The Validation and Application of New Methods in Microplastics Research

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Dedication

I am dedicating this dissertation to my friend, Jane Greenberg, who taught me that everything, even sorrow, especially sorrow, can be spun into compassion, empathy, and love. I am a better person because of Jane. I am also dedicating this work to the Palestinians in Gaza who, according to articles published by the Lancet[1], by the United Nations[2], and by Amnesty International[3] are experiencing genocide while those in positions of power and privilege know this and do nothing to stop it. Witnessing the courage and resilience of the Palestinian people as they face unspeakable horror and suffering, who am I to be afraid to speak out against all forms of injustice?

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[1] Salmiya, Muhammad Abu. "Stop the Gaza Genocide Immediately." *The Lancet* 403, no. 10441 (May 25, 2024): 2286–87. https://doi.org/10.1016/S0140-6736(24)00135-1.

[2] Jayasuriya, Jayantha, Ahmad Faisal Muhamad, and Coly Seck. "Report of the Special Committee to Investigate Israeli Practices Affecting the Human Rights of the Palestinian People and Other Arabs of the Occupied Territories." UN General Assembly, September 20, 2024. https://docs.un.org/en/A/79/363.

[3] "YOU FEEL LIKE YOU ARE SUBHUMAN' ISRAEL'S GENOCIDE AGAINST PALESTINIANS IN GAZA." Amnesty International, December 5, 2024. https://www.amnesty.org/en/documents/mde15/8668/2024/en/.

Abstract

Plastic products subjected to natural weathering and anthropogenic activities splinter into progressively finer particles throughout their entire lifecycle. Eight decades of this material's continual degradation, combined with an ever-expanding global plastics market, results in the gradual accumulation of small (1 – 5,000 µm) synthetic particles known as microplastics. These fossil fuel-derived materials settle and flow between environmental matrices such as air, water, soil, and biota as fragments, fibers, foams, and films. While their presence is known and thoroughly documented, questions remain about the source, fate, and transport of microplastics, the human and ecological health implications, and the most effective means of constraining their planetary circulation. These questions persist, in part, because released contaminant particles are vastly heterogeneous. Their original fabrication involves an essential synthetic polymer or co-polymer resin merged with myriad chemical additives subjected to a unique set of degradative forces that may involve mechanical stress and/or distinct enzymatic and chemical conditions. In addition to these challenges, the methods applied to isolate, identify, and characterize contaminant particles are not fully standardized, making results difficult to replicate and leaving uncertainty about findings. This dissertation seeks to enhance sample processing methods for the detection, enumeration, and physical-chemical characterization of microplastic contamination for three applications: 1) fate and transport research, 2) consumer exposure research, and 3) efforts to enhance science literacy.

While this work focuses on advancing laboratory processing methods for the extraction of microplastics from filtered lab water and potable tap water, they can easily extend to other environmental media such as air, raw water, and wastewater. Method enhancement centers on improving quality assurance and quality control measures and generating a comprehensive chemical and physical profile of contaminant particles. This validated method, applied to a 52-week examination of urban atmospheric fallout in a humid continental climate, elucidates particle fate and transport by comparing abundance, size, morphology, and chemical composition of microplastic contaminant particles in weather conditions that vary in terms of precipitation and temperature. Next, the improved methods are applied to a study involving the potential release of microplastics from reusable sports water bottles as they are used and cared for by members of a local cycling team over the course of 92 days. Once again, microplastic abundance, size, morphology, and chemical composition are examined to see if there are any changes linked to bottle composition and design, frequency of use, and washing habits. Finally, these verified methods are adapted for use by non-scientists in a statewide citizen science project that seeks to educate high school students about microplastic contaminants, improve overall science literacy, foster civic engagement, while generating rigorous data across a broad temporal and geographic expanse.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Definition
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared
BIPOC	Black, Indigenous, and People of Color
DDT	Dichlorodiphenyltrichloroethane
DI	Deionized
EPS	Expanded polystyrene
FTIR	Fourier Transform Infrared
GC/MS	Gas Chromatography-Mass Spectrometry
GI	Gastrointestinal
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HYSPLIT	HYbrid Single-Particle Lagrangian Integrated Trajectory
IRB	Institutional Review Board
LDIR	Laser Direct Infrared
LDPE	Low Density Polyethylene
LOD	Limit of Detection
LOQ	Limit of Quantification
MIPAR	Material Image Processing & Automated Reconstruction
MP-VAT	Microplastic Visual Analysis Tool
NOAA	National Oceanic and Atmospheric Administration
PA	Polyamide
PAH	Polycyclic aromatic hydrocarbons
PAN	Polyacrylonitrile
PBDE	Polybrominated Diphenyl Ethers
PC	Polycarbonate
PCB	Polychlorinated Biphenyl
PE	Polyethylene
PES	Polyether sulfone
PET	polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PSU	Polysulfone
PUR	Polyurethane
PVC	Polyvinyl Chloride
QA/QC	Quality Assurance and Quality Control
RO	Reverse Osmosis
SEM-EDS	Scanning Electron Microscopy Energy Dispersive X-ray Spectroscopy
SOP	Standard Operating Procedure
TPU	Thermoplastic polyurethane
UV	Ultraviolet
WPO	Wet Peroxide Oxidation

