Plastic Microbead Accumulation in our Freshwaters:

North American Great Lakes Assessments and Perspective

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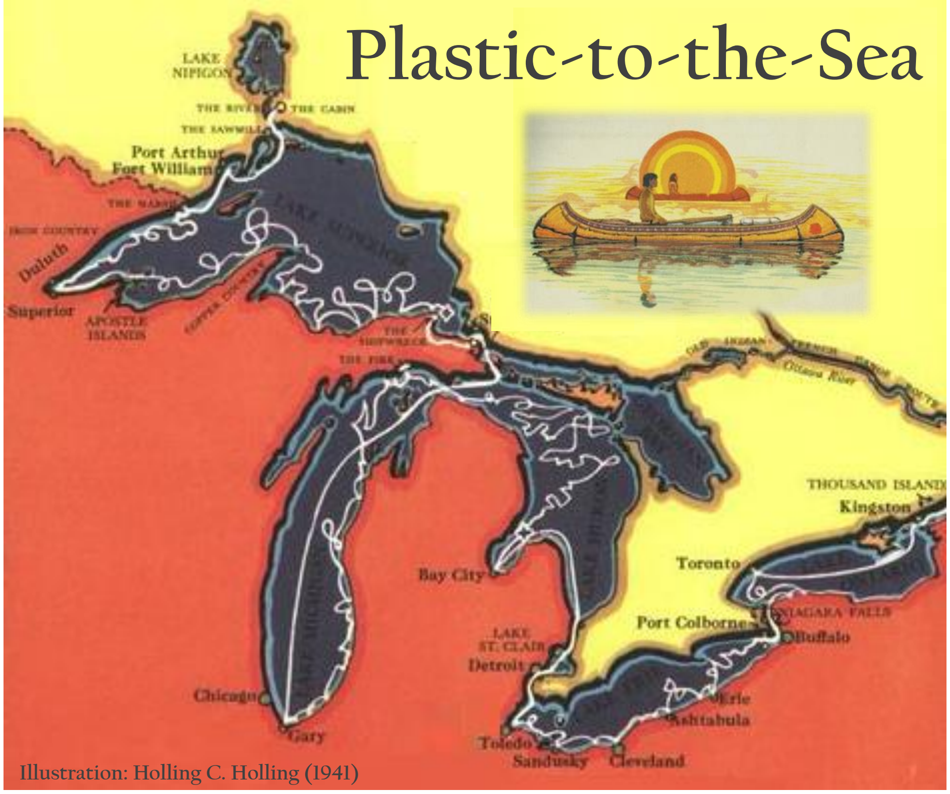

Illustration: Holling C. Holling (1941)

The authors review recent studies conducted across the Great Lakes of North America to assess the quantity and type of microplastic waste found in these waters, sediments, and beaches. Findings from their own studies are shared, sampling plastic pollution from remote and secluded Nature Reserves in Lake Erie (ON), and the Ottawa River watershed (QC), showing significant accumulation of microbeads.

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Abstract

Spherical ‘microbeads’ made of plastics are now ubiquitous in a wide range of personal healthcare and cleansing products, used by the average North American consumer now at upwards of *quadrillions per day*. Designed to be flushable, these plastic microbeads inevitably end up in municipal wastewater streams, and then to a large extent leak into our freshwater ecosystems. Recent studies throughout the important Great Lakes system of North America have reported microbeads at essentially all locations examined. On the shorelines, in surface waters, throughout water columns, and in sediments of these freshwater systems, microbeads are now ever-present, and are accumulating in significant amounts. Their small and stable shape and composition, and limited pathways to degradation produce a long lifespan, with the capacity to remain in the freshwater environment for potentially hundreds of years. This review collects and compares initial microbead studies between 2013–2021 in the Great Lakes region to provide a snapshot of the current levels and locations, and to serve as a baseline for future tracking to assess progress as the microbead contamination and accumulation problem is addressed. We as well present findings from our own local study of microplastic/bead accumulation downstream of the Great Lakes, in the St. Lawrence and Ottawa rivers near Montreal. Aspects of microbead contamination represent a unique subset of the worldwide microplastic problem, in that much control remains over their life cycle and eventual fate. Consequently, the power to address this microbead problem can rest with polymer chemists and engineers, who, armed with a better understanding of the relevant physical polymer properties of the beads that govern their movement into the aquatic environment, hold the ability to rationally redesign microbead composition and develop removal techniques.

1. Introduction

Lightweight, durable, and inexpensive, plastic is produced prodigiously today worldwide [1], for widespread applications in the packaging, construction, transportation, recreation, and medical industries [2]. Plastics provide diverse benefits: they protect foods from contamination, save fuel during transportation, improve health and medical care, and help provide clean water [2]. However, rather than decompose, most fossil fuel-derived hydrocarbon-based polymers tend to build up in the environment, and discarded plastic has accumulated alarmingly now in our natural environment, flushed from land to sea, and will persist for hundreds to thousands of years. Annual worldwide plastic production is now estimated to exceed 300 million tonnes/year [3], much of which ends up in bodies of water after use. Hundreds of marine species have suffered documented lethal contact with marine debris, and 90% of these encounters involve plastic [4]. As plastic pollution gains media and political attention, governments have started to impose bans on the material. For example, Canada has proposed a ban on single-use plastics by 2021, targeting items such as single-use grocery bags, straws, and disposable cutlery. In addition to outright bans, other promising strategies for dealing with microplastic pollution accumulation include enhanced recycling programs, improved ecolabelling, behavioural change, and some emerging biotechnology solutions [5].

Plastic refers to the family of organic polymers derived from petroleum. Such polymers include polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PETE), and polyurethane (PU) [6]. Although much of the plastic that we use in our daily lives is meso-scale in size (centimetres to metres), the majority of plastic that accumulates in the environment exists as much smaller broken-down plastic particles. Though precise definitions differ, macroplastics are commonly defined to be larger than five millimetres in their longest dimension, while microplastics measure less than five millimetres in any dimension, and nanoplastics under 100 nanometres [7]. Microplastics have been found to account for 92% of plastic debris found at the surface of oceans [8], and include a wide range of small plastic pieces that can be differentiated usefully based on a number of factors such as shape, size, and origin. Origin refers to whether the microplastic is primary or secondary; primary microplastics are those that are intentionally manufactured to be micro-sized, while secondary microplastics are those that are manufactured to be larger but become micro-sized after degradation [9]. Primary microplastics include industrially-manufactured microbeads and virgin plastic pellets, which are used in personal care products and as raw materials for product manufacturing, respectively [10]. Secondary microplastics, on the other hand, originate from larger plastics and include synthetic clothing microfibres and fragments of plastic packaging [6].

Most plastic accumulates first in landfills or on surface environments, then can later partition into soils, freshwater systems, and eventually oceans [1], with lakes and rivers serving as the most important microplastic carrier pathways [11]. For aquatic systems in general, microplastics can derive from point sources like wastewater treatment plants (WWTPs), non-point sources associated with runoff and accidental effluent, and tributaries [11]. While WWTPs can be effective at removing most microplastics, they need to process such large volumes of effluent that many current filter capture designs miss the smaller particles [11], a fact confirmed by observations of increased concentrations of microplastics at downstream sampling sites compared to upstream concentrations [11]. Microplastics may also become re-suspended and even become airborne during the sludge treatment process, thus also making their way directly into terrestrial ecosystems, often over surprising distances [11].

Microplastics tend to build up and accumulate near regions with high anthropogenic influence. One recent study found that high population density near sampling locations was correlated with more abundant plastic debris, while conservation areas, on the other hand, had the lowest abundance [12]. Still, microplastics have been detected ubiquitously in nearly all environments studied, and can even be found in locations with seemingly little direct human influence [6], such as Arctic ice sheets [13] and remote far-north Canadian lakes [14]. Marine studies have found microplastics in surface waters, deep pelagic water columns, deep-sea trench bottoms, and coastal, deep-sea, and beach sediments [[15], [16], [17], [11]]. While there is a growing awareness of microplastics in surface ocean environments, various studies have also found their presence in freshwater, with rivers also potential sinks of microplastics [11]. Microplastic concentrations in 29 Great Lakes tributaries have been found to range from 0.05 to 32 items per cubic metre [18]. Remote freshwater bodies, such as Lake Hovsgol in Mongolia, were also found to contain microplastics [19]. Microplastics have recently been confirmed present in mosquitoes, and can be transferred from the water-residing pupal stage into the flying adult stage, thus potentially impacting terrestrial organisms that feed on this life stage [20]. In general, factors such as size, type, chemical structure, mechanical strength, and weatherability all impact the fate, behaviour, and environmental impact of microplastics [10]. For example, microplastics measured in the reef systems of Australia's Great Barrier Reef were found to be significantly different in chemical composition, shape, and colour to those detected in surface waters [21]. The density of different polymer types can also vary and affect where they are distributed, so reporting a depth of sampling when conducting field studies is important. For example, low-density plastics like PP and LDPE are relatively buoyant, while those that are high density such as PVC, PS, HDPE, and PA are much more likely to sink and accumulate in river sediments [6]. In surface waters, microfibres are often the dominant type of microplastic found [[22], [21], [12]]. This observation could be due to the fact that more effective removal techniques often exist for other microplastic types with more uniform shape such microbeads [11], or that environmental factors such as hydrology are another significant determinant governing microplastic distribution [11].

With such widespread abundance in practically all of our freshwater systems, microplastics are also now inevitably ingested by humans at significant levels. Depending on age and gender, it is estimated that the average American consumes between 39,000 and 52,000 microplastic particles each year [23]. When microplastic inhalation is accounted for, this number—and likely an underestimate—increases to between 74,000 and 121,000 particles per year [23]. In fact, a single plastic tea bag has been shown to release billions of microplastic particles into the hot water on steeping [24]. Although there is still limited research on the effect of microplastics on organisms and food chains, there has been concern that microplastics' ability to transport pollutants adsorbed to their surface, as well as the tendency of chemical additives added in manufacturing to leach out upon ingestion, will lead to an increase in pollutants entering the food chain, particularly endocrine-disrupting chemicals (EDCs) and persistent organic pollutants (POPs) [25]. Plastics themselves are largely biochemically inert and thus do not interact directly with cell membranes, but surface adsorbed pollutants, on the other hand, can interact strongly with living cells. Aside from being ingested by aquatic animals such as barnacles, mussels, copepods, and fish, microplastics are thought to affect sediment-dwelling species, since they can impact the permeability and insulation of sediment environments, thereby disrupting organisms and their eggs [26]. This could affect the gender balance of sea turtles, since the sex of sea turtle eggs depends on temperature [26].

There are numerous other studies showing that exposure to microplastics can trigger toxic effects on the feeding, reproduction, energy metabolism, genetics, and physiology of organisms [27], including reports of microplastics depleting the energy reserves of marine worms by up to 50% [28]. Microplastics can also cause direct effects on prey species, such as tubular dilation in clams, and indirect effects on predator species such as white sturgeon [29]. Schessl et al. in 2019 stipulated that microplastic ingestion may result in gut blockages and that adsorbed contaminants pose a potential risk [30]. The presence of microplastics, for example, reduced chlorophyll concentrations and growth in algae, while in crustaceans, chronic exposure resulted in reproductive and developmental effects [6]. Fish and aquatic invertebrates have been shown to be negatively affected by ingestion of microplastics [31], and other studies have shown that microplastic particles can be transferred from fish to marine mammals**—***ie*: plastic can cross trophic levels [32]. Due to their propensity to bioaccumulate and cross trophic levels, microplastics also carry potential ecological and food web implications. However, it is important to note that the dose of the contaminant, as well as the test species, is particularly important when examining the toxicological effects. For example, at concentrations of 0.008, 10, and 100 milligrams per litre, microplastics were not observed to significantly affect biomarkers in the Pacific oyster *Crassostrea gigas* [33].

One particular group of microplastics that has received recent media attention is the microbead. In contrast to broken-down macro- or meso-plastics, microbeads, also termed "microspheres," "nanospheres," or "plastic particulates" [34], are deliberately produced (primary) microplastic particles that are added to personal care and cosmetic products. They can be as large as 5 mm in diameter and exist at nearly any diameter smaller [35]. Microbeads within the same product can exist in a variety of shapes, and can be uniform in size or range from 10 μm to 2000 mm. While they are generally most easily produced as spherical, they can also be present as ellipses, ribbons, threads, or other fragmented, irregular shapes [36], if easier and/or cheaper to produce that way. Dyes can be added during bead fabrication to produce a variety of different colours, the most common being white or blue, and sometimes pink [34]. Figure 1 presents images of typical microbead additives from several different personal care products, showing common shapes and colours of microbeads, including 'e' and 't' – ellipses and threads, respectively. One study found that microbeads' variable colours and sizes were the most important factors in determining the probability of underestimating microbead numbers [37]. Overall, underestimation of microbead counts in sediment samples was found to exceed 78%, likely the result of observer and technical error [37].

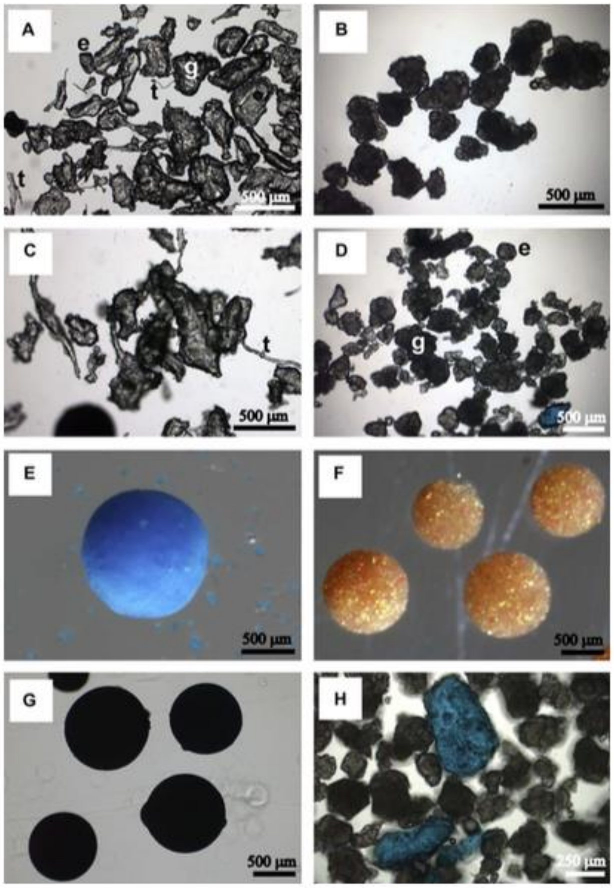


Figure 1: Photomicrographs of various microbeads extracted from personal care products [36].

Applications of microbeads are now ubiquitous, and since microbeads can act as abrasives, use now extends well beyond personal care products to include myriad other products, such as household and industrial cleaning supplies [38]. Approximately 93% of microbeads used in cosmetic products are made from polyethylene (PE), while other common polymers employed include polypropylene (PP), polyethylene terephthalate (PETE), polymethyl methacrylate (PMMA), and nylon [34]. The polymer used to synthesize the microbead influences the properties and behaviour of microbeads in different surroundings, and can have implications on particles moving between disparate environments.

After describing household use of microplastics and their disposal in wastewater and providing an overview of microplastic composition and degradation, this paper reviews ten representative studies conducted across the five Great Lakes of North America conducted between 2013 and 2019, which together represent a comprehensive geographical summary overview of findings on current microbead pollution found across the Great Lakes. In addition, we present the detailed results of a study of our own to observe and measure microplastics/microbeads in sampling locations downstream from all of the Great Lakes, in three contrasting waters: firstly in small fresh rivers in Quebec flowing through a large protected nature preserve remote from human habitation and activity before feeding into the Ottawa River, secondly in the larger Ottawa River itself downstream from increased human habitat, and lastly in the St. Lawrence Seaway at the Island of Montreal, downstream from the effluent of more than 40 million North Americans.

2. Household use of plastic microbeads and disposal in wastewater

Microbeads are designed to be readily discarded down the household drain [38], and therefore possess a uniquely direct route to freshwater contamination. Microbeads rinsed down the drain first enter municipal water collection systems and then wastewater treatment plants, where wastewater is filtered, sedimented, and then chemically treated through several sequential processes. First, large items and particulates are screened out *via* screen filters of successively smaller mesh size. There is an inherent size limit to this filtering, however, as employment of mesh size near that typical of microbeads is prohibitive due to clogging and unacceptable flow reduction. Insoluble contaminants of any size that float can more easily be skimmed off the surface without reducing waste stream flow or clogging filters that require expensive replacement or tedious cleaning, a distinction important to the ‘floating microbead’ discussion that is to follow. After these first two ‘size’ and ‘flotation’ removal steps, what generally remains are soluble contaminants, or particles small enough to remain suspended colloidally in the waste flow. A further wastewater physical treatment step allows the remaining mixture to settle in tanks to reduce flow turbulence, necessary to separate sludge or other suspended heavy solids from lighter liquid phase effluent [8, 37]. This is generally effective at removing the larger and more dense (faster sinking) contaminant particles. In this process, up to 95–99.9% of microbeads may be trapped in the sludge, yet the rest remain in the effluent. Clearly, the smaller and more closely density-matched a particle is to water, the more difficult its removal. For a given size of plastic microbead then, its density becomes a crucial parameter in passing undeterred through municipal wastewater treatment processes and into eventual freshwater contamination. Thus, the microbead manufacturer's choice of polymer is highly consequential, as will be discussed in later sections.

From an American perspective, a conservative estimate in 2017 reported that at least 8 trillion microbeads are emitted *via* effluent into aquatic habitats in the U.S. every day [39], with New York State residents alone washing approximately 20 tonnes per year of microbeads down the drain. Assuming that 99% of microbeads washed down the drains are trapped in the sludge and eliminated from outflow, this estimate implies that 800 trillion microbeads per day remain in this solid sludge from wastewater treatment plants. This sludge is often used for agricultural purposes for which it is spread over the land, resulting in microbeads readily re-entering freshwater ecosystems directly *via* farmland runoff [38].

In response to these initial microbead lifecycle findings, several countries, including the U.S, Canada, and the U.K., aided by companies such as Unilever and L'Oréal [40], have enacted legislation in an effort to reduce or remove microbeads from personal care products and thus control their entrance into the environment. The U.S., for example, passed the Microbead-Free Waters Act of 2015, which prohibits the manufacture and interstate commerce of microbeads in what is termed "rinse off cosmetics" [39]. However, the wording of the act allows for loopholes, as it specifies that a microbead is "any solid plastic particle that is less than five millimeters in size and is *intended to be used to exfoliate or cleanse* the human body or any part thereof" [39]. The efficacy of this legislation may be proven minimal after creative legal interpretation of "*intent to exfoliate*", and while encouraging in its introduction, it is not currently regarded as aggressive or effective legislation for the elimination of microbead pollution in the environment. It seems that simply investing in policies might not be sufficient, as an Australian study showed that investing in campaigns and outreach programs led to a greater reduction of waste than investing in policies [41].

Microbeads are now readily found in freshwater systems around the world. Results from North American studies conducted in San Francisco, California [42] bear similarities to those from South America in Santa Fe, Argentina [43], and provide statistics showing microbeads as contributors to the significant microplastic pollution problem in freshwater systems. While the first of these two studies draws attention to tributaries as a main source, the latter reports on their presence in a lake ecosystem. A study on a remote lake in Mongolia also reported the presence of plastic pellets of undetermined origin [19]. Comparing the results of these studies reveals that the presence of microbeads is dependent on the population of people using microbead-containing products and on the methods used at the treatment plants, which act as the gateway to freshwater habitats.

3. Microbead composition and degradation

Before summarizing the ten studies overviewing the current microbead pollution in the Great Lakes, it is useful to introduce basic microbead composition and life-cycle behaviour once arrived to the environment, and in particular, microbead degradation pathways.

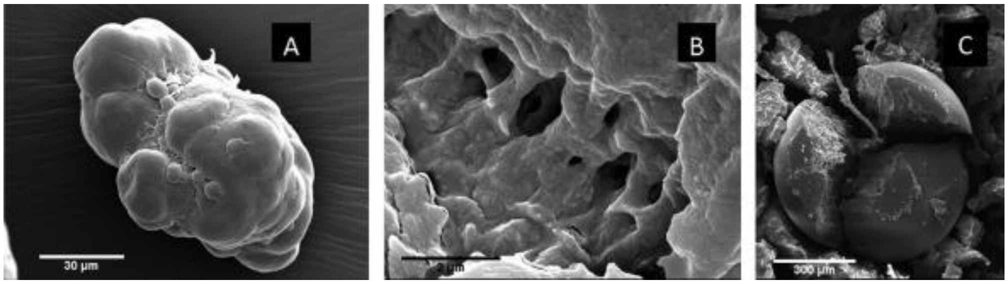


Figure 2: Scanning electron microscopy images of PE pellets found in commercially available face washes, a) rough topography microbead pellet, b) surface microbead topography, c) cracked smooth spherical microbead pellet [34].

While biodegradable microbeads do exist and are receiving recent interest, with various preparations and studies now being reported [[44], [45], [46]], in general these materials are not yet cost competitive with traditional oil-derived commodity plastics such as polyethylene. Thus, while encouraging in development, there has yet to be seen such ‘green’ microbeads make their way to industry on any appreciable scale [44]. The vast majority of microbeads exist therefore as petroleum-derived polymers, the same as our inexpensive and thus plentiful meso- and macro-plastic products. Possessing no natural environmental chemical or biological degradation pathways, these polymers degrade mainly only through deep-blue visible and ultraviolet sunlight photo-degradation, a slow process occurring through electronic excitation from absorbed high-energy photons, causing decomposition or dissociation of the molecular bonds upon absorption of short-wavelength ultraviolet light [47]. The smaller the surface area-to-volume ratio, the slower the photo-degradation, hence spherical microbeads enjoy an especially long lifetime in the environment. However, multiple studies have cited organisms such as bacteria, fungi, worms, and biofilms as being potentially capable of degrading plastics [[48], [49], [50], [51], [52], [53], [54]].

Similar to its dominant market share in meso- and macro-plastic products, polyethylene (PE) accounts for 93% of microbeads found in cosmetic products [34]. A 2015 study confirms the abundance of PE pellets found in six of the best-selling commercial facial scrubs [34], characterizing and reporting the different sizes, shapes, number of particles, and mass of the particles present. These physical characteristics all influence the potential for these particles to pass through typical wastewater processing systems, with density being perhaps the most crucial factor. The researchers showed that the size of particles varies within the different facial scrubs studied, but that the distribution of sizes seems consistent, ranging from 10 to 1000μm in diameter for five of the six scrubs studied, with one of the scrubs having a larger range between 10 and 2000μm [34]. This diameter range is compatible with a similar study performed in 2009 [36]. The number of particles in the cosmetic products was determined to be less than 10,000 per mL for five of the six scrubs, while the remaining scrub contained more than double this amount of particles [34]. Additionally, all brands of face wash were comprised of a range of particle shape and surface topography, including ellipses, ribbons, threads, and fragments. Finally, it was found that surface topography affects sorption of organic pollutants: rough forms of the PE spheres showed greater sorption capacity than the smooth forms for both PHE and DDT pollutants [34].

A study published in 2016 that sampled microbeads in the Great Lakes region investigated microplastics composition through Raman spectroscopy [55]. Of the 90 particles studied, 60 were determined to be synthetic polymers, 3 were identified as non-plastics, 5 contained plasticizers, and 22 could not be identified. Of the analyzed particles, 31% were confirmed to be PE, while 10% was polystyrene (PS) and 4% were polyurethane (PU) [55]. While PE was the most abundant polymeric material found, one might expect the percentage of PE to be far higher considering that 93% of all microbeads in commercial cosmetic products are made of PE [34]. This leads to two possible conclusions: either PS or PU are more likely to be transferred to aquatic environments, or these microbeads are in fact secondary microbeads. As with nearly all of the most common commodity plastics, all of the various PE varieties are slightly more dense than freshwater, so they sink, but slowly—especially with small size, spherical shape, and weak self-aggregation forces—combining into a worst-case scenario for easy removal from effluent. The same slight and unfortunate density advantage over water is true for five of the other most common seven plastics: poly(ethylene terephthalate) (PETE), poly(vinyl chloride) (PVC), polystyrene (PS), polycarbonate (PC), and polyurethane (PU). Density values of the plastics used for microbeads range from 0.91 g/cm^3 (for PP) and 1.01 g/cm^3 (for PS) at lower-density, to 1.39 g/cm^3 for PETE and 1.41 g/cm^3 (for PVC) on the higher-density end of range [56]. In comparison, the density of freshwater ranges from 0.997 g/cm^3 to 0.999 g/cm^3, depending on the temperature (25 °C to 15 °C). In general, then, these small spherical microbead particles sink very slowly in wastewater, if at all, and thus resist separation during municipal treatment and pass as effluent, yet eventually sink in the Great Lakes and their associated connecting rivers and the Saint Lawrence Seaway outflow. Other fates include floating at or just below the lake surface (if photo-degradation leads to bubbling of the material or other decomposition effects that lower the density to near or below that of water), or partial sinking to a depth at which the bead density equals that of water at that lower sub-surface temperature, such as resting at a thermocline layer interface. The one notable exception to this density-driven determination of fate is PP, the only ‘plastic that floats’ with a significantly lower (~10%) density than fresh water, and hence PP’s inherent advantage and widespread use in watercraft and watersports applications. To determine the exact compositions and morphologies of beads most likely to reach aquatic environments, and to determine where in these environments they will eventually partition, more detailed examination must be undertaken. Studies that link the size and shape of beads found in the environment to clues as to where, how, and why they were initially produced by the manufacturer will be particularly useful.

4. Great Lakes regions studied

An important system to study is the Great Lakes of North America: Lake Superior flows into Huron, then into Erie, and finally, Ontario, straddling and forming much of the border between Canada and the United States (Figure 3). Lake Michigan in the US empties into Huron, so all 5 lakes eventually outlet *via* the St. Lawrence seaway into the Atlantic Ocean [57]. The population in both Canada and the U.S. together that depends on these Great Lakes as a life source now exceeds 40 million people, providing drinking water directly for roughly 10% of the U.S. population and more than 30% of the Canadian population [58]. The system further provides 56 billion gallons of water per day on average for agricultural, municipal, and industrial use. Comprising 95,000 square miles, the Great Lakes represent the largest surface area and repository of freshwater in the world, making up 84% of North America’s surface fresh water, and together hold 21% of the world’s supply of surface fresh water [58].

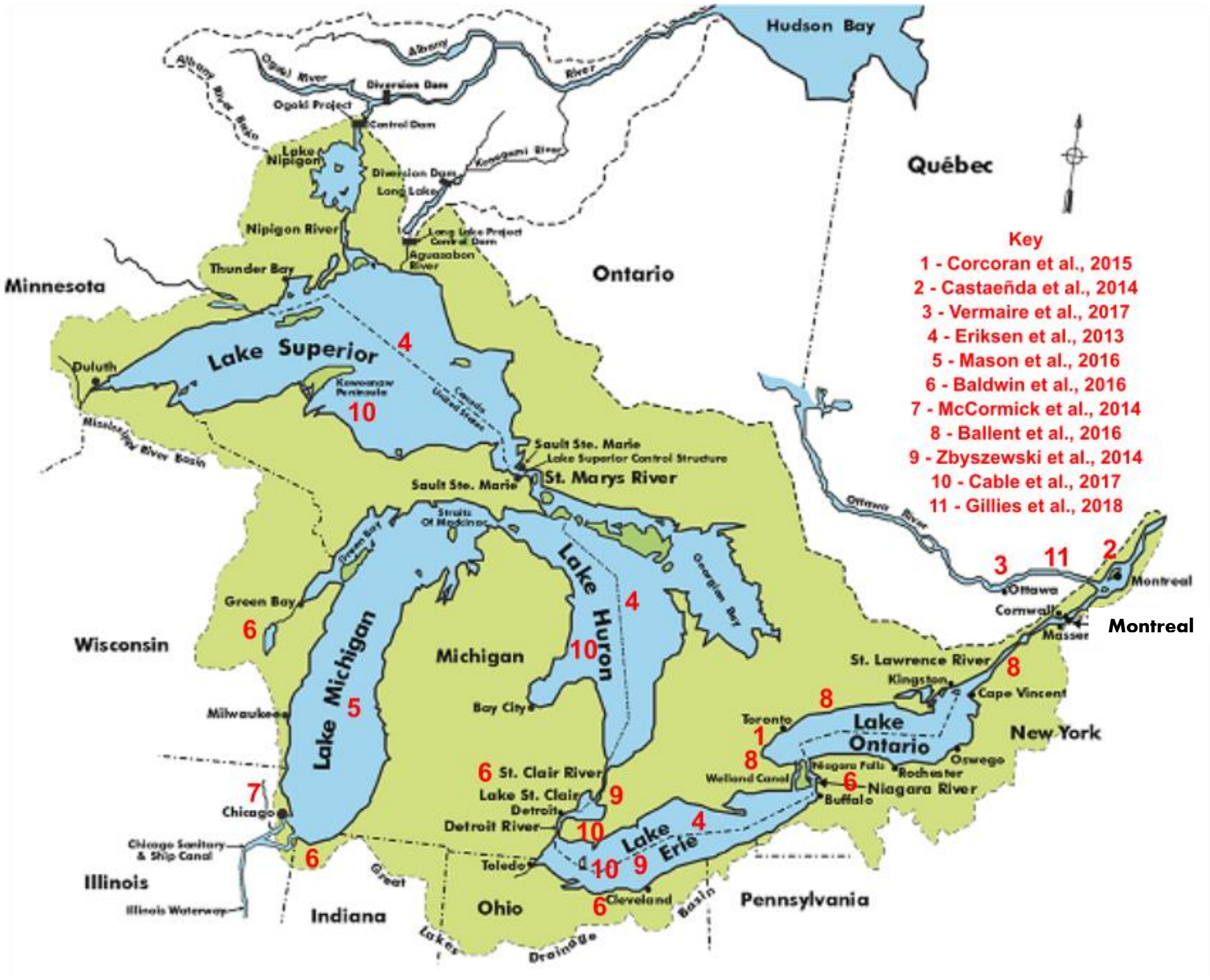


Figure 3: Map of the Great Lakes watershed, with numbers identifying the locations of the studies reviewed in this paper, as the watershed flows from Lake Superior in the Northwest, past Montreal to the East *via* the St. Lawrence River. Map Source: U.S Army Corps of Engineers, Detroit District.

Since each lake possesses a different waste effluent inflow, size, shape, cross-sectional profile, and thus retention time (i.e. the amount of time it takes for each lake to rid itself of pollutants), it is imperative to conduct studies at different parts of all five lakes to compile a full picture of microbead pollution and its effects. For these reasons, this paper reviews ten large studies conducted across the five Great Lakes mainly over five years (2013 – 2017), with some added sampling and analysis collected and conducted by the authors in 2018 and 2019 near Montreal, Quebec, at the outflow, and in 2020 and 2021 in Long Point Bay on Lake Erie. The map in Figure 3 displays the location of the studies, which together represent a substantial body of research and a comprehensive examination of current microbead pollution in the Great Lakes.

While there is growing evidence of microbead contamination throughout the Great Lakes, specific ecotoxicological consequences are as yet less clear. It has been shown in some experimental trials that plastic debris can transfer chemical additives/plasticizers that cause detrimental physiological effects to marine life [[59], [60]]. Many recent reviews now thoroughly address the chemical and toxicological effects of microplastic waste in general, so for the purpose of this review we focus on the question of sources and current extent of microbead contamination and accumulation in the Great Lakes, as opposed to environmental consequences. The following section overviews a comparison of ten recent and extensive studies reporting microplastic concentration in different regions of the Great Lakes watershed. Included in this comparison are the various contrasting regions of the Great Lakes that were sampled in each study, the techniques used to perform sampling and obtain data, and the findings from each study. Firstly, studies will be presented in chronological order; in the sections comparing techniques and findings, they will be presented based on similarity of the methods employed.

In 2013, a study was conducted to test for microplastic pollution in the surface waters of Lake Superior, Lake Huron and Lake Erie [61]. The expedition began in Lake Superior and ended in Lake Erie, extracting five samples from the former and eight from the latter and Lake Huron. In 2014, three notable studies were conducted in different regions of the Great Lakes in Canada, a study of microplastic pollution in the St. Lawrence river was performed, testing sediment specifically at ten different sites along the river [62]. Notably, four of these sites were located downstream of municipal or industrial plants, and one was located downstream of a nuclear power plant. Across the border in the U.S., a similar study was undertaken in the North Shore Channel in Chicago, which links Lake Michigan with the Mississippi River [63]. Notably, this channel also contains several wastewater treatment plants along its stretch. Finally, a third study was conducted in 2014, but this time widely surveying a number of the Great Lakes [64], including Lake Huron, Lake Erie, and smaller Lake St. Clair that connects them and that also makes up part of the Great Lakes system. In 2015, Lake Ontario, more specifically the Humber Bay region near metropolitan Toronto, was tested for plastic pollution [65]. Samples were taken from the beach, along the bank of the Humber River, and from the lake sediment. In 2016, researchers tested Lake Ontario again, this time sampling from nearshore, tributary, beach sediment, and even parts of the St. Lawrence river connected to the lake [55]. A more extensive study was done, sampling from 29 of the Great Lakes tributaries, spanning six different states and accounting for 22% of total tributary contribution to the Great Lakes [18]. This report included a map highlighting the tributaries sampled, and went further to indicate the uses of the tributaries in the different states, shown in Figure 4. Finally, in 2016, a study examined plastic pollution in the surface waters of Lake Michigan, in the lake’s open water and at a variety of sites on the lake, as is identified in Figure 5 [66].

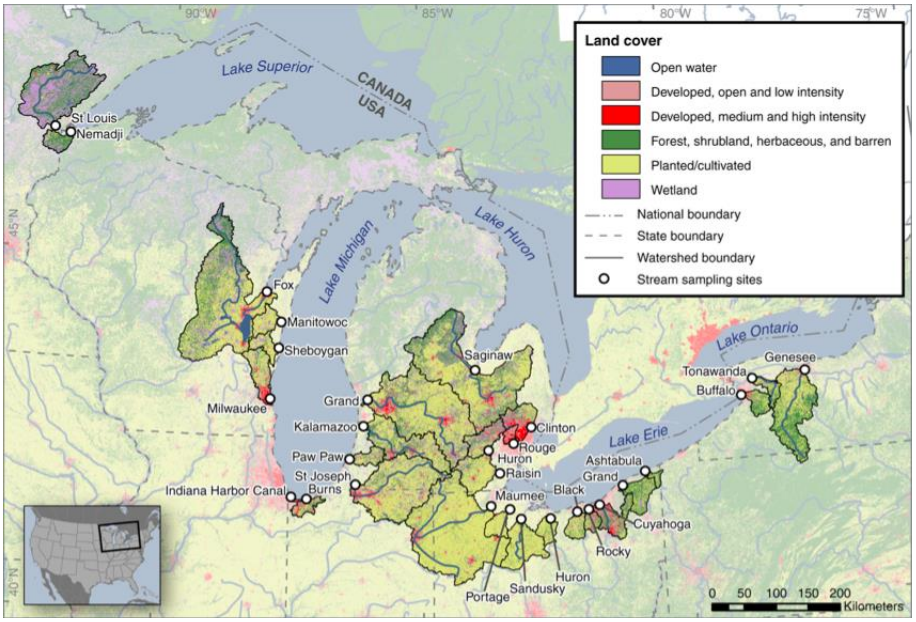


Figure 4: Map of Great Lakes tributaries studied, and uses of land in each US watershed [18].

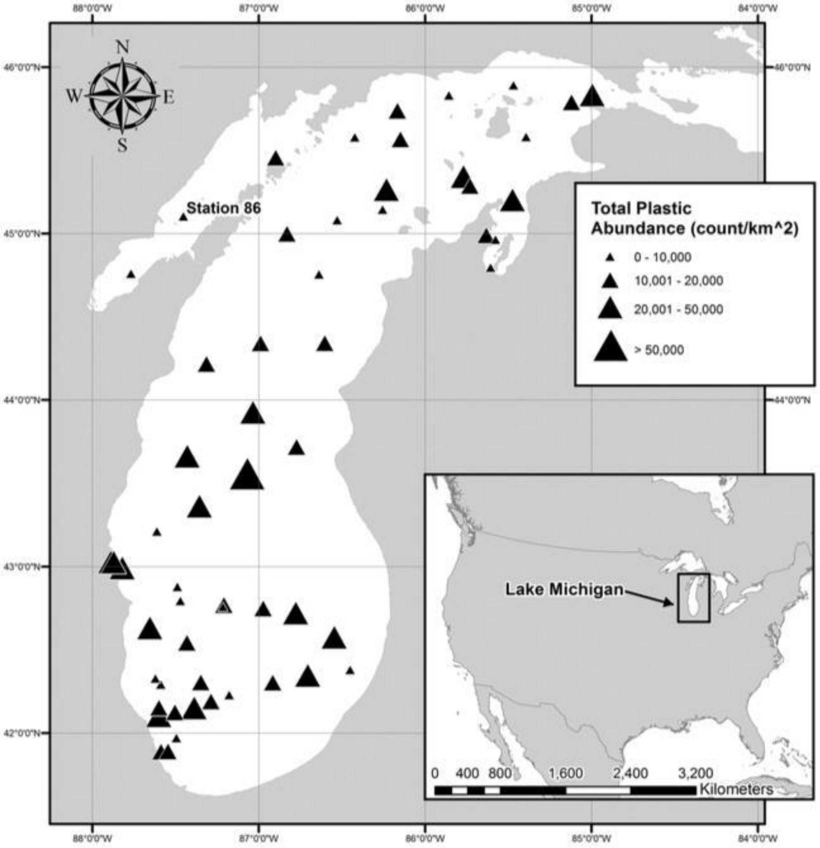


Figure 5: Distribution of plastic found in Lake Michigan surface water samples during summer 2013 [66].

A 2017 study examined the distribution of microplastic pollution across 38 different stations in the surface waters of Lake Superior, Lake Huron, Lake St. Clair, and Lake Erie [67]. The second study finished in 2017 observed microplastic pollution in the Ottawa River (draining much of Eastern Ontario and flowing into the St. Lawrence River at Montreal) and several of its tributaries, from the water column and surface water [68]. Finally, our studies conducted in the summers of 2018–2021 examined microplastic abundance observed in Long Point Bay Biosphere of Lake Erie, and across 29 sampling sites in the surface waters of the Kenauk Nature Reserve, a large human-free protected wilderness watershed area in Quebec whose rivers empty into the Ottawa River near the village of Montebello; secondly, the Ottawa River, downstream from habitat for approx. 1–2 million humans; and lastly in the St. Lawrence River, which drains the entire Great Lakes Region, home to more than 40 million human inhabitants in the United States and Canada.

5. Techniques used for water sampling

One of the largest challenges facing direct comparisons between various field studies is the use of diverse field sampling techniques, lab methods, and measurement units, making it challenging for clear comparison [69]. Sampling methods from surface waters include pumping, trawling, or filling bottles or buckets, followed by sieving to isolate particles of the desired size [70]. All of the studies showed techniques used to sample plastic from the water that were similar to at least one other study. The most commonly used technique was the manta trawl, which is a large net that can sample greater volumes of water but tends to collect samples primarily from the surface or the top of the water column [[61], [66], [67], [68]]. Concentration of microplastics in the water can be calculated using a flowmeter and measurements conducted over a set time interval. The mesh size of the net can vary, with the smallest being 100 mm [67] and the largest and most common being 333 mm [61]. After collection, manta trawl nets are rinsed into a vial that is then brought to the laboratory for further inspection.

Another similar sampling method is the use of a neuston net. Two studies included in this review deployed neuston nets to collect samples, one in some of the tributaries of the Great Lakes watershed [18], and the other in the North Shore Channel in Chicago [63]. Neuston nets are similar in theory to manta trawls, as they collect samples from the surface and upper water column of aquatic systems. Both studies used neuston nets with mesh sizes of 0.333mm, identical to that of the manta trawls used in some aforementioned studies. Again, concentration can be calculated by measuring water flow with a flowmeter and taking measurements in set time intervals. The key difference is that neuston nets can be deployed behind or beside a boat, or even by wading, whereas the manta trawls were deployed exclusively by boats.

Wading could allow access to sampling shallower waters that would not be possible with the use of only manta trawls, thus providing a better geographically unrestricted representation of microplastic, and effectively microbead, concentration in fresh water.

Three of the studies chose to focus on microplastics found present in sediment, therefore requiring a different technique than nets for sampling. The study conducted along the St. Lawrence River used a combination of two sample collection techniques: first with a petite ponar grab, then with a Peterson grab [62]. The former allows for sampling of depths up to 10 cm, while the latter allows for sampling further to depths of 15 cm. The second study, published two years later, extracts sediment samples using a petite ponar grab and stainless steel split spoon corer [55]. Samples that were taken from shallower depths were completed using a Glew gravity corer and a Shipek grab sampler. The final study included sediment sampling and used a mini box corer at different depths of Lake Ontario: one closer to shore, and the other in a deeper, more central location [65]. Finally, two of the studies collected plastic from land, on the beaches of several Great Lakes. Collection was carried out by simply measuring out a quadrant and taking samples to later be inspected in the lab [[64], [65]]. One of the studies specified the instrument used to collect plastic debris on the beach: a stainless steel trowel [64].

In general, the steps to identifying and analyzing microplastics are separation from organic matter, microscopic visual observation, and then chemical analysis [71]. Firstly, organic matter must be separated from microplastics before identification. This is normally accomplished with acidic, oxidative, alkaline, or enzymatic digestion using chemicals such as hydrogen peroxide, sodium hydroxide, hydrochloric acid, nitric acid, or potassium hydroxide [72]. Other methods include density separation [73], and oil extractions that take advantage of the oleophilic properties of plastics [74]. The latter methods can offer some benefits, in that they do not cause discolouration or physical alteration of plastic particles as does acid digestion, which can render visual assessment difficult [75].

Laboratory treatment was generally similar across all studies. Most generally followed the procedure outlined in "*Sources and sinks of microplastic in Canadian Lake Ontario nearshore, tributary, and beach sediments*" in which samples were dried, weighed, and sieved several times through filters of varying mesh sizes to divide plastics into size classes [75]. Further separation can be accomplished with a procedure based on density—for example, separating plastic that floats from plastic that sinks [55]. Microplastic can then be further examined for chemical composition and by morphology. The wet peroxide oxidation technique is used to remove organic material from microplastic so it can be observed under a variety of microscopes, including stereo microscopes, which allow microplastics to be more easily quantified and separated into different morphology types.

Following extraction, microplastics can be identified with light microscopy and visual sorting [73]. Visual keys often describe identifying microplastics based on the absence of organic or cellular structures, homogeneity across particles, and consistent colours or gloss [73]. While visual identification is quick and relatively available and inexpensive, it is prone to high error rates, so secondary analyses are often employed to confirm the polymer's identity. Such analyses include the 'hot needle test', polarized light microscopy [73], thermal analytical techniques [76], or more advanced chemical techniques, including vibrational spectroscopy, densitometry, and gas chromatography-mass spectrometry [77]. Two major spectroscopic methods are FTIR (Fourier Transform Infrared Spectroscopy) and Raman, both of which employ vibrational spectroscopy to obtain the frequency spectrum of a particle, specifically the tell-tale 'fingerprint regions' unique to each polymer's molecular structure. Both methods exploit the fact that molecules absorb frequencies characteristic of their structure. A particle's IR spectrum can thus be used to positively and conclusively identify the chemical polymer type of microplastic found [72]. Across these studies, FTIR and Raman spectroscopy represent the most frequently used techniques to determine polymer composition [55], while additionally, Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) has also been used successfully to assess composition of beads [61].

Other useful analysis techniques reported included selective fluorescent staining using Nile Red dye, which adsorbs to plastic surfaces, rendering them fluorescent under blue light [78]. Once the Nile Red dye is applied, highly-sensitive fluorescence microscopy can be used to characterize resulting emissions to precisely detect and count microplastics [78]. Other studies have also used Nile Red dye to stain the microplastics, followed by observation with fluorescence microscopy and image analysis software [79]. Scanning electron microscopy (SEM), a powerful high-resolution type of electron microscopy that produces visual images of a sample by scanning the surface with a beam of electrons, has been shown to quickly screen for plastic against non-plastic pellets, and can also be used for the identification of smaller fragments [80]. Machine learning has even been applied to assist microplastic identification, where machine learning algorithms were able to correctly identify spectra of some the most prevalent polymers such as PE [81]. Such advanced techniques are still relatively new however, and were not used in any of the Great Lakes studies discussed in this paper.

6. Findings of the Great Lakes studies

For studies focusing on surface water and the upper water column, the best comparison comes from juxtaposing average abundances or concentrations of plastic particles per unit of volume. However, units used for volume varied greatly, so a next best way of comparing results from studies is through percentages; specifically, comparing the fraction of plastic microbead pellets or spheres to that comprising the total amount of microplastic found from sampling. For visual comparison, Table 1 depicts these percentages of microbeads—or equivalently "pellets," as they are called in a handful of the studies—associated with their study and year.

Location of Study	**Percentage of microbeads found**	**Year**
Lake Ontario [65]	shoreline 74%; sediment <6%	2015
St. Lawrence River [62]	80%	2014
Ottawa River and Tributaries [68]	6%	2017
Lake Superior, Huron, & Erie [61]	48%	2013
Lake Michigan [66]	4%	2016
29 Great Lakes Tributaries [18]	<2%	2016
North Shore Channel, Chicago [63]	2.26%	2014
Lake Ontario [55]	<10%	2016
Lake St. Clair & Erie [64]	St. Clair – 13%; Erie – 39%	2014
Lakes Superior, Huron, St. Clair, & Erie [67]	0.2%	2017

In order to draw meaningful conclusions, it is important to link the findings of these studies, where in each sampling locale (i.e. sediment, surface water) samples were collected, and when they were collected. Results will be grouped and presented by the region of the water that was tested. Firstly, the studies sampling from surface water of the different Great Lakes bodies of water will be compared. Then, studies analyzing sediments will be compared, followed by studies that sampled from shorelines/beaches.

From the study conducted on the Great Lakes of Superior, Huron, and Erie in 2013, it was determined that approximately 48% of all plastic collected during sampling was composed of pellets, while non-spherical plastic fragments made up the other majority [61]. In this study, it proved crucial to assess the composition, which was completed using electron dispersive X-ray spectroscopy and scanning electron microscopy (SEM/EDS). This was because what was at first identified as a microplastic (particle labelled B) based on its size, shape, and colour—and thus hypothesized as likely a microbead—was found instead after chemical analysis to be coal fly ash, shown in Figure 6. This distinction will become crucial to confirm in future studies, as the Great Lakes region has also been home to much fallout from coal-fired electricity generation plants in the past, and still some in the present.

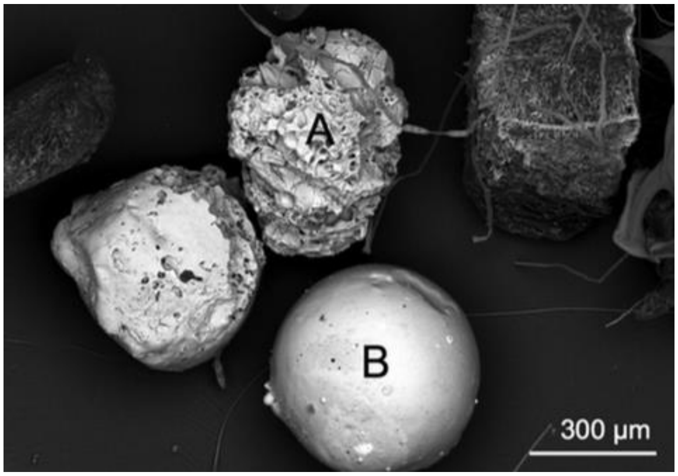


Figure 6: Coal Ash in two forms, identified by composition confirmed with SEM/EDS, originally mis-identified as microplastic [61].

Comparatively, the remaining studies and our own that tested surface waters for plastic pollution found that pellets/microbeads contribute much less than that found from the 2013 study. The other five studies that sampled surface water were conducted post-2013, and of these studies, the highest percentage of pellets/microbeads reported was 6% [68], followed by 4% [66], 2.26% [63], <2% [18], with the lowest percentage reported of 0.2% [67]. It may be important to note that plastic pollution overall was significant; in every study, plastic was found in either all samples or all except for one. However, there are large differences compared to the microbead percentages reported in the 2013 study on Lakes Superior, Huron, and Erie [61]. This discrepancy could be the result of events such as mistaking other particles for plastic microbeads, as described with the coal ash similarity problem. Lake Erie is reported to be especially polluted with plastic debris, considering the multiple points of high population along its shore and relatively low volume compared to its neighbouring lakes. However, the study in 2017 also sampling from Lake Erie [67] reported the lowest of all concentrations of microbeads. A final possibility for this discrepancy could be inconsistent methodology used for both sampling and counting microbeads, which has been cited as an issue for comparison of studies, and which therefore poses a general problem in estimating microbead pollution across the Great Lakes watershed. The depth of sampling, as well as more localized factors such as water flow, could also explain the discrepancy in microbeads, since microplastic fate heavily depends on properties such as density. Microfibres are now often the most prevalent microplastic in surface water samples, due to both their density and the fact that better removal techniques exist for lower aspect ratio microplastic types such as microbeads [42]. Nonetheless, it is crucial to understand that the data reported above together comprise a consensus of a significant plastic microbead accumulation in these aquatic environments, and though pellets/microbeads may not make up a large percentage of what is found, they still can cause adverse effects to the ecosystem and represent a significant new contribution to the already immense problem of plastic pollution in freshwater environments.

In the study reported on the surface water of the North Shore Channel in Chicago [63], the difference between surface water concentration of microplastic upstream of an urban wastewater treatment plant was compared with concentrations from downstream of the plant. Not only did pellet/microbead concentration increase from upstream to downstream locations, but overall microplastic concentration increased further downstream from the treatment plant. The study included a bar graph, which is reproduced here as Figure 7, and which compared the upstream and downstream microplastic absolute concentrations in the Great Lakes with the concentrations found off the California coast and in parts of the Pacific Ocean. Overall, a greater amount was found in the Great Lakes than in these ocean locales.

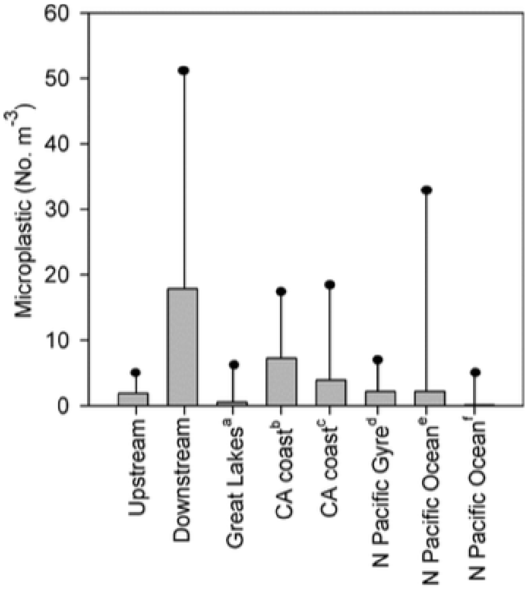


Figure 7: Mean and maximum microplastic concentrations of the study sites upstream and downstream of a Great lakes wastewater treatment plant, compared to some Pacific Ocean concentrations reported in previous literature [63].

The data in Figure 7 clearly suggest that wastewater treatment plants can act as a direct and major route of plastic debris' entrance into freshwater systems. The comparable microplastic concentration upstream to those present in, for example, the North Pacific Gyre, indicates that although wastewater treatment plants act as a source of entry, they are not the only conduit for plastics to enter the environment. Gyres are large systems of rotating ocean currents where ocean plastics have been shown to collect and accumulate, making them regions of some of the highest plastic concentrations observed in the ocean [82]. The comparison of river microplastic concentrations to oceanic concentrations suggests that research must focus not only on plastic accumulation in oceans but also in freshwater systems, since freshwater has shown higher microplastic concentrations and is likely a major source of plastic debris in the ocean.

Figure 8: Typical plastic microbeads recovered from bottom sediments along the St. Lawrence river [62].

The studies that sampled from sediment did not consistently report a breakdown of morphologies that made up all the plastic extracted from the sediment. Instead, the study completed in the St. Lawrence River looked only for microbeads in the sediment [62]. To note from these reports is that microbeads were recovered at eight of the ten sites sampled, which was labeled numerically as 80% in Table 1, and the mean density of microbeads at a site was approximately 13,800 microbeads per m^2 of bottom surface area [62]. Figure 8 provides a visual confirmation of the microbeads recovered from some of the sites tested along the St. Lawrence River.

Microbeads found in sediments are not often uniform; as can be seen in Figure 8, they exist in different shapes, diameters, colours, and transparencies. In the study that reported microplastic pollution in sediments at different bottom locations of Lake Ontario, including the figure comparing abundance of different microplastics at the different sites is more revealing than reporting the numerical values of microbeads [55]. In Table 1, the average percentage of microbeads of the total microplastic found in the sediments was estimated from the graph to be less than 10%. Figure 9 provides a comparison in abundance of fiber, fragment, and bead in the three different sediments tested.

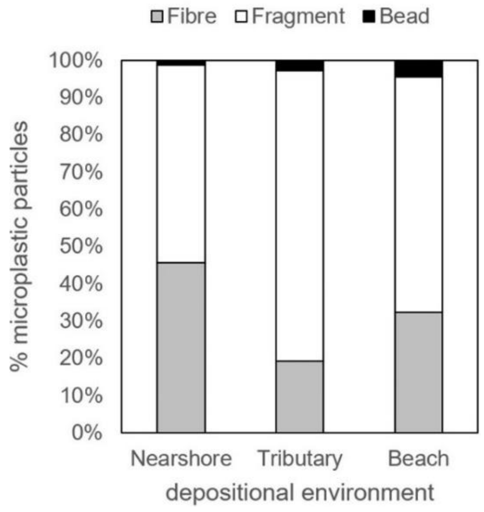


Figure 9: Chart of various microplastic morphology categories found present in three different sedimentary environments in Lake Ontario [55].

Figure 9 suggests that of all microplastic present in the sediments sampled in Lake Ontario, microbeads make up a small percentage. Microbeads are most abundantly found in beach sediments, likely making up no more than 5% of total microplastic extracted. The other study that sampled from Lake Ontario in 2015, and specifically in the Humber Bay region near Toronto, also found low levels of microbeads/pellets in its sediments. In fact, the only numerical report of microbeads or pellets from samples is in a table noting that only two out of 35 plastic particles from the sediment displayed a “rounded” shape [65], which could be considered as possible microbeads. This means that microbeads accounted for less than 6% of the microplastic recovered from these sediments, and overall microbeads seem to have a greater tendency to remain in the sediments of rivers than lakes, based on the studies in review. Consequently, it is perhaps more important to focus on the presence of wastewater treatment plants as a source. Along the stretch tested of the St. Lawrence River, there were five large treatment plants, whereas the the lake sediment sampling locations were located near fewer/smaller treatment plants. Considering proximity of treatment plants with current and behaviours of river ecosystems, perhaps sedimentation in the river is follows a pathway in a manner dissimilar to that in lakes. If true, this again however provides a strong implication for wastewater treatment plants as a major source of microbead pollution in the environment.

Two studies surveyed beaches of the Great Lakes for plastic debris. In a 2014 study, Lake Erie and Lake St. Clair beach sediment of differing compositions—sandy, gravelly, and muddy—were examined [64]. Of the debris recovered from Lake Erie beaches, 39% were found to be pellets. Interestingly, not only was the greatest abundance of plastic debris observed along the shoreline of the most populated regions—

Presque Isle (Erie, PA) and Fairport Harbor (Cleveland, OH)—but also, some of the highest concentrations of pellets. This can be seen clearly in Figure 10, where plastic debris abundance is given by *n*, and where pie charts present the types of plastics that made up this debris, with pellets in black.

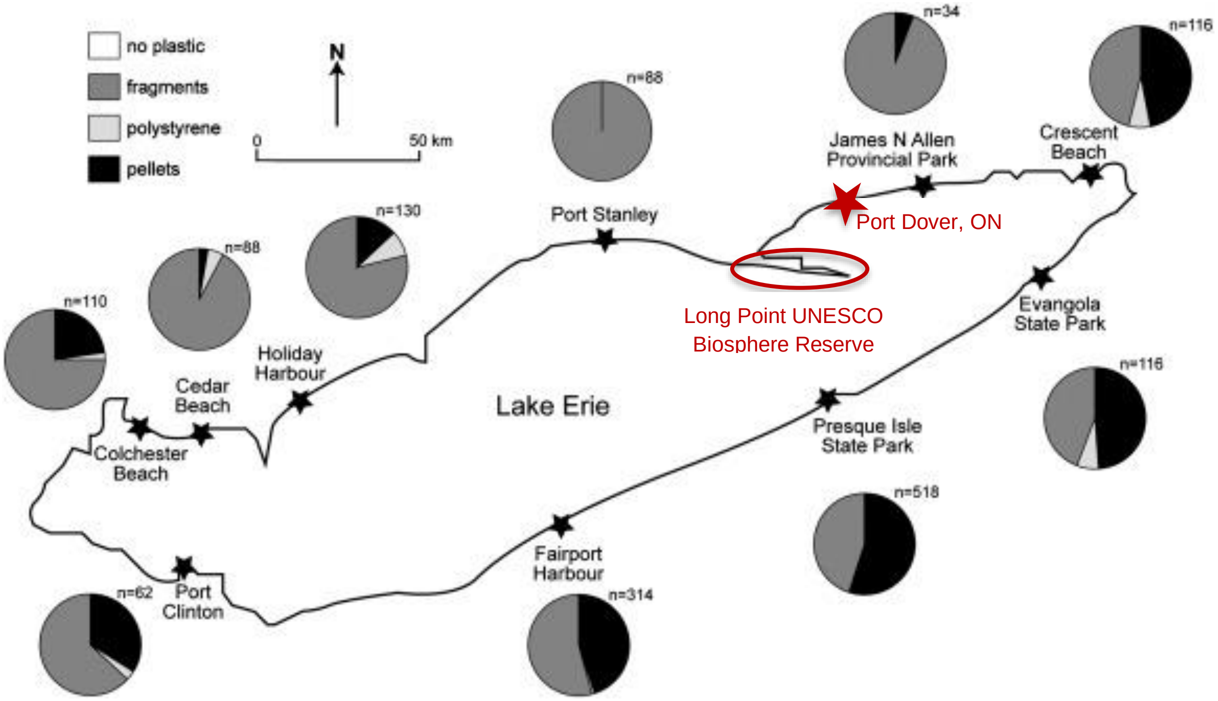


Figure 10: Analysis of plastic debris found on various shoreline locations of Lake Erie, with pie diagrams illustrating the relative abundance of each of the different types of plastic found [64].

Only approximately 13% of the plastic debris recovered from the beaches of Lake St. Clair were pellets. These results contrasted to those of a study previously conducted on Lake Huron, which reported that 94% of plastic debris recovered from the beaches were pellets. In the first part of a study conducted on Lake Ontario [65], a section of the Humber Bay beach was sampled to see what types and how much plastic contributed to pollution on the beach, and here also pellets dominated, comprising 74% of all plastic debris collected on the beach. Although Lake Erie is centered in a generally high-density urban environment on both sides of the USA-Canada border, an important opportunity is presented by Long Point, a 40km sandspit extending from the northern Canadian shore, that is protected from influence as a UNESCO World Biosphere Reserve, with restricted access by boats and pedestrians. Sampling of beach wash *via* kayak can be achieved however from Long Point Bay locations such as Port Dover ON (map Fig. 10), with strong confidence that beach artifacts found here were deposited by the lake, and not by human visitors from shore. A typical sample of microplastics sampled from these secluded beaches is presented in Fig 11, confirmed as plastic microbeads by spectroscopic and NMR analysis (Chemistry Department, McGill U. Montreal).

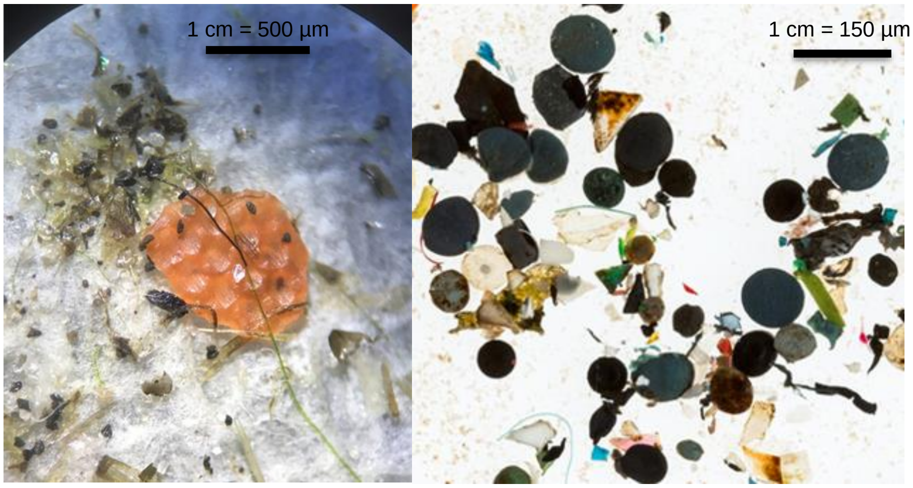
Since such high percentages of microbeads/pellets are found washed up on beaches of lakes, it can be inferred that much of these microplastics float, thus responding to the question of lesser abundance of microbeads in the sediments of lakes than rivers. As with most particulates and debris that can wash up on a given beach for sampling, results can vary greatly depending on the size, shape, and grade of beach (and thus mean water velocity for settling), and also on recent wind, wave, and weather patterns. However, in general, the highest concentrations of plastics were found nearest high population densities, which are usually accompanied by a greater number of wastewater treatment plants that could respond to the waste. If microbead pollution is to be reduced, catching microbeads by these treatment plants is a key.

While studies have reported varying amounts of microbeads on the shorelines, in surface waters and water columns, and in sediments of freshwater systems, overall microbeads are present and accumulating in significant amounts. Simply by being present in an ecosystem, microbeads are expected to have an impact, though this will likely vary depending on abundance. Their composition and limited means of degradation indicate a long lifespan, meaning that they have the capacity to remain in the environment for potentially hundreds of years and have detrimental effects. There appear to be clear population and down-stream effects: the studies completed at Fredonia in 2012 and 2013 estimated that in the northernmost Lakes, Superior and Huron, there were about 7,000 plastic particles per square kilometer. Lake Michigan exhibited 17,000, then the combined flow into Lake Erie found 46,000, and last-in-stream and most highly-populated Lake Ontario sported 248,000 particles per square kilometer, nearly 40 times that of Superior.

7. Downstream study of 3 contrasting sample locations near Montreal

In the summer of 2018, we carried out sampling in the Great Lakes watershed, downstream of the Great Lakes system. Sampling was conducted over 29 sites in three distinct but connected freshwater systems: firstly in rivers and lakes in the Kenauk Nature Reserve, a 65,000-acre restricted access protected wilderness reserve near Montebello, Quebec that drains into the Ottawa River directly or indirectly through the Riviere Rouge; secondly along the Ottawa River itself downstream from modest development, after draining large areas of Eastern Ontario and Western Quebec; and lastly in the St. Lawrence River on the banks of the island of Montreal, where the Ottawa River joins the Saint Lawrence to drain the Great Lakes eventually into the Atlantic Ocean. Surface water samples were collected using a ‘scoop and filter method’ (see supplementary information for details), after which an oil extraction was performed to isolate the microplastics, which were then examined and characterized at McGill University’s Chemistry Department.

In summary, microplastics were found in every sample collected, including the attempted control consisting of municipal water taken from a kitchen faucet nearby. We observed an average of 6.7 microplastics per site, or approximately 0.0084 pieces/L. Fibre was the most frequently occurring type, representing 67% of all pieces found. Only 0.003% were found to be microbeads at this location. Sampling sites near boat and recreational activity generally resulted in higher microplastic abundance, and sites with high microplastic abundance were located in all study regions (Kenauk, the Ottawa River, and the St. Lawrence River). While study location was predicted to have an effect on microplastic abundance (since Kenauk has far less human influence than the other areas), it was not observed in fact to have a significant effect on microplastic abundance, although the difference in abundances between Kenauk and the other two regions was more significant than those between the Ottawa and the St. Lawrence. A similar relationship was reported for population level and microplastic abundance as higher populations resulted in higher microplastic abundance. *Ie*: waters sampled in the protected nature reserve appeared to be similarly polluted with plastic as those locations downstream from either modest, or significant human habitation. The plastic appears to be partitioning everywhere one thinks to look.



Figures 11 (left) and 12 (right): Optical microscope images of typical samples we collected. (left): orange microplastic fragment overlaid with a black microfibre, from the St. Lawrence River in QC, 2018. (right): microplastics including suspected microbeads sampled from Lake Erie near Port Dover ON, and from secluded beaches in the Long Point UNESCO Biosphere Reserve, 2020–2021 [map Fig. 10].

8. Conclusions, perspective, and outlook

Various studies across the Great Lakes Regions report varied amounts and compositions of plastic microbeads on the shorelines, in surface waters, water columns, and sediments of freshwater systems. What is clear is that overall, microbeads are present and accumulating in significant amounts. They have been found at essentially every location sampled, including large nature preserves supposedly isolated from human activity. The stable shape and composition of microbeads and limited pathways to degradation produce a long lifespan, with the capacity to remain in the freshwater environment for potentially hundreds of years. There appear to be clear population-links and down-stream effects from treatment plant effluent, as the studies completed at Fredonia in 2012 and 2013 estimated that in the northern first two Lakes Superior and Huron there were about 7,000 plastic particles per square kilometer. Lake Michigan exhibited 17,000, then the combined flow into Lake Erie found 46,000, and last-in-stream and most highly-populated Lake Ontario sported 248,000 particles per square km, nearly 40 times that of Superior.

Clearly, the most direct and far-reaching solution to reduce and even reverse microbead accumulation in fresh waters might be legislative, restricting or banning use entirely. With microbead incorporation into personal care now so prevalent and ubiquitous however, a more immediate and perhaps even more effective solution might be replacing current microbeads with an equivalently performing, available, and affordable alternative. With greatest ease, for example, there is some suggestion from these studies that simply using lower-density polypropylene (PP) beads, or other floating plastics in place of PE, PS, PMMA, PETE, etc., might be effective in pollution reduction, as they could more easily be separated in wastewater treatment plants by surface skimming, and otherwise be readily substituted yet perform identically to current more neutral-density alternatives. Similarly, microbeads that more easily sink could also be more readily removed during municipal wastewater treatment. Polymer-based microbeads are still fundamentally challenging spheres to sink quickly, but inorganic substitutes such as silicon oxides may provide an easily-separated viable alternative. Indeed, the use of well-rounded ('soft') beach sand to clean skin, clothes, and dishes has seen millennia of successful and non-toxic employment as part of many traditional beach and island cultures, cheaply and easily sourced, and then easily removed and recycled for re-use, or simply returned from whence it came to lakebeds and beaches.

Several other clever and creative solutions have been proposed recently that could also prove to be easily implementable and effective, including an optimistic and viable proposal of making microbeads out of natural chitin derived from waste shrimp shells, which would be easily biodegradable in an aqueous marine environment, being a natural product already [45]. Oat husk cellulose-based waste from food production is another possibility inviting exploration, as is using sawdust waste from the forest and lumber industry. These proposed solutions would allow the use of bio-derived-microbeads to continue, yet without creating long-lived waste, and in fact, even being manufactured from waste [45]. Attention could also be drawn to current wastewater treatment techniques, to address specifically the problem of plastic microbead separation by filtering of smaller mesh size, or the removal of microbeads from wastewater using electro-coagulation, effectively enlarging their effective size for easier filtering and reducing their potential to contaminate freshwaters through wastewater treatment plant discharge [83]. Lastly, other solutions proposed could potentially remove microbeads that have already been polluting the environment, such as using the fungus *Zalerion maritimum*, where microbeads made of polyethylene were eliminated *via* biodegradation [44]. Perhaps a combination of all these approaches—bead replacement, improved separation methods, and exploration of new bio-degradation pathways for the enormous backlog of current accumulation, might provide a best overall approach to this significant problem of plastic microbeads now building up in our fresh and marine waters. There is encouragement however, in noting that if this problem can be addressed now in early stages, it may well still be readily remedied, reduced, and even reversed.

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Supplementary information

Plastic Microbead Accumulation in our Freshwaters: North American Great Lakes Assessments and Perspective

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Materials and methods for sample collection

Study sites

Field sampling was carried out in Kenauk Nature, a 65,000-acre protected wilderness reserve near Montebello, Quebec. It was also conducted along the Ottawa River leading to Montreal and in the St. Lawrence River around Montreal. These are three distinct freshwater systems, but they are all connected: In Kenauk Nature, Papineau Lake flows into the Kinonge River, which empties into the Ottawa River. The Ottawa River later flows into the St. Lawrence River near Montreal.

Ten sample sites were located in Kenauk (the ten sample sites consisted of one or two in northern Papineau Lake near where people live, then further down into Whitefish Lake and along the Kinonge River), ten along the Ottawa River, and ten in the St. Lawrence River. Within Kenauk, both slow-moving streams and the edges of lakes were sampled. In the Ottawa and St. Lawrence Rivers, the edges of the rivers were sampled. At all sites, only the surface water was sampled. A control was also taken, using water from a faucet in Kenauk.

Sampling time and period

Sampling occurred from July 23, 2018 to August 7, 2018. Samples in Kenauk were collected from July 23 to July 26; samples in the Ottawa River were collected from July 26 to July 31; and samples in the St. Lawrence River were collected from August 1 to August 6. Depending on the site, sampling took between 30 minutes and two hours. Each site was sampled only once, no matter the weather. Samples were stored in the refrigerator, and underwent oil extraction and microscopic identification in August 2018.

Sampling methods

At each site, GPS coordinates, water temperature, air temperature, weather, date, and time were noted. Sites were chosen for location and accessibility. Water was sampled three times at each site using the scoop and filter method. For each of the three samples, a 3.5L plastic jug was used to filter 262.5L of water through a filter (made of a Nalgene water bottle and 100-micrometer Nylon mesh), totaling 787.5 L of filtered water at each site. The filter trapped the microplastics, though it could not trap anything less than 100 micrometers in diameter. After each sampling, the mesh was put into a Whirlpak bag to prevent contamination, and the process was continued for the remaining two meshes. At each site, the water was tested between 5 and 10 metres from the shoreline at a depth of 0.5 metres. The 3.5L plastic jug was used 75 times so that 262.5L of water was filtered and the mesh was collected; this was repeated until three mesh pieces had been collected (a total of 787.5 L filtered at each site). This was carried out for each of the 30 sites. After the samples had been collected, they were stored in a refrigerator until further analysis.

Sample processing and characterization

Oil extraction

To examine the microplastics, they had to first be extracted from the sediments that had been caught in the mesh. To do this, each mesh was washed with 30 ml of distilled water into a beaker. The inside of the Whirl Pak bag was also rinsed with distilled water; this was added to the beaker to ensure that microplastics stuck to the side of the Whirl Pak were included. The contents of the beaker were then added to a 60 ml syringe. The bottom of the syringe was covered in Parafilm and was then shaken. The contents were left to settle for a short period. Next, five ml of canola oil were added to the syringe, which was shaken for 30 seconds to allow the entire sediment fraction to come into contact with the oil. The mixture was left to settle for two minutes so that the oil layer was completely separated on top of the water layer. Since plastics are oleophilic, they were attracted to the oil layer and in theory would remain in that layer. The water and sediment layer in the syringe was emptied to leave the top oil and plastic layer, which was then emptied into a vacuum filtration device, leaving just the microplastics on a filter paper. The syringe and beaker used earlier were then rinsed with soapy water and emptied into the vacuum filtration (to ensure that plastics stuck to the walls of the syringe and beaker were included). Then, the filter paper was put in a petri dish and the lid was closed to prevent contamination.

Lab analysis and microplastic identification

The mesh and filter paper from each site were examined under a stereomicroscope at 40X magnification. The fragments from each mesh sample were counted and identified using the key ‘Guide to Microplastics Identification.’ Polymer verification was attempted with Raman spectroscopy, but this method burnt some samples, and overall the samples were too “messy” to return a result. Spectroscopic analysis is increasingly used as it is important to verify that the microplastics that were recorded were actually plastics. However, it was not possible here.

Geographic interpretation

The effect of several variables—sampling site, location, population, water flow, and water quality—on microplastic abundance was examined. Overall trends of microplastic type were also analyzed. While other variables such as weather and ecosystem type were also recorded, such variables were affected by many other factors, and their effect on microplastic abundance was subsequently not examined.

Effect of sampling site on microplastic abundance

Sampling site had a relatively significant effect on microplastic abundance, $F(29, 523) = 1.44$, $p = 0.06623$ (Figure 1). The relationship was very weak ($R^2 = 0.02258$). Sampling sites with the greatest influence on microplastic abundance were sites 4, 11, 14, 23, and 28. Figure 1 shows that the sites with the highest microplastic abundance were sites 3, 4, 11, and 24. The sites with the lowest abundance were sites 7, 8, 10, 16, and the control. Rather than larger scale factors such as population, it seems that nearby boat and recreational activity were the biggest drivers of microplastic abundance based on sampling site. In Kenauk, the sites with the highest average abundance were 3 (near the marina) and 4 (on Whitefish Lake). These were the sites with the highest human influence—the marina has many boats and boat ropes, while Whitefish also has boats and swimmers. Site 11 in Montebello along the Ottawa River had lots of sediments in the samples, likely due to the fact that it had recently rained heavily. Sediments and organic matter could potentially attract microplastics, resulting in a higher abundance. Site 24 was in Beaconsfield in the West Island of Montreal along the St. Lawrence River. It was a sandy shoreline next to a park and yacht club. Finally, site 28 was by a marina in Montebello—again, the site of heavy boat traffic.

Other sites with relatively high microplastic abundances were site 12, which was on the shore of Lac St-Louis in the St. Lawrence River, and which had lots of organic matter; 14, which was in the St. Lawrence River, with lots of sediments, boat traffic, and visible plastic pollution; 19, in the Ottawa River near a ferry crossing; 20, which was near a boat launch in the Ottawa River; 22, again near a boat launch in the Ottawa River; and 26, located in the St. Lawrence River in the east of Montreal, near a park with visible plastic pollution. The lowest recorded plastic pollution was in more secluded sites in Kenauk; at some sites in the Ottawa River where samples were taken off of docks; and some parts of eastern Montreal and near the port of Montreal. Most of these sites were not sites of very high human activity, which could explain their lower microplastic abundance.

Effect of location on microplastic abundance

Location (whether the sample was taken in Kenauk, the Ottawa River, or the St. Lawrence River) did not have a significant effect on microplastic abundance, $F(2, 550) = 0.5439$, $p = 0.5256$ (Figure 2). The relationship was also very weak ($R^2 = -0.001292$). Although none of the locations had a significant effect on microplastic abundance, there were differences between the abundances found in each region. The difference in microplastic abundance between sites in the Ottawa River and the St. Lawrence was the least significant ($p = 0.9122$). The difference between the St. Lawrence River and Kenauk was slightly more significant ($p = 0.7565$), while the difference between the Ottawa River and Kenauk was the most significant of the three ($p = 0.4973$).

While the differences in microplastic abundance between locations was not significant as expected, this could be due to the higher than expected influence of localized factors, such as hydrology, water quality, and proximity to human activity. That being said, the least significant difference was between the Ottawa River and the St. Lawrence River, which is an interesting result, as it speaks to the similarity of these two regions in terms of microplastic abundance. The pristine region of Kenauk was hypothesized to have a much lower microplastic abundance relative to the other two regions due to its overall lower human influence, and this was supported by the fact that the difference in abundances between Kenauk and the Ottawa and St. Lawrence were more significant than those between the Ottawa and St. Lawrence.

Nonetheless, the effect of regional location on microplastic abundance was overall not significant, which was a surprising result considering that other studies found that abundance can vary with population. There are indeed many factors to account for, and when comparing population gradients, future studies should compare microplastic abundance while making other factors—such as ecosystem type, water quality, and proximity to human activity—as normalized as possible. In this study, there were simply far too many varying factors at each sample site.

Effect of population on microplastic abundance

An online population count map was used to rank population levels and to see in which population levels the sampling locations fell. Some sampling locations in Ottawa, for example, fell into 3 (high population), but others were in 2 (medium population). Kenauk's sites were all in the lowest population level. The St Lawrence's sites were also in the highest population level. Overall, population did not have a significant effect on microplastic abundance, $F(2, 550) = 0.838$, $p = 0.4331$ (Figure 3). The relationship was, again, very weak ($R^2 = -0.00059$), but there was a difference in microplastic abundance between population levels. The difference in abundance between population levels 2 and 1 was the least significant ($p = 0.8821$), while the difference in abundance between population levels 3 and 2 was more significant ($p = 0.7371$). The difference between population levels 3 and 1 was the most significant ($p = 0.4112$). Again, while this wasn't a significant result, the population levels that were the furthest apart—3 and 1—had the greatest difference in microplastic abundance. This supports the hypothesis that microplastic abundance varies along a population gradient, although not as strongly as expected.

Effect of water flow on microplastic abundance

Water flow did not have a significant effect on microplastic abundance, $F(2, 550) = 1.273$, $p = 0.2808$ (Figure 4). The relationship was weak ($R^2 = 0.0009886$). However, fast water flow seemed to have a slightly higher effect on microplastic abundance, and it resulted in a higher average of microplastics. The difference in microplastic abundance between areas of slow water flow and the control was the least significant ($p = 0.5826$), while the difference between areas of fast water flow and the control was the most significant ($p = 0.4238$).

This result could be explained either because samplers were shoveling more 'new' water through the mesh when sampling faster flow areas, rather that recycling much of the water, as happened in slower flow areas. This makes study design essential to reducing sampling bias. It could also be that more microplastics are transported when water flow is higher. If such is the case, times of very high water flow (such as after heavy rainfall) could be important drivers of microplastic abundance.

Effect of water quality on microplastic abundance

Water flow did not have a significant effect on microplastic abundance, although the p-value was still quite low, $F(3, 549) = 1.605$, $p = 0.1871$ (Figure 5). The relationship was weak ($R^2 = 0.003278$). However, there was a difference in the significance between different treatments. For example, samples with lots of organic matter differed very little in microplastic abundance to sample with lots of sediments ($p = 0.9999$). The difference in microplastic abundance between organic matter-heavy samples and clear samples was more significant (p = 0.4745), while the difference in abundance between sediment-heavy samples and clear samples was most significant ($p = 0.4745$).

Samples with high amounts of plants and sediments were expected to yield higher microplastic abundances based on the assumption that microplastics might be attracted to these particles and ‘stick’ to them. Had the oil extraction protocol not been used during the microplastic extraction, which got rid of a lot of organic matter and sediments, the difference between microplastic abundance in sediment or organic matter filled waters and clearer waters might have been greater.

Overall microplastic type

Microplastics were found in every sample, including the control. There was an average of 6.73 microplastics per site (per roughly 800 L of water). This means that the average concentration across all sites was 0.0084 pieces/L. The sites with the highest concentration had 0.04 microplastic pieces/L, while the sites with the lowest concentration had about 0.006 pieces/L. Fibre was the most frequently occurring type (371 fibres, 167 fragments, 6 styrofoam, 5 film, 2 pellets, 2 unidentified) (Figure 6). 67% of all pieces were fibres. 0.003% were pellets.

Of the few freshwater microplastic studies, very few used the scoop and filter method used on the small scale in which it was used in this study. The varying methodologies used to sample for microplastics has already been identified as a problem, as it makes comparisons of concentrations between studies, and thus statistical analyses such as meta-analysis very difficult. Compared to a study by Vermaire et al. (2017)

in the Ottawa River, the average number of microplastics was much lower (0.0084 pieces/L compared to 0.1 pieces/L). However, Vermaire et al. (2017) sampled 100 L of water, while this study sampled 800 L of water. There is the potential that some of this water was recycled when sampling, which would reduce the concentration of microplastics. Nonetheless, similar to Vermaire et al. (2017), microfibres were the dominant microplastic type, and microplastics were found in every sample. Microfibres often make up the majority of surface water plastic fragments due to their density and their small size, which makes it easy for them to slip through wastewater treatment (unlike larger fragments such as pellets). This is similar to the findings of other studies, both freshwater and marine. Still, the lack of consensus regarding microplastic concentration remains a problem, and established field sampling and lab techniques must be established so that future researchers can use reliable microplastic concentration methodology.

Sources of uncertainty and error

There are a few potential sources of error associated with this study that could bias the results. Firstly, many of the independent variables, such as water quality and water flow, were picked up in the metadata collected at each site, and thus could be subject to observational error. Secondly, there is always some element of error in field sampling (such as precision error, user error, etc.). The oil extraction protocol could have reduced microplastics in each sample by discarding some when getting rid of the plant matter and sediments, or it could have added some, due to the possibility of microplastics from the air contaminating the sample. Contamination in both the field and the lab could have skewed the results, since at the time of processing, it had not been confirmed that microplastics can take to the air. Microscope identification was also subject to human error, as certain particles—such as sand grains and plastic fragments—can be very difficult to tell apart. Furthermore, microscope identification could not be verified using spectroscopy. However, since only one person examined the samples under the microscope, this eliminated the error associated with multiple people doing it.

Comparison of plastics concentrations

The methods in which microplastics are collected and analyzed varies greatly, making concentration calculations very difficult to standardize. However, quantifying microplastic concentrations and trends in freshwater environments that have been largely ignored is very important. This study found that while regional factors such as population and location do not seem to affect microplastic abundance as much as previously thought, localized factors such as sampling site and proximity to human activity significantly affect microplastic abundance. Other factors such as water quality and water flow also affected abundance, though not significantly. Microplastics were found in each sample, including the control, and were mostly composed of microfibres, which is similar to what many other marine and freshwater studies have found. It is clear that microplastic pollution is a problem even in remote locations, and that localized factors could play more of a driving role than regional ones. Future research should pay particular attention to increasing the reliability and consistency of methodologies used in microplastic studies.

Figures

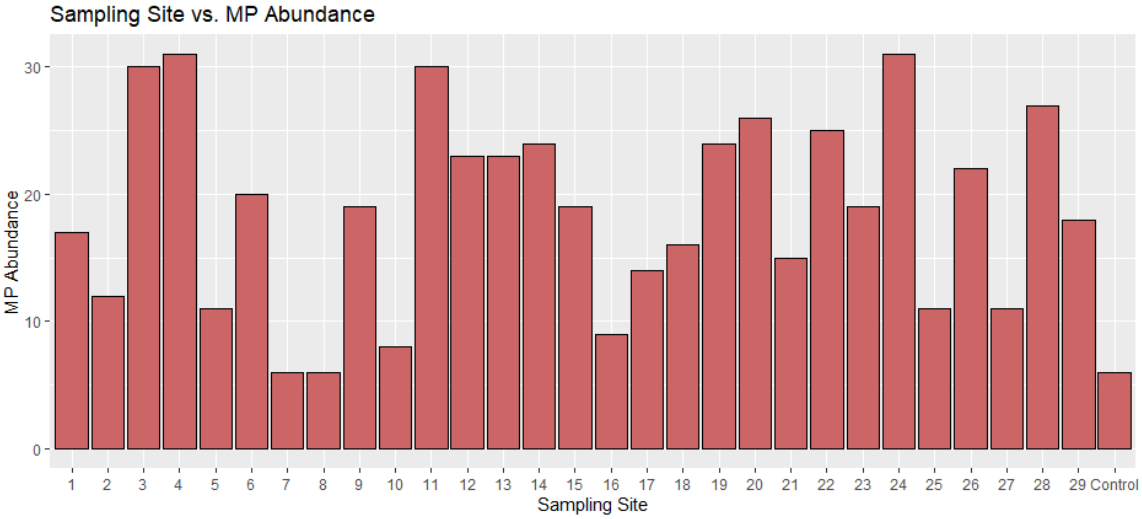

Figure S1. The effect of sampling site on microplastic abundance. Sites 1-10 were in Kenauk; sites 11, 16-22, 28, and 29 were in the Ottawa River; and sites 12-15 and 23-27 were in the St. Lawrence River. In Kenauk, the sites with the highest average abundance were 3 (the marina) and 4 (Whitefish Lake). Other sites with high microplastic abundance were site 11 in Montebello along the Ottawa River and site 24 in Beaconsfield along the St. Lawrence River. Sites with relatively high abundances were sites 12, 14, and 26 in the St. Lawrence River and sites 19, 20, and 22 in the Ottawa River. The lowest plastic pollution was more secluded sites in Kenauk, at some sites in the Ottawa River, and some parts of eastern Montreal and near the port of Montreal. A table describing each sampling site is also available (Table S1).

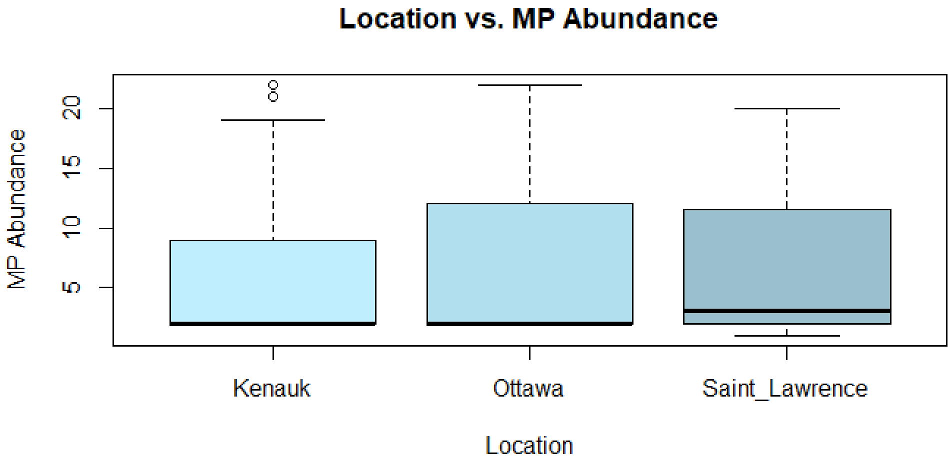

Figure S2. The effect of location on microplastic abundance. There were no significant effects of location on abundance. Each location had a mean of 2.5 microplastics per site, although the St. Lawrence's mean was slightly higher, and the maximum number of microplastics found in the Ottawa and St. Lawrence were higher than the maximum of Kenauk.

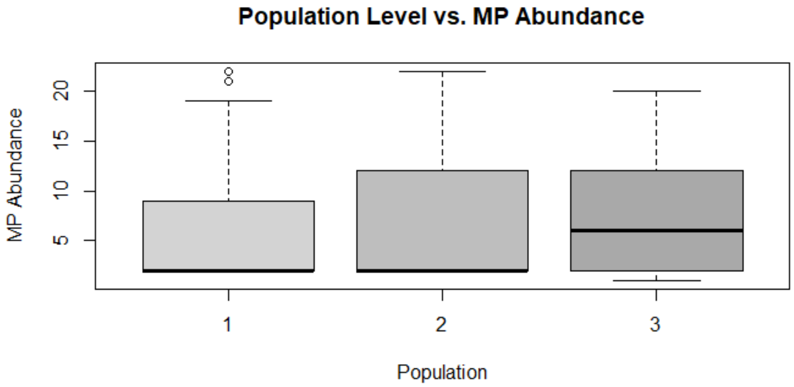

Figure S3. The effect of population on microplastic abundance. Overall, the effect was not significant. However, population levels 2 and 3 resulted in a higher maximum microplastic abundance than population level 1, and population level 3 resulted in a higher mean than population levels 1 and 2.

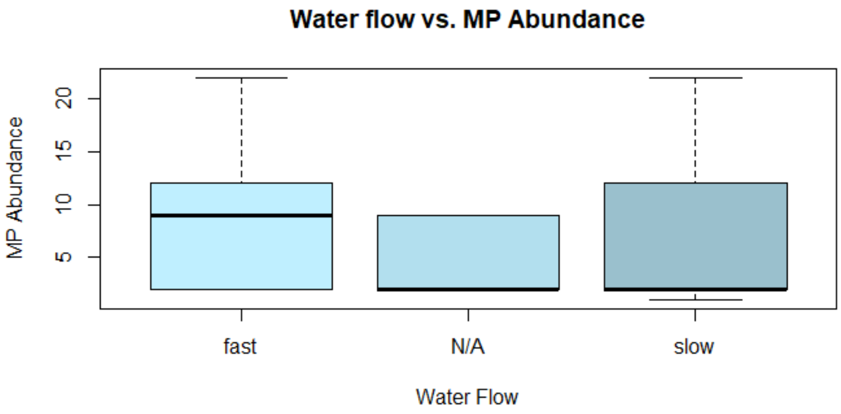

Figure S4. The effect of water flow on microplastic abundance. The effect was not significant, although fast water flow resulted in a higher mean microplastic abundance than the control (N/A) and sites with slower water flow.

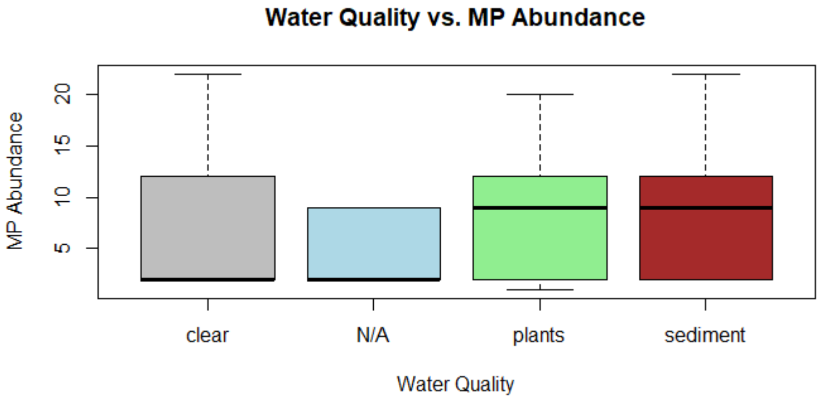

Figure S5. The effect of water quality on microplastic abundance. There was a large difference between samples that had lots of sediment and samples that were relatively clear, as well as between samples that had lots of plant matter and samples that were relatively clear. Clear samples had a mean microplastic abundance of less than 5, as did the control. Samples with more plant matter and sediments had a similar mean microplastic abundance of nearly 10.

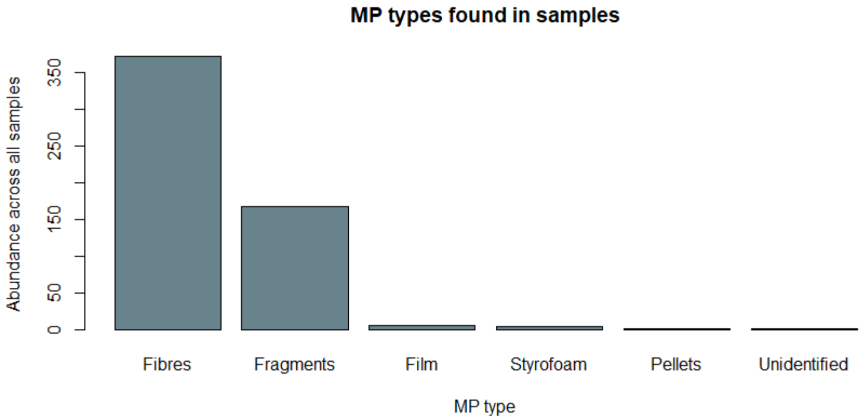

Figure S6. Microplastic types across samples. Microplastics were found in every sample, including the control. There was an average of 6.73 microplastics per site. Fibre was the most frequently occurring type (371 fibres, 167 fragments, 6 styrofoam, 5 film, 2 pellets, 2 unidentified). 67% of all pieces were fibres, while 0.003% were pellets.

Site	**brief description of location**
1	Kenauk. Pumpkinseed Chalet docks in Papineau Lake.
2	Kenauk. North of the marina in Papineau Lake, near the road.
3	Kenauk. By the marina in Papineau Lake.
4	Kenauk. Whitefish Lake docks.
5	Kenauk. Chalet des Rapides in the Kinonge River.
6	Kenauk. Bridge in the Kinonge River.
7	Kenauk. Kinonge River.
8	Kenauk. Pine Chalet docks (Kinonge River).
9	Kenauk. Northern Lake Papineau.
10	Kenauk. Kinonge River, very near to Ottawa River and residential areas.
11	Ottawa River. Montebello, Quebec.
12	St Lawrence River.
13	St Lawrence River. Shore of Lac St-Louis in Ste-Anne-de-Bellevue.
14	St Lawrence River. Boat traffic nearby.

15	St Lawrence River in Verdun. Next to a park.
16	Ottawa River. Off the docks by the little lighthouse in Grenville.
17	Ottawa River. Off the docks in a park in Grenville.
18	Ottawa River. Off the docks in Grenville-sur-la-Rouge.
19	Ottawa River. Fassett by the ferry crossing.
20	Ottawa River. Fassett by a boat launch.
21	Ottawa River. Papineauville by a boat launch.
22	Ottawa River. Neighborhood in Papineauville.
23	St Lawrence River. Baie d'Urfe, Parc Berfold.
24	St Lawrence River. Beaconsfield, Centennial Hall Park (park and yacht club).
25	St Lawrence River. Near Parc St-Louis.
26	St Lawrence River. Near Parc du Bout de l'Ile (very far east on the island of Montreal).
27	St Lawrence River. Parc de la Promenade-Bellerive, near the port of Montreal.
28	Ottawa River. By the marina in Montebello.
29	Ottawa River. By the wastewater treatment plant in L'Orignal, Ontario.

Table S1. List of sampling sites and description.