated by converting the number of pixels measured to a known length in millimetres. All non-fibres were referred to as fragments as there were few to no films or foams identified; see Supporting Information (Section A) for further details on the size, shape, and colour of anthropogenic particles. A minimum of 20% of the anthropogenic particles were randomly selected and tested using the hot needle test to determine the proportion that were plastic (i.e., polymers with a

petrochemical base). Following Roblin et al. (2020) and Hidalgo-Ruz et al. (2012), a hot needle was pressed against the edge of the selected particle and if the particle melted, it was classified as plastic. Within the lake water and sediment samples, the proportion of plastic (i.e., the proportion of anthropogenic particles that melted) were used to report the number of microplastics.

2.8. Raman Spectroscopy

Similar to Loppi et al. (2021), micro-Raman spectroscopy (WITec, operated by WITec Control) was used to characterize the polymer type of the plastic particles. Fibres were analyzed using 785 nm laser and fragments were analyzed using 532 nm laser at 100x objective and adjustable power (ranging from 0 mW to approximately 85 mW). To confirm polymer identity, spectra were recorded in 0–1800 cm^{-1} wavelength and analyzed through a commercial library (Open Specy; Cowger et al. 2021; Figure SI-2).

2.9. Quality Control and Assurance

This study was built onto an existing long-term monitoring program that routinely used plastic containers for collection. To determine potential contamination, lake blanks ($n=12$) were collected in the field using filtered B-pure™ or DI water with the routine sample containers; plastic particles were not identified in the lake blanks. Furthermore, procedural open-air blanks, i.e., open petri dishes with filter papers, were routinely collected and used to determine potential contamination during the extraction and analysis process in the laboratory. Open-air blanks were exposed during filtering and particle identification and the period of exposure was recorded. The average potential contamination was less than one microplastic per sample based on the average contamination from the open-air blanks and the time samples were exposed to the air (Table 2). Accordingly, samples were not blank corrected as potential contamination was low. Procedural B-pure™ water, DI water, zinc chloride and hydrogen peroxide blanks were also collected by vacuum filtering a known quantity through 1.6 μm Fisherbrand™ G6 glass-fibre filter papers and analyzing them following the same method as the field samples (Table 2). The average potential contamination in these blanks ranged from 0.16 mp/L to 7.38 mp/L, therefore, all solutions (i.e., DI water, B-pure™ water, zinc chloride, hydrogen peroxide) were vacuum filtered prior to use in the Fe (II) solution, sediment digestion, density separation, rinsing and cleaning to prevent or limit potential contamination.

Approximately 21 microplastics were identified in the blanks, nine were found in solutions prior to filtration (i.e., all solutions were filtered prior to use), 11 were found in open air blanks with an average exposure of 6.5 hours and one was found in a microscope blank with an average exposure of 42 hours. The field samples had an average exposure of 0.60 hours during the filtering process and 0.17 hours during microscope analysis. Although some plastic was used during the sample procedure, i.e., the sample bottles were comprised of polyethylene terephthalate, the petri dishes and weigh boats were comprised of polystyrene, and the Whirl-Pak® bags were comprised of polyethylene, potential contamination was limited. There were no microplastics identified in the lake field blanks ($n=12$) and the polystyrene, polyethylene and polyethylene terephthalate polymers identified in the field samples were coloured indicating that they did not originate from laboratory sources. In addition, all equipment was triple rinsed with filtered B-pure™ or filtered DI water prior to use and immediately covered in aluminium foil when not in use. The Buchner funnel was triple rinsed with filtered B-pure™ or filtered DI water, cleaned with Kimwipes™ to remove any remaining material, and triple rinsed again in between each sample. Finally, all samples were collected, processed and analyzed using nitrile gloves and 100% cotton laboratory coats were worn when processing and analyzing the samples.

2.10. Data Analysis

The concentration of microplastics in lake water is reported as the number of microplastics per litre (mp/L) and for sediment as the number of microplastics per gram of dried sediment (mp/g). For the lake water samples collected 1-metre below the surface between May and June 2019, the average concentration of microplastics in the lake was multiplied by the total lake volume to determine the total number of microplastics in the lake. A Spearman's correlation test was used to determine the association between the average microplastic concentration and lake attributes (number of shoreline residences, lake area, watershed area, mean depth and max depth; Table 1). The variability between replicate (n=3) lake water samples was determined as the coefficient of variation. For the temporal samples collected between May and October 2019, the average concentration of microplastics was determined for each month by lake. The concentration of microplastics in the epilimnion, metalimnion and hypolimnion for each lake was calculated and multiplied by the percent of the total lake volume that each stratum comprised to determine the volume-weighted concentration of microplastics. Statistical comparisons between temporal periods or lake strata were generally based on a paired T-test or repeated-measures ANOVA with Tukey's pairwise test carried out in Past 4.06b (Hammer et al. 2001).

The concentration of microplastics (mp/g) in each sediment core section was calculated and the average microplastic concentration was determined for duplicate cores. The average variability between duplicate sediment samples was based on their relative percent difference. As individual cores were not dated, the average sedimentation rate for lakes in Muskoka-Haliburton (Mills et al. 2009) and microplastic concentration was used to determine a rough microplastic accumulation rate (mp/m²/day) in the top section of the sediment core for each lake. The percent of microplastics that are removed annually via sedimentation was determined using the microplastic accumulation rate, lake area and total number of microplastics in the lake. A Spearman's correlation test was then used to determine if there was a correlation between the average microplastic concentration and lake attributes (number of shoreline residences, lake area, watershed area, mean depth and max depth).

3. Results and Discussion

3.1 Spatial and Temporal Variability in Microplastic Concentration

The concentration of microplastics in the study lakes (n=12) ranged from 1.02–2.39 mp/L between May and June 2019 with an average concentration of 1.78 mp/L (Table 3; Table SI-5); microplastics were normally distributed across the lakes (Figure 2) with a coefficient of variation of 22%. The average coefficient of variation for replicate samples (n=3) was generally consistent across the study lakes at 53%. When lake volume was taken into account, the total number of microplastics in lakes across the region ranged from 0.45 billion to 34.59 billion microplastics, with an average of 8.18 billion microplastics per lake, and a coefficient of variation of 115%.

The abundance of microplastic (mp/L) in the study lakes was weakly correlated with lake area (rs: 0.469), the number of shoreline residences (rs: 0.399), and watershed area (rs: 0.350). Given the low variability in the concentration of microplastics between lakes (coefficient of variation of 22%), this suggests that diffuse inputs such as atmospheric deposition, which is influenced by lake and catchment size, was the dominant source of microplastics to the study lakes. While the number of shoreline residences may be an indicator of direct anthropogenic inputs, the number of residences is also correlated to lake size, which is the likely association in the study lakes given the weaker correlation to microplastic abundance. Furthermore, lakes which had very few or no residences (see Table 1) and consequently limited road

access, had similar microplastic concentrations compared with lakes that had numerous homes and cottages and year-round road access (Table SI-5). This further suggests that atmospheric deposition is likely the dominant source of microplastics to the study region and is an important vector in the transport of microplastics to remote or rural regions. Several studies have observed that microplastics found in remote areas such as the Arctic, French Pyrenees Mountain, Atlantic coastline, the Alps and Muskoka, are linked to atmospheric deposition (Bergmann et al. 2019; Allen et al. 2019; Ambrosini et al. 2019; Roblin et al. 2020; Welsh 2022). Furthermore, the concentration of microplastics detected in atmospheric deposition in the French Pyrenees mountain, a remote site with no development within 95 km, was comparable to microplastic concentrations in atmospheric deposition in large cities (Allen et al. 2019; Dris et al. 2016; Cai et al. 2017; Wright et al. 2020).

The average concentration of microplastics in the lake samples collected between May and October from the study lakes (n=9) ranged from 0.91 to 2.09 mp/L, with the highest concentration generally being observed between May and June (Figure 3a; Table SI-6). The average monthly microplastic abundance was significantly lower in August compared with May and June 2019 ($p < 0.05$). Further, the average microplastic abundance during May–June 2019 in the study lakes (n=9) was significantly greater than the six-month (May–October 2019) average abundance (1.43 mp/L; $p < 0.05$), suggesting a distinct seasonal variability (Figure 3b). There was no significant difference in the abundance of microplastics between lakes during May to October 2019, except for Chub Lake, which had a significantly higher abundance compared with Harp (Figure 3c; $p < 0.05$).

Comparing the concentration of microplastics in lakes across published studies can be difficult due to differences in sampling methodologies; net or trawl versus pump, mesh size, sample depth, identification techniques (visual identification, FT-IR, Raman, size class) as well as the use of different units (mp/L, mp/km², mp/m³) for reporting microplastics concentration (ECCC 2020; Dusaucy et al. 2021). The average concentration of microplastics in the study lakes during May–June (1.78 mp/L) was much higher than the concentration of microplastics observed in Lake Simcoe (0.4 mp/L) and the concentration of particles in Lake Ontario, near Toronto (0.8 particle/L), both of which are urbanized lakes (Felismino et al. 2021; Grbić et al. 2020). The average microplastic concentration in the study lakes, however, is generally consistent with stream samples collected from Mount Everest during April and May 2019, which ranged from 0–2 mp/L with an average concentration of 1 mp/L (Napper et al. 2020).

3.2. Variability in Microplastic Concentration with Depth

Across the stratified layers, there was no significant difference in the concentration of microplastics, but generally higher concentrations were observed in the upper layers (Figure 4a; Table SI-7). The average microplastic concentration in the epilimnion and metalimnion was 0.67 mp/L and 0.70 mp/L, respectively, compared with 0.53 mp/L in the hypolimnion. Further, the coefficient of variations for microplastics was greater in the metalimnion (63%) compared with the epilimnion (33%) and hypolimnion (51%) potentially suggesting a slight preferential settling of microplastics within the study lakes based on plastic type (fibre versus fragment) or density.

The average volume-weighted microplastic concentration (0.66 mp/L) was very similar to the concentration in samples collected at a depth of 1-m (0.68 mp/L), suggesting that a 1-m depth sample is representative of the concentration of microplastics in the lake, although the 1-m samples showed greater variability between lakes (49% versus 26%; Figure 4b). It is worth noting that the abundance of

microplastics in the study lakes was significantly lower ($p < 0.05$) in August 2020 compared with August 2019, again highlighting the importance of capturing temporal observations (Figure 4c; Table SI-8).

The lack of a distinct vertical distribution in the concentration of microplastics in the study lakes is similar to Tamminga and Fischer (2020), who examined a dimictic lake in northern Germany and found that the concentration of fibres did not display a noticeable vertical distribution. They did, however, find that the concentration of irregular particles displayed a distinct vertical pattern, decreasing with depth, but this vertical difference was not significant (Tamminga and Fischer 2020). Similarly, Lenaker et al. (2019) found that the concentration of microplastics at five out of the six river and lake sites, were not significantly different between the surface and subsurface.

3.3. Sediment

In the top 0–3 cm section, the concentration of microplastics ranged from 2.17 to 5.45 mp/g with an average of 3.66 mp/g and a coefficient of variation of 35% (Figure 5; Table SI-9).

There was a weak correlation between the concentration of microplastics in the top 0–3 cm section and the number of cottages on the lake (r_s : 0.487). In the bottom section of the sediment core, the abundance of microplastics was significantly lower ($p < 0.05$) than the top section, with an average concentration of 0.72 mp/g and a coefficient of variation of 47%. The presence of microplastics in the bottom 30–33 cm section of the sediment core, which corresponds temporally to the pre-industrial era, suggests that microplastics can migrate or mobilize down the sediment column.

The average relative percent difference between duplicate cores ($n = 3$) for the top 0–3 cm section was 20% and for the bottom 30–33 cm section it was 42%, suggesting slightly higher variability in microplastics abundance in sediment between the lakes than between duplicate cores. Furthermore, the bottom section of the sediment core had greater variability in microplastic abundance compared to the top. Given an average rate of sedimentation for the study lakes (Mills et al. 2009), the average accumulation of microplastics in the top lake sediment was 1.78 mp/m²/day (1.06–2.65 mp/m²/day) with a coefficient of variation of 34%. As such, the percent of microplastics buried annually into the lake sediment ranged from 1.1–63.6% with an average of 14% and a median of 5.3%. This suggests that for most lakes (Table SI-9), lake sediment is a small sink for microplastics with the majority of microplastics leaving the lake via the outflow.

The concentration of microplastics in the top section of the sediment core for the study lakes (2,170–5,450 mp/kg) is much higher than the concentration of microplastics in sediment observed in urban lakes such as Lake Simcoe (1.24–160 mp/kg), Lake Erie (0–391 mp/kg), Lake Ontario (40–4,270 mp/kg) and lakes located in Lake Mead National Recreation Area (87.5–1,010 mp/kg) (Felismino et al. 2021; Dean et al. 2018; Ballent et al. 2016; Baldwin et al. 2020). This is likely due to methodological differences as these studies used a Petite Ponar to collect surficial sediment samples. If the concentration of microplastics in the 0–3 cm section and 30–33 cm section of the study lakes is averaged (Table SI-5), the range is reduced to 1,730–3,220 mp/g but it is still higher than observations from other studies. The decrease in microplastic abundance with sediment depth is similar to Turner et al. (2019) who collected a sediment core from an urban lake in London, UK; in their study, the concentration of microplastics in the 0–5 cm section was 539 particles/kg and decreased to a concentration of 78.2 particles/kg at a depth of 30–35 cm.

3.4. Microplastic Characterization

In the lake water and bottom section of the sediment core, fibres were the dominant anthropogenic particle shape identified (>70%) whereas in the top section of the sediment core, fibres were only slightly more common than fragments (57%; see Supporting Information Section A). There was no noticeable difference in the proportion of fibres and fragments across the lakes depths with fibres being relatively evenly distributed (51–67%).

Of the anthropogenic particles that were identified in the lake samples and tested using a hot needle, 14% melted and were identified as plastic. In the lake sediment, 36% of the anthropogenic particles identified in the top 0–3 cm section melted while only 13% in the bottom 30–33 cm section melted. Thermoplastics were the predominant plastic type identified in the study lakes with polypropylene being most abundant (26%) followed by acrylonitrile butadiene styrene (21%) and polyethylene (16%). In the lake samples, there was an equal proportion (22%) of polypropylene, polyethylene and polyisoprene identified with polyamide, polyacrylamide and polyethylene terephthalate also being identified. In the lake sediment, the majority of particles were acrylonitrile butadiene (40%) followed by polypropylene (30%). Polyamide, polyethylene, and styrene acrylonitrile were also identified but were less common.

4. Conclusion

There was limited spatial variability in the concentration of microplastics across the study lakes with microplastic abundance being weakly correlated with lake area (r_s : 0.469), the number of shoreline residences (r_s : 0.399), and watershed area (r_s : 0.350). This suggests that atmospheric deposition is the dominant source of microplastics to the study lakes, and therefore, limited spatial monitoring can adequately quantify microplastic abundance in headwater catchments in rural regions. In contrast, temporal monitoring is required as a distinct seasonal variability in microplastics abundance was observed, with August 2019 having a significantly lower concentration compared with May and June 2019. This suggests that seasonal changes in diffuse sources or hydrological processes influence the abundance of microplastics within headwater lakes in rural areas.

Sediment has been identified as an important long-term sink for microplastics in freshwater environments; however, in this study, the percent of microplastics buried in the lake sediment annually was relatively low. This suggests that lake sediment is not a dominant sink for microplastics, but rather that small rural lakes are reservoirs for export to downstream rivers or lakes. Few studies, however, have evaluated the residence time of microplastics in lakes, which is an important factor in the microplastic cycle as it identifies the length of time microplastics can stay (reside) in a lake. Future monitoring and research in rural lakes should focus on a catchment balance approach for microplastics, including atmospheric inputs, in order to better understand the fluxes and cycle of microplastics in lakes. This will help to improve our understanding on microplastics and will allow for effective policy decisions to address microplastic pollution.

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490 Statements and Declaration

491 Funding

492 This research was carried out with the support from ECO Canada Co-op Student Work Placement program
493 and an NSERC CGS-M scholarship to Brittany Welsh.

494 Competing Interests

495 The authors declare no competing financial or personal interests

496 Author Contribution

497 **Brittany Welsh:** Study design, particle extractions and identification, microscopy, interpretation
498 of data, writing – original draft

499 **Julian Aherne:** Study design, interpretation of data, writing – original draft

500 **Andrew M. Paterson:** Study design, interpretation of data, writing – review and editing

501 **Huaxia Yao:** Supported field sampling, provided background information on study sites, writing
502 – review and editing

503 **Chris McConnell:** Supported field sampling, provided background information on study sites,
504 writing – review and editing

505 **Table 1** Physical characteristics for the 14 study lakes in Muskoka-Haliburton, Canada including: location (UTM Zone 17), type of samples collected
 506 (W = lake water, S = lake sediment, D = depth stratification, T = Temporal samples), number of shoreline residences on the lake, lake area (ha),
 507 watershed area (ha), lake volume (10^4 m^3), mean and max depth (Hall and Smol 1996; Girard et al. 2007; Nürnberg et al. 2018; Wong et al. 1997)

Lake	Easting (m)	Northing (m)	Samples				Number of Shoreline Residences	Lake Area (ha)	Watershed Area (ha)	Lake Volume (10^4 m^3)	Mean Depth (m)	Max Depth (m)
			W	S	D	T						
Blue Chalk	661837	5007066	X		X	X	11	52	158	447	8.5	23
Chub	638398	5017348	X		X	X	24	34	306	306	8.9	27
Crosson	654512	4994145			X	X	0	57	578	522	9.2	25.0
Dickie	650192	5001333	X	X	X	X	133	94	500	468	5.0	12.0
Fawn	637717	5003574	X	X			51	11	1,462	300	3.5	7.9
Harp	645976	5026855	X	X	X	X	96	71	542	949	13.3	37.5
Heeney	649092	4998918	X			X	11	21	93	71	3.3	5.8
Mouse	669338	5005773	X	X			0 ^b	9	185	44	4.9	9.0
Plastic	671097	5005242	X	X	X	X	0	32	128	254	7.9	16.3
Ranger	668976	5001928	X	X			1 ^c	86	260	63	5.6	13.0
Ridout	658756	5004543			X		1	47	54	314	6.7	20.4
Red Chalk East	661809	5006310	X		X	X	0	13	589	74	5.7	19.0
Red Chalk Main	661108	5006047	X		X	X	3	44	589	736	16.7	38
Three Mile ^a	620114	5005350	X		X	X	145	230	1,320	1,549	6.7	12.0
Young	613953	5006692		X			4	106	502	1,274	12.0	21.1

508 ^a Hammell's Bay

509 ^b There is an abandoned ranger's camp next to the lake

510 ^c There is also an abandoned sawmill next to the lake

511

Table 2 Average concentration of microplastics (mp/L; mp/hr) in each of the blank samples collected and their coefficient of variation

QA/QC Sample (liquid)	Average Microplastic Concentration (mp/L) ^a	Coefficient of Variation (%)
Unfiltered B-Pure (n=16)	0.46	65
Unfiltered Deionised (DI) water (n = 8)	0.16	90
Unfiltered H ₂ O ₂ (n=6)	1.26	64
Unfiltered ZnCl ₂ (n=5)	7.38	9
Lake field blank (n=12)	0.00	68
	Average Microplastic Concentration (mp/hr) ^a	Coefficient of Variation (%)
QA/QC Sample (open-air)	0.00	83
Oven (n=12)	0.20	178
Filtering (n=51)	0.24	100
Fume hood (n=11)	0.07	78

^a The average concentration of microplastics in the liquid and air QA/QC samples have been normalized to MP L⁻¹ and MP hr⁻¹ respectively

Figure Captions

Fig. 1 Location of the 14 study lakes in Muskoka-Haliburton, Canada. Inset shows the location of the 14 sites in south-central Ontario, Canada

Fig. 2 Distribution (histogram and box-plot) in the concentration of microplastics (mp/L) in the lake water samples collected from the study lakes (n=12) in Muskoka-Haliburton, Canada, during May and June 2019. Box-plot: The black line represents the median, the box represents the 25th and 75th percentile and the whiskers represent 1.5 times the interquartile range

Fig. 3 Distribution in the concentration of microplastics (mp/L) identified in lake water samples collected from (a) each of the study lakes (n=9) in Muskoka-Haliburton; (b) during each month (May to October) that samples were collected; and (c) May and June 2019 versus May to October 2019. Box-plot: The black line represents the median, the box represents the 25th and 75th percentile and the whiskers represent 1.5 times the interquartile range. Notation: BC=Blue Chalk, CB=Chub, DE=Dickie, HP=Harp, HY=Heeney, PC=Plastic, RCE=Red Chalk East, RCM=Red Chalk Main, TM=Three Mile

Fig. 4 Distribution in (a) the concentration of microplastics (mp/L) observed in the epilimnion, metalimnion and hypolimnion; (b) the volume-weighted average compared with the microplastic concentration and microplastic concentration from samples collected 1-metre below the surface; and (c) the concentration of microplastics (mp/L) in samples collected in August 2019 and August 2020 from the study lakes in Muskoka-Haliburton, Canada

Fig. 5 Distribution in the concentration of microplastics (mp/g) in the top 0–3 cm section and bottom 30–33 cm section of the sediment cores collected from the study lakes (n=7) in Muskoka-Haliburton, Canada between June and September 2019

538 Fig. 1

539

Fig. 2

Fig. 3

Fig. 4

548 Fig. 5

549

Supporting Information

Microplastics in headwater lake catchments in Muskoka-Haliburton, Canada

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Environmental Earth Sciences

Section A

Fibres were the dominant shape identified in lake water and in the bottom 30–33 cm section of the sediment core with more than 70% of the identified anthropogenic particles being fibres (Figure SI-1). In the top 0 – 3 cm section however, fibres were only slightly more dominant than fragments (57%). Across the different lake depths, there was no noticeable difference in the proportion of fibres and fragments across the water column with fibres being relatively evenly distributed (51-67%). The even distribution of fibres across the water column suggests that the specific density of polymers may be less important for fibres (UNEP 2020).

Anthropogenic particle size ranged from 20 µm to 4,990 µm across the study sites, with lake water having the smallest fibre and fragment sizes while lake sediment had the largest (Table SI-1). This suggests that large sized particles have a tendency to sink to the lake bottom while smaller particles remain suspended in the lake. Within the lake sediment, the size of fibres in the 0–3 cm and 30–33 cm sections were generally similar; however, the size of fragments in the 30–33 cm section were about 2 times greater than the size of fragments in the 0–3 cm section. The dominance of smaller particles (fibres and fragments) in the study suggests that particles are likely secondary anthropogenic particles that arose through the weathering and breakdown of large plastic pieces currently in the environment (Kooi and Koelmans 2019).

Across the depth samples, the mean and median anthropogenic particle size generally increased down the water column (Table SI-2). The proportion of anthropogenic particles that had a size less than 400 µm also decreased down the water column while the proportion of particles that had a size greater than 1,000 µm increased (Table SI-3a). At a depth of 1-metre and in the epilimnion, approximately 64% of anthropogenic particles had a size less than 400 µm while only 14% of particles had a size greater than 1,000 µm while in the metalimnion and hypolimnion, less than 62% of particles had a size less than 400 µm and more than 17% had a size greater than 1,000 µm. There was also a significant difference in the proportion of anthropogenic particles. There did not appear to be a difference in the proportion of fragments that fell within each size across the lake depths (Table SI-3b) but for fibres, there was a larger proportion of fibres which had a size less than 400 µm in the epilimnion (57%) and at 1-metre (46%) whereas there was a larger proportion of fibres that had a size greater than 1,000 µm in the metalimnion (33%) and hypolimnion (43%; Table SI-3c).

Blue was the most common coloured anthropogenic particle identified across all sample media with more than 72% of fibres and 60% of fragments being blue (Table SI-4). White was also commonly identified for fragments (15%) but was less common for fibres (0.12%). White fragments were common in the sediment

589 samples with 21% of the fragments identified in the 0–3 cm section and 52% in the 30–33 cm section
590 being white. This could suggest that white fragments are a specific type of polymer that tends to sink or
591 that the fragment lose their colour during the digestion process. Bright eye-catching colours, such as blue,
592 may be more easily recognized during visual identification compared to dull colours and thus
593 overestimated (Hidalgo-Ruz et al. 2012; Dris et al. 2015). Furthermore, transparent or uncoloured
594 microplastics may be easily overlooked as they are harder to identify (Dris et al. 2015).

595 **Fig. SI-1** Proportion of fibres and fragments identified in lake water, sediment (top 0-3cm section and
596 bottom 30-33 cm section) and depth samples (1-metre, epilimnion, metalimnion, and hypolimnion)
597 collected from the study headwater lakes in Muskoka-Haliburton, Canada

598 **Table SI-1** The mean and median size (μm) as well as the range (μm) and coefficient of variation (CV) (%)
599 for anthropogenic particles (microfibres and fragments combined) microfibres only, and fragments only,
600 collected from atmospheric deposition, lake water, and sediment collected from headwater lakes in
601 Muskoka-Haliburton, Canada

602 **Table SI-2** The mean (μm), median (μm) and range (μm) in anthropogenic particles observed in the depth
603 samples collected from the epilimnion, metalimnion, hypolimnion and 1-metre below the surface from
604 each of the study lakes in Muskoka-Haliburton, Canada as well the coefficient of variation (%)

605 **Table SI-3a** The proportion (%) of anthropogenic particles identified in lake water samples collected from
606 the epilimnion, metalimnion, hypolimnion and 1-metre below the surface that fall within each size class

607 **Table SI-3b** The proportion (%) of fragments identified in lake water samples collected from the
608 epilimnion, metalimnion, hypolimnion and 1-metre below the surface that fell within in each size class

609 **Table SI-3c** The proportion (%) of fibres identified in lake water samples collected from the epilimnion,
610 metalimnion, hypolimnion, and 1-metre below the surface that fell within in each size class

611 **Table SI-4** The proportion (%) of fibres and fragments and their colour that were identified in lake water
612 and sediment samples (0-3cm and 30-33 cm sections) collected from the headwater lakes in Muskoka-
613 Haliburton, Canada

Fig SI-1 Proportion of fibres and fragments identified in lake water, sediment (top 0–3 cm section and bottom 30–33 cm section) and depth samples (1-metre, epilimnion, metalimnion, and hypolimnion) collected from the study headwater lakes in Muskoka-Haliburton, Canada

Table SI-1 The mean and median size (μm) as well as the range (μm) and coefficient of variation (CV) (%) for anthropogenic particles (microfibres and fragments combined) microfibres only, and fragments only, collected from atmospheric deposition, lake water, and sediment collected from headwater lakes in Muskoka-Haliburton, Canada

Sample Type		Number of Particles	Median (μm)	Mean (μm)	Range (μm)	Coefficient of Variation (%)
Lake Water	Particles	882	260	529	20 – 4,990	124
	Fibres	616	450	700	20 – 4,990	101
	Fragments	266	60	132	20 – 2,200	158
Sediment ^a 0–3 cm	Particles	215	290	649	20 – 4,860	127
	Fibres	123	770	1,035	110 – 4,860	91
	Fragments	92	160	327	20 – 4,140	163
Sediment ^a 30–33 cm	Particles	170	660	918	30 – 4,890	94
	Fibres	124	815	1,026	120 – 4,890	81
	Fragments	46	310	622	30 – 4,850	142

^a Some of the anthropogenic particle pictures were lost and therefore the size of the particles were not measured

Table SI-2 The mean (μm), median (μm) and range (μm) in anthropogenic particles observed in the depth samples collected from the epilimnion, metalimnion, hypolimnion and 1-metre below the surface from each of the study lakes in Muskoka-Haliburton, Canada as well the coefficient of variation (%)

	Number of Particles	Median (μm)	Mean (μm)	Range (μm)	Coefficient of Variation (%)
1-metre	50	210	452	20 – 2,770	136
Epilimnion	45	160	427	40 – 2,860	147
Metalimnion	47	230	443	30 – 2,040	117
Hypolimnion	35	680	790	50 – 3,530	98

Table SI-3a The proportion (%) of anthropogenic particles identified in lake water samples collected from the epilimnion, metalimnion, hypolimnion and 1-metre below the surface that fall within each size class

	1-metre	Epilimnion	Metalimnion	Hypolimnion
<200	46.00	60.00	46.81	28.57
200-400	18.00	11.11	14.89	14.29
400-600	12.00	8.89	12.77	5.71
600-800	6.00	4.44	6.38	14.29
800-1,000	4.00	2.22	2.13	8.57
$\geq 1,000$	14.00	13.33	17.02	28.57

Table SI-3b The proportion (%) of fragments identified in lake water samples collected from the epilimnion, metalimnion, hypolimnion and 1-metre below the surface that fell within in each size class

	1-metre	Epilimnion	Metalimnion	Hypolimnion
<50	41.67	20.00	34.78	0.00
50-100	16.67	40.00	17.39	25.00
100-150	16.67	20.00	17.39	33.33
150-200	4.17	0.00	8.70	16.67
200-250	4.17	6.67	0.00	8.33
250-300	0	13.33	4.35	0.00
≥ 300	16.67	0.00	17.39	16.67

Table SI-3c The proportion (%) of fibres identified in lake water samples collected from the epilimnion, metalimnion, hypolimnion, and 1-metre below the surface that fell within in each size class

	1-metre	Epilimnion	Metalimnion	Hypolimnion
<200	15.38	50.00	16.67	4.35
200-400	30.77	6.67	25.00	13.04
400-600	7.69	13.33	12.50	8.70
600-800	11.54	6.67	8.33	17.39
800-1,000	7.69	3.33	4.17	13.04
$\geq 1,000$	26.92	20.00	33.33	43.48

Table SI-4 The proportion (%) of fibres and fragments and their colour that were identified in lake water and sediment samples (0-3cm and 30-33 cm sections) collected from the headwater lakes in Muskoka-Haliburton, Canada

Colour	Fibre (%)	Fragment (%)
Black	6.84	0.00
Blue	72.07	59.65
Blue-white	0.00	2.48
Brown	0.12	0.00
Clear	0.35	0.99
Green	1.51	7.92
Grey	8.00	1.49
Orange	0.23	1.24
Pink	1.97	0.50
Purple	0.70	0.00
Red	8.00	8.42
Red-white	0.00	0.50
White	0.12	14.60
Yellow	0.12	2.23

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Section B:

Fig SI-2a Spectra from micro-Raman spectroscopy analyzed through commercial library Open Specy for polyethylene terephthalate microfibre observed in a sample collected from Red Chalk East in Muskoka-Haliburton, Canada

Fig SI-2b Spectra from micro-Raman spectroscopy analyzed through commercial library Open Specy for polypropylene fragment observed in the top 0-3 cm section of the sediment core collected from Dickie Lake in Muskoka-Haliburton, Canada

Table SI-5 The average concentration (mp/L) and total number of microplastics identified each of the study lakes in Muskoka-Haliburton, Canada during May–June 2019

Table SI-6 The average, range and median concentration of microplastics (mp/L) in the study lakes in Muskoka-Haliburton, Canada between May and October 2019 as well as the coefficient of variation, 5th and 9th percentile

Table SI-7 Concentration of microplastics (mp/L) identified at each depth in each of the study lakes in Muskoka-Haliburton, Canada (taking into account the percent total volume that each stratum comprises)

Table SI-8 The concentration of microplastics (mp/L) in samples collected 1-metre below the surface in August 2019 and in August 2020 from the study lakes (n=10) in Muskoka-Haliburton, Canada as well as the relative percent difference

Table SI-9 Microplastic concentration (mp/g) in the top 0–3 cm section and bottom 30–33 cm section of the sediment cores collected from seven of the study lakes in Muskoka-Haliburton, Canada between June and September 2019 as well as the microplastic accumulation rate (mp/m²/day), percent of microplastics buried in the lake sediment and the average microplastic concentration (MP/g) between the 0–3 and 30–33 cm section.

Fig SI-2a Spectra from micro-Raman spectroscopy analyzed through commercial library Open Specy for polyethylene terephthalate microfibre observed in a sample collected from Red Chalk East in Muskoka-Haliburton, Canada

Fig SI-2b Spectra from micro-Raman spectroscopy analyzed through commercial library Open Specy for polypropylene fragment observed in the top 0-3 cm section of the sediment core collected from Dickie Lake in Muskoka-Haliburton, Canada

Table SI-5 The average concentration (mp/L) and total number of microplastics identified each of the study lakes in Muskoka-Haliburton, Canada during May–June 2019

Lake	Average Concentration (mp/L)	Total Number of Microplastics (x10 ⁹ MP)
Blue Chalk	1.72	7.68
Chub	2.39	7.27
Dickie	2.21	10.34
Fawn	1.84	5.53
Harp	1.37	12.97
Heeney	1.52	1.07
Mouse	1.02	0.45
Plastic	1.61	4.09
Ranger	1.93	1.22
Red Chalk East	1.91	1.42
Red Chalk Main	1.57	11.56
Three Mile	2.23	34.59

Table SI-6 The average, range and median concentration of microplastics (mp/L) in the study lakes in Muskoka-Haliburton, Canada between May and October 2019 as well as the coefficient of variation, 5th and 95th percentile

Lake	Average (mp/L)	Range (mp/L)	Median (mp/L)	5 th Percentile	95 th Percentile	Coefficient of Variation (%)
Blue Chalk	1.68	0.82 – 2.62	1.63	0.90	2.51	46
Chub	2.09	0.94 – 2.96	2.23	1.11	2.88	41
Dickie	1.09	0.37 – 2.21	0.88	0.40	2.06	76
Harp	0.91	0.37 – 1.40	0.78	0.43	1.39	49
Heeney	1.49	1.04 – 1.81	1.55	1.11	1.78	22
Plastic	1.42	0.95 – 2.27	1.23	0.98	2.13	41
Red Chalk East	1.41	0.28 – 2.49	1.42	0.44	2.35	64
Red Chalk Main	1.53	0.74 – 2.24	1.57	0.86	2.15	40
Three Mile	1.26	0.20 – 2.23	1.35	0.31	2.15	81

Table SI-7 Concentration of microplastics (mp/L) identified at each depth in each of the study lakes in Muskoka-Haliburton, Canada (taking into account the percent total volume that each stratum comprises)

Lake	Epilimnion	Metalimnion	Hypolimnion	VW average	1-metre	Relative Percent Difference (%)
Blue Chalk	1.05	0.77	0.45	0.95	1.01	7
Chub	0.45	0.92	1.06	0.60	1.21	67
Crosson	0.59	0.89	0.32	0.60	0.46	26
Dickie	0.73	0.14	0.43	0.65	0.75	14
Harp	0.62	0.30	0.46	0.53	0.31	53
Plastic	0.42	1.42	0.75	0.57	0.59	3
Red Chalk East	0.59	0.00	0.15	0.53	0.58	9
Red Chalk Main	0.93	0.88	0.60	0.80	0.72	10
Ridout	0.91	1.10	0.77	0.92	1.03	11
Three Mile	0.44	0.59	0.30	0.45	0.15	101

Table SI-8 The concentration of microplastics (mp/L) in samples collected 1-metre below the surface in August 2019 and in August 2020 from the study lakes (n=10) in Muskoka-Haliburton, Canada as well as the relative percent difference

Lake	2019 Concentration (mp/L)	2020 Concentration (mp/L)	Relative Percent Difference (%)
Blue Chalk	1.65	1.01	48
Chub	0.94	1.21	25
Crosson	0.67	0.46	37
Dickie	0.37	0.75	68
Harp	0.66	0.31	72
Heeney	1.04	0.60	54
Plastic	1.12	0.58	64
Red Chalk East	1.51	0.58	89
Red Chalk Main	2.24	0.72	103
Three Mile	1.35	0.15	160

Table SI-9 Microplastic concentration (mp/g) in the top 0–3 cm section and bottom 30–33 cm section of the sediment cores collected from seven of the study lakes in Muskoka-Haliburton, Canada between June and September 2019 as well as the microplastic accumulation rate (mp/m²/day), percent of microplastics buried in the lake sediment and the average microplastic concentration (mp/g) between the 0–3 and 30–33 cm section

Lake	Top 0–3 cm			Bottom 30–33 cm	Average 0–3 and 30–33 cm (mp/g)
	Concentration (mp/g)	Accumulation Rate (mp/m ² /day)	Percent Burial (%)	Concentration (mp/g)	
Dickie	3.83	1.86	6.15	0.67	2.25
Fawn	2.99	1.46	1.09	0.69	1.84
Harp	5.45	2.65	5.32	0.99	3.22
Mouse	3.66	1.78	12.99	0.34	2.00
Plastic	2.17	1.06	3.04	1.28	1.73
Ranger	5.11	2.48	63.57	0.35	2.73
Young	2.40	1.17	2.89	0.69	1.55