Organization

This dissertation is organized with an introduction to the history, hegemony, and profusion of plastic and its ongoing emergence as a global contaminant of concern. This is followed by a literature review of the atmospheric fallout of microplastic pollution, human exposure, and human health consequences. Finally, this is tied together with an explanation/justification for the research performed. Chapters 3 – 6 are four, stand-alone research papers. Chapters 3 is published and chapter 6 is being submitted for peer-review.

Chapter 1: Introduction

In her book, *Plastic: A Toxic Love Story*, author Susan Freinkel describes traditional construction and fabrication materials such as wood, leather, stone, wool, and metal as having intrinsic properties defined by their utility. While each category exhibits a degree of natural variation as well as material limits expandable through chemical or physical manipulation, they remain bound by their distinct and essential properties. Simply stated, it is impossible to erect a skyscraper from worsted wool, just as it is impossible to weave a cozy infant bunting from stone. This is where Freinkel differentiates plastic from other conventional materials by highlighting its most important and singular quality, its plasticity. She succinctly describes its most vital characteristic as its "protean ability to be whatever we need them to be" (Freinkel, 2011). Indeed, modern plastic is like an exceptionally talented thespian, capable of flawlessly performing nearly any role cast.

Such an accomplishment would be impossible without the curiosity and ingenuity of early chemists, engineers, and inventors like Alexander Parkes, John Wesley Hyatt, and Leo Baekeland. These pioneers of polymer chemistry originally filled niche markets with their inventions. Celluloid for example, a material that emerged in the 1860s, offered a cheap alternative to luxury items conventionally crafted from nature, such as tortoise shell, ivory, coral, and pearl. Also, due to its transparency and ease of mass production into thin sheets, celluloid served as an inexpensive substrate for a light-sensitive chemical coating capable of capturing images, for still and moving pictures until 1950 (Friedel, 1976). Since industrial feedstock

for celluloid is camphor and nitrocellulose derived from cotton, this polymer prototype is now more accurately classified as semi-synthetic. However, these initial forays into polymer chemistry eventually paved the way to the first true synthetic polymer, Bakelite, conceived in 1907 and commercially viable in 1909. This fully synthetic polymer was unique in the way its inventor harnessed the potential of synthetic organic chemistry and found applications that were both technically and commercially relevant (Friedel, 1976). Even more consequential, with construction now taking place on a molecular level, this invention shattered the limits of human imagination around building and fabrication.

In the context of this new and seemingly boundless material frontier, an almost frenzied proliferation of discoveries followed in the wake of Bakelite. While some inventions failed to achieve commercial success, others easily landed footholds in myriad consumer products that offered comfort, convenience, and style. Polymers that reached some degree of recognition in either brand or chemical nomenclature include isoprene (1910), cellophane (1912), polyvinyl chloride (1926), acrylic (1927), polyethylene (1933), low-density polyethylene and nylon (1935), polystyrene and polyurethanes (1937), Teflon (1938), Formica (1947), Velcro (1948), Lycra (1949), Styrofoam (1954), polypropylene (1957), Kevlar (1965), and polyethylene terephthalate bottles (1973)(Decker, 2014). The popularity of these polymers accelerated and soon rivaled conventional materials. For example, aggregated global plastic production exceeded steel in volume as early as 1979 (Freinkel, 2011). Moreover, between 1952 and 1980 the compounded annual growth rate of synthetic polymers surpassed that of

aluminum, steel, and paper, making plastic the fastest growing material in the modern era (W. E. Brown, 1992).

This highly innovative and productive area of industrial science has been expanding for over a century. It now offers in excess of one thousand synthetic polymer varieties, many of which are termed 'boutique polymers' produced in small batches and customized to carry out specific functions (J. Nelson, personal communication, April 2019). For example, sequence-controlled polymers are tailored for specific biomedical therapies and can be designed to release drugs or deliver genes (Barnes et al., 2015). These types of polymers can also be used as code banks to store vast amounts of data in compact spaces, much like DNA and RNA (Lutz et al., 2013). Modern polymer chemists are also actively pushing against the physical parameters originally set by natural and modified anthropic materials. For example, 2DPA-1 is twice as strong as steel (Zeng et al., 2022), polycarbonate has 100 times the impact strength compared to glass (Kyriacos, 2017), the 'super fiber' Zylon has a tensile strength that rivals cotton, wool, silk, and hemp (Bunsell, 2021), and woven Kevlar fibers are strong enough to distribute the force of a bullet (Nair et al., 2020). In addition to these extraordinary feats of strength and durability, many synthetic polymers maintain low density, thus serving their primary structural function without a commensurate addition of weight. In many ways, the current era of material research is as exciting and inventive as ever.

Apart from the innovative potential exhibited by the polymer chemistry vanguard, the global plastics industry has established itself through the mass production of a narrow set of materials with quotidian applications. For example, 40.5% of the average global market share for

plastics from 2019-2023 was dedicated to packaging, while 20.32% went into building and construction, and another 23.48% devoted to automotive, electrical, agriculture, houseware, leisure, and sports (Plastic Europe, 2023). In fact, a mere seven categories of plastic together comprised nearly three-quarters (74.6%) of the industry's manufacturing base in 2023. These include PP (18.9%), LDPE (14.1%), PVC (12.7%), HDPE (12.2%), PET (6.2%), PUR (5.3%), and PS (5.2%) (Plastic Europe, 2023).

These and other plastics started out as material novelties, but most did not reach industrial scale manufacturing until World War II when demand spurred a 285% increase in production between 1939 and 1945 (Freinkel, 2011). Interestingly, this eruption in growth was intimately tied to geopolitics and material scarcity during the first and second world wars (Immerwahr, 2019). This initial surge was followed by seven decades of protracted growth, with global plastic yields doubling every 11 years (Wilcox et al., 2020). The key to sustaining this progress is plastic's relatively inexpensive feedstock of ethylene and propylene monomers derived from natural gas, or indirectly from naphtha distilled from crude oil. Many of the elemental components needed to generate plastic were originally byproducts of fossil fuel refinement (EIA, 2023). Through the mature technology in petroleum extraction and processing, a well-established transportation infrastructure of raw materials, and global fossil fuel subsidies topping $7 trillion in 2022, plastic has been able to maintain its material monopoly (Black, 2023). Today, fossil fuels supply 90.5% of globally manufactured plastics, while the remaining 9.5% comes from mechanical and chemical recycling (9%) and bio-based plastics (0.5%) (Plastic Europe, 2023).

In summary, a century of research and development transformed plastics into versatile, lightweight, and durable products, while a bounty of raw materials and economies of scale keep most plastic products inexpensive. They have become an integral component of contemporary life, offering safety (i.e. sterility), convenience, and entertainment. According to the latest report released by the prominent trade organization Plastic Europe, global plastic production in 2023 reached an all-time high at 414 million tonnes (Plastic Europe, 2024). Clearly, the market success of plastic is unequivocal, and by all accounts continued expansion in global manufacturing is anticipated, with some forecasting a tripling in production by 2060 (Watkins, 2022). Since many developed nations are approaching market saturation with plastic products (Mordor, 2025d), manufacturers and distributors are looking to expand sales in the developing nations of Latin America, Asia and Africa (Mordor, 2025b, 2025c, 2025a). There is no question that plastics unlocked a vast expanse of possibilities. Not all possibilities are enthusiastically celebrated or embraced, as some may carry significant risks.

It is ironic when contemplating the hegemonic rise and material dominance of plastic, that the very same properties that make this modern material desirable, also make it an intractable global pollutant. Its durability resists mineralization, its low-density facilitates transport, its versatility makes it difficult to define and identify, and its low cost makes it exceedingly abundant and easy to discard. An estimated 10.6 billion tonnes of accumulated legacy items are in existence today (Geyer et al., 2017) and roughly 10-40 million tonnes of plastics are discharged into terrestrial and marine

environments each year (Thompson et al., 2024). Both discarded *and* new plastics shed particles known as microplastics, which collect in, and flow between, environmental media. Recent evidence of this pollutant's ubiquity and global reach brings a palpable urgency to a discipline that has, until now, quietly existed for nearly seven decades.

Once mass production of plastic was in full swing, it enjoyed roughly two decades of uncomplicated adulation, eliciting only wonder and excitement. Then, as far back as 1958, plastic was formally documented as a pollutant for the first time in history when 3 - 5 mm PE pellets were recovered from the GI tracts of three species of seabirds from genus *Pachyptila* that were found dead or dying on New Zealand beaches (Harper & Fowler, 1987). Four years later, another team of scientists on the other side of the globe (*Antipodes Map*, 2024) found 20 - 50 mm jagged plastic fragments in the upper GI tract of storm petrels on the Atlantic coast of Canada (Rothstein, 1973). Curiously, these and other early records documenting wildlife entanglement and ingestion of plastic surfaced *after* several publications revealed plastic in abiotic environmental media (Ryan, 2015). One paper characterized 25 - 50 mm plastic fragments subjected to varying degrees of weathering by the waters in the Western Sargasso Sea (Carpenter & Smith Jr, 1972) while another described 0.5 mm PS spheres off the coast of New England (Carpenter et al., 1972). These publications combined with a 1971 paper by Buchanan reporting fibers and fragments from the Northumberland coast of the North Sea (Buchanan, 1971), comprise the seminal works of plastic and microplastic pollution in abiotic environmental media. Together, they illustrate an early understanding of plastic's planetary reach as well as prescient

concerns over the leaching of PCBs and plasticizers (Carpenter et al., 1972) and the "increasing production of plastics, combined with present waste-disposal practices" that could result in an accumulation of synthetic polymers in marine habitats (Carpenter & Smith Jr, 1972).

As this area of research evolved from a loose collection of sporadic novelty findings into a mature discipline, a more concrete definition took shape. The term 'microplastic' was used for the first time in a scientific publication in 2004 (Thompson et al., 2004). In a 20-year reflection on that work, the lead author defines microplastics as solid, fossil fuel derived synthetic polymers and their intentionally added functional additives and unintentionally sorbed chemicals (Thompson et al., 2024). To be considered *micro*plastic, particles must be less than 5mm in size, which is incongruent with the International System of Units convention. However, this upper limit was determined by a 2009 convening organized by NOAA's Marine Debris Program. The steering committee intentionally chose to include a larger size fraction out of concern for the ecological effects that readily ingestible particles up to 5 mm might pose (Arthur et al., 2009).

Since the first emergence of plastic and microplastic pollution, this area of research now occupies an interdisciplinary space that includes engineering, chemistry, biology, toxicology, ecology, and public health. Not only has it expanded in scope, but it has also grown considerably in bulk. A bibliometric analysis by Li et al. arranged 2,872 publications temporally from 2004 to 2020 and divided them into two phases (Figure 1.1). The initial 'development phase' spans 2004 to 2011 and is characterized as steady and uniform while the 'rapid growth

phase' spans 2011 to March, 2020 and it is distinguished by accelerated growth in publications (M. Li et al., 2022a).

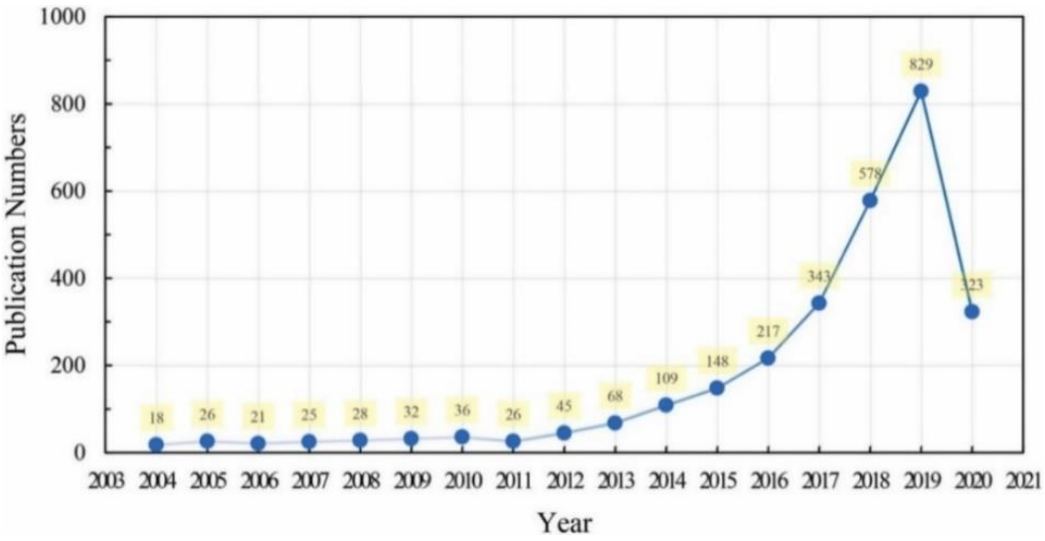

Figure 1.1 Temporal distribution of microplastic publications from 2004 to May of 2020 (M. Li et al., 2022a)

While publications in the 'development stage' and the transition to the 'rapid growth stage' were narrowly confined to research in marine habitats (Browne, 2015) they still represented a wide geographic range. For example, an abundance of conspicuously colored synthetic polymer fibers and fragments were discovered in sea ice core samples collected in the Arctic Ocean (Obbard et al., 2014), while another team of scientists recovered microplastics from deep-sea sediment some 1,200 to 4,800 meters below the water's surface in the Atlantic Sector of the Southern Ocean (Van Cauwenberghe et al., 2013). Gradually, this narrow focus on marine habitats gave way to the exploration of previously untested abiotic media such as soil (Rillig, 2012), freshwater (Eriksen et al., 2013), and ambient air (Dris et al., 2015). This marked the beginning of the 'rapid growth phase' as the results from these tests

spawned new questions about the reach and ubiquity of this particle contaminant and the mechanisms underlying its global passage.

However small the individual particles, some research indicates the vast worldwide expanse of microplastics may carry consequential planetary impacts in the aggregate. For example, particles that can access the Earth's atmosphere can absorb or scatter UV light from the sun (Aeschlimann et al., 2022; Revell et al., 2021; Y. Wang et al., 2023). In addition to particles suspended aloft, those settling at the poles threaten to reduce the Earth's albedo. According to analysis employing two models, total global tire wear emissions are between 2380 and 2910 kilotonnes $year^{-1}$, with an estimated 8.1 kilotonnes $year^{-1}$ settling on ice and snow (Evangeliou et al., 2020). Particles deposited on the surface of high-altitude glaciers also carry the potential of altering light reflectance. Moreover, they can release particles into valleys and rivers as the glacier melts (Ambrosini et al., 2019; Cabrera et al., 2022; Y. Zhang et al., 2021), which is concerning as mountain ranges are an important source of fresh water for habitats below. The Himalayas, for example, provides water for some 240 million human inhabitants. The presence of microplastics in oceans also threatens to disrupt carbon sequestration by compromising phytoplankton's ability to photosynthesize, creating a toxic environment for zooplankton, and disrupting the biological pump that brings carbon rich fecal pellets to settle on ocean floors (Cole et al., 2016; Shore et al., 2021; Wieczorek et al., 2019).

As research in this discipline progresses, distinct lines of inquiry take shape. One line, held primarily by those who are actively conducting research in the field concerns the development of techniques

and advancement of methods that have been validated and conform to the discipline's QA/QC standards. This work is key to generating high quality results that are accurate and reproducible. It is necessary as there are currently no universally accepted standardized methods for the collection, processing, enumeration, and characterization of contaminant particles. Another vital line of inquiry involves identifying environmental sources and understanding material degradation, particle release, transport, and global environmental sinks. This work advances knowledge in the distribution and accumulation of microplastic particles, both locally and globally. Related inquiries include exposure research, particularly studies that involve human exposure through inhalation and ingestion and the potential risks and consequences involved in the uptake of these contaminant particles. It should be noted that exposure research is an area of keen public interest. This segues into yet another line of inquiry within the field that, although less prominent in the literature, should not be marginalized or ignored. This involves evaluating the public's perception of microplastic pollution, and meaningfully engaging community members in studying the issue and finding solutions. By elevating critical thinking and fostering science literacy, members of the public will have an opportunity to develop their scientific aptitude, advance their understanding of environmental pollutants, and strengthen their agency and self-efficacy, allowing them to advocate for themselves and their community members.

This dissertation seeks to reach across the multidimensional problem of global microplastic contamination by plumbing each line of inquiry described above in four discrete chapters including methods

(Chapter 3), fate and transport (Chapter 4), human exposure (Chapter 5), and public engagement (Chapter 6). A literature-rich background in current research concerning fate and transport of microplastics, human exposure, and human health implications can be found in Chapter 2. Finally, a case is made for the critical importance of educating and engaging the public about environmental contamination in general, and microplastic pollution in particular.

Chapter 2: Literature Review

Review of Literature: Atmospheric Transport and Deposition

The first study to capture the atmospheric fallout of microplastics took place on the rooftop of a university building in Paris, France (Dris et al., 2015). Results were expressed in flux, which in an environmental chemistry context is a unit that describes the rate that a material/substance flows across a boundary over a specific unit of time. In Paris, the mean flux of microplastic contamination over a 93-day period was 118 #/m²/day. In this first paper, the authors suspected atmospheric fallout was linked to rainfall since the samples with the fewest particles (29 #/m²/day) took place over a long stretch of dry weather, while the sample yielding the most particles (280 #/m²/day) occurred after a stretch of consecutive rainy days. However, this was no more than a conjecture as associations with precipitation were not part of their original hypothesis. It is important to note that over 90% of the suspected particles were fibers which was an unusual morphology in 2015. Also, no chemical characterization was carried out in this first study. All particles were identified as plastic through visual sorting, which is a problematic method when dealing with particles that are smaller than 1mm. According to prior work, up to 70% of smaller size fraction particles are misidentified as synthetic (Hidalgo-Ruz et al., 2012). Since investigators were counting particles as small as 100 µm, errors and bias in counting should be assumed.

The following year, Dris et al. continued to collect atmospheric samples in Paris, France, using the same collection device and methods, only in this follow up study, two sites were examined for the sake of

comparisons. One site was a densely populated urban location and the other, a less densely populated suburban location, both in Paris, France. For this second study, the analytical instrument μ-FTIR was used to determine chemical composition of the particles captured. This time, 100% of the particles collected were fibers and 29% of them were confirmed as fully derived from petrochemicals. The mean flux in the urban setting was similar to the first study (110 #/m²/day) while the suburban setting yielded roughly half the amount (53 #/m²/day). Once again, the authors stated rainfall may influence the fallout since the range in flux was lower (2 – 32 #/m²/day) when daily rainfall was between 0 and 0.2 mm, but wider (11 – 355 #/m²/day) when daily rainfall was between 2 – 5 mm. It is important to note that both studies employed passive bulk sampling, where both wet and dry fallout were collected through a single stainless-steel funnel (0.325 m^2) for a duration as low as seven days and as high as thirty days. Through a bulk sampling method, the proportion of particles that settle out on their own versus those driven out of ambient air through precipitation remains unknown.

The emergence of these seminal publications inspired others to examine the entrainment, deposition, conveyance, and source of atmospherically suspended microplastics. Many early studies took samples from urban areas. For example, one study collected samples in Dongguan, China, a city with 8.3 million residents (Cai et al., 2017). Similar to findings reported by Dris et al. (2015), 90% of suspected particles were fibers. Using the same chemical characterization techniques, 33% of the fibers were identified as synthetic. The range in flux for non-fiber microplastics and fibers was 175–313 #/m²/day, in

a city that has a population nearly four times that of Paris, France. Another urban study involved the collection of passive air samples in London, a city of nearly 9 million (Wright et al., 2020a). Again, 92% of particles collected were fibers but only 17% were synthetic. Mean fallout of confirmed microplastic fiber was 712 #/m²/day, which far exceeds previous studies. Yet another study involved the collection of passive fallout in Guangzhou, China, a subtropical urban environment with 15 million residents. A flux of 114 #/m²/day, matched Paris, France, but fewer particles (79%) were identified as fibers (Huang et al., 2021). Since this study spanned a full year, the authors found a higher rate of deposition in spring (141 #/m²/day) and winter (140 #/m²/day) compared to summer (102 #/m²/day) and autumn (78 #/m²/day). In addition, there was no correlation between cumulative rainfall and deposition, but other meteorological factors, such as wind, did appear to play a role in fallout.

Atmospheric sampling also took place in rural areas, such as a meteorological station in the French Pyrenees. Here wet and dry collection vessels were positioned in an area 6km from a village of 540 residents and 25km from a town of 9,720 residents (Allen et al., 2019). The dominant morphology of particles found significantly departed from studies involving urban fallout as a higher proportion of fragments were collected compared to fibers. In addition, the flux of fragmented plastic particles was higher in this rural mountain catchment than fragments in any of the urban studies described above, with a mean flux of 365 #/m²/day. Once again, meteorological data collected in this study links microplastic deposition with maximum rainfall intensity and snowfall events. However, they found the average and maximum duration

of rain and snow events negatively correlated with microplastic deposition, which suggests that the occurrence and intensity of precipitation may be more important than duration. This is consistent with other research involving atmospherically entrained particulate matter, where ambient air is washed of pollutants in the early stages of precipitation. Therefore, a large volume of precipitation collected for analysis would be expected to yield diluted contaminants (Franz & Eisenreich, 1993).

Since sampling took place far from obvious sources, such as residential and industrial areas, the authors decided to use a modeling method called HYSPLIT4 that traces parcels of air backwards to indicate its trajectory. Through this technique they found no directional connections to densely populated urban centers or heavy industrial areas. The authors state in their discussion that a transport model capable of tracking longer distances might yield more promising results in identifying possible sources. While the source of microplastic fragments in the remote regions of southern France remain unknown, HYSPLIT was used by another team of researchers who collected active air samples 2,800 meters above ground level across central Spain (González-Pleiter et al., 2021). Here, samples were gathered directly from the Planetary Boundary Layer with a research plane. Back trajectory modeling showed that airborne particles could travel 400 km within 24 hours.

Other rural and remote areas include Mount Everest where sampling took place at several sites, including the Balcony, which sits 8,440 meters above sea level (Napper et al., 2020). Since fallen snow and nearby stream water was sampled, results were reported in units of

particles per volume of snow. The mean concentration in melted snow was 30 ± #/L with the highest levels in samples collected at the Everest Base Camp. Of all particles extracted, 95% were fibers. Although Mount Everest has no permanent residents, it is heavily trafficked by tourists with some 45,000 visitors in 2016 during the climbing season. Therefore, it is likely that tourist clothing contributes to the contamination, although a proportion of particles could come from long range atmospheric transport. This remains unknown. Microplastics have also been extracted from glacier snow in Italy, Ecuador, and the Tibetan Plateau (Ambrosini et al., 2019; Cabrera et al., 2022; Y. Zhang et al., 2021). These studies represent some of the uppermost limits in altitude. A study that represents the upper-most limit in latitude involved nine snow samples from Arctic ice floes in the Fram Strait and five samples from the island of Svalbard (Bergmann et al., 2019). Mean particles and fibers were two orders of magnitude higher in the Arctic than those found on Mount Everest with concentrations of 1.76 X 10^3 #/L for particles and 1.38 X 10^3 #/L for fibers. All the studies involving snow and ice collect wet and dry fallout together. It is uncertain what the rate of fallout might be as snow samples could potentially hold months or even years of accumulation. A concern involving snow and sea ice, however, is the reduction of the Earth's albedo.

Other studies explored the distribution of microplastics suspended in ambient air, revealing valuable information about source and transport. For example, one study used a total suspended particulate sampler, which is an active sampling method where entrained particles are pulled directly from ambient air (K. Liu, Wang, et al., 2019). Each sample involved roughly 6m^3 of air. Across four locations,

from a densely populated city center to a sparsely populated coastal region, investigators found a gradient of entrained microplastics. This same study also examined parcels of air sampled at varying heights (1.7m, 33m, 80m) and found the highest densities at the lowest height where most humans are breathing. The same lead investigator used the total suspended particulate instrument on a research vessel through the West Pacific Ocean (Liu, Wu, et al., 2019). Here, a similar gradient was discovered with more particles near the coast compared to air sampled above the pelagic ocean. Investigators also found a lower abundance of particles at night, positing the relative high humidity in the evening might cause the particles to settle to the sea surface. Another study employing two separate active sampling devices took place on the French Atlantic coast. This study looked at the ocean to atmospheric transmission of microplastics from wave action and bubble burst ejection (Allen et al., 2020). One active pumped air sampler gathered 18 m^3 of air per sample while the other, an air water droplet sampler, collected 6.410 m^3 per sample. Estimations based on their findings indicate a global total of 136,995 tons/year of microplastics blown onshore from oceans.

As seen in the studies described above, many urban locations reveal a preponderance of fiber, while rural and remote regions yield a mix of fiber and fragment (Padha et al., 2022). There are exceptions to this rule. A study of three rural and three urban sampling sites in Hamburg Germany, home to 1.8 million residents, identified 95% of plastics as fragments overall and only 5% as fibers (Klein & Fischer, 2019). There were three bulk samplers positioned at each of the six locations, each consisting of a PVC pipe, and a PE funnel and bottle.

To account for particles from the sampling vessel itself, blanks were run with a sampling vessel that was covered in aluminum foil. The authors found a higher mean flux in the Beech/oak forests, Douglas fir forest, and open field compared to the more densely populated university campus, residential area, and industrialized area. They suggest a 'comb out' effect to explain the unexpected results in the two forests. Here, particles that had adhered to the leaves and needles over time, would get washed out in a rain event, resulting in a higher count. The open field had the second highest mean flux, and the authors believe this may be due to the fact that samplers were stationed close to a road where tire wear particles, road paint, and dust is more likely to be suspended and collected by the passive sampler.

In examining prior work, it is abundantly clear that some combination of environmental conditions, such as land cover, precipitation, surface water turbulence, and/or wind aids in the movement of microplastic particles. However, another possible feature of particle movement is the particle size, shape, and density (which is related to the particle's chemistry). A team of researchers that were interested in knowing how different microplastic morphologies moved from land to air, treated both fibers and beads to wind tunnel experiments (Bullard et al., 2021). They found that the density of microplastics will affect their entrainment potential, or their ability to get into the atmosphere, as well as their settling velocity, which determines their potential for long-range transport. They also found sphere morphologies may have an easier time sieving down - or falling between crumbs of soil - compared to fibers. This leaves fibers more likely to be atmospherically re-entrained than beads. They also find

that particles with a high aspect ratio, or length to width ratio, have a higher surface to volume ratio which helps them remain aloft. In short, fibers have a lower settling velocity which allows them to remain entrained in the atmosphere for longer periods of time, and thus potentially traveling farther than spherical particles. In fact, previous work (Ginoux, 2003) has shown that a particle with a length of 100 µm and an aspect ratio (λ) of 10, has a settling velocity that is nearly an order of magnitude slower than a 100 µm sphere. Through wind tunnel experiments, the authors conclude that a 250 µm particle of PE with a density of 1.2 g/cm^3 could travel 126 km with a wind speed of only 7m/s. This empirical evidence is important as atmospheric transport modeling shows that in the United States, roads make up 84% of microplastic emission sources, oceans make up 11%, soil dust makes up 5% and urban centers make up < 1% of emissions sources (Brahney et al., 2021).

Together, the findings previously described provide ample evidence of microplastics suspended in and falling out of ambient air. Still, questions remained about the types of weather conditions that are more likely to drive particles out of the air and how rates of fallout might be linked to climate. To examine these very questions, one study used a team of Aerochem Metrics model 31 wet/dry collectors stationed in or near 11 remote national parks in the Western/Southwestern United States. These devices were able to separate wet and dry deposition, which was a first for microplastic research (Brahney et al., 2020). While the study extended approximately 1.5 years, and the data set was comprised of 236 wet samples and 103 dry samples, no detailed season variations were shared. All first-pass

analysis was carried out with visual sorting alone and only colorful particles and fibers were counted. Deposition rates range from 48 – 435 #/m^2/day, which is comparable to fallout in the French Pyrenees. Fibers made up 66% of wet and 70% of dry, and surprisingly, a full 30% of plastic captured were 5-30 µm spheres that the authors suspect came from industrial coating and paint applications (Brahney et al., 2020). Dry deposition accounted for more than 75% of what they found by estimated mass. Finally, using HYSPLIT, they concluded wet deposition shows stronger correlation to population density and dust deposition, while dry deposition is negatively correlated with dust deposition and may be more linked to long-range transport and "broad scale atmospheric patterns." Also interesting, particles from wet deposition were not only fewer in number but also larger in size.

Four more studies that separated wet and dry fallout soon emerged, but only one of them, (Xiong et al., 2022), used a Loda Electronics Model 2001 wet/dry collector, which is similar to the one used by Brahney et al. (2020). This investigation involved air and water samples collected from the Flathead Lake watershed, which is home to 121,000 residents. However, the single location where ambient air was sampled was 41 km from Kalispell (population: 23,241) and 23 km from Polson (population: 4,918). A total of 20 air samples were collected over the course of a year, but only 10 contained measurable precipitation and were counted as such. Each sample represented one week. Dry deposition flux was 69 #/m^2/day (range: 4-140 #/m^2/day), which is lower than the figures reported by Brahney et al. Here, some seasonal variations were given, with dry fallout being the highest in the fall, while wet fallout was highest in winter and lowest in summer.

Wet deposition was not reported as flux but in terms of precipitation density (0.006 – 0.050 particles/mL).

The first studies to separate wet and dry deposition took place in arid or semi-arid regions. For example, Polson, the town closest to the sampling site in Flathead Lake, MT receives about 409 mm of precipitation each year. This is not a dramatic departure from the areas sampled by Brahney et al. (2020) in the Western/Southwestern U.S. Yet another study that involves air sampling in the relatively arid region took place in Shiraz, Iran and Mount Derak, at a location 20 km NW of the city (Abbasi & Turner, 2021). Shiraz is home to 1.9 million making this sampling station more urban, but it receives roughly 330 mm of precipitation each year. Sampling spanned one year with each sample representing a whole month. This study collected fewer wet deposition samples (n = 29) than Brahney et al. (Brahney et al., 2020) (n = 236), but more than Xiong et al. (Xiong et al., 2022) (n = 10). However, all samples were collected manually as there was no automated instrument, which means collections depended on rain forecasts, which are not always accurate.

Of all particles recovered, over 99% were fibers. Units were reported as (particles/m^2) instead of flux, which is a function of time. However, the authors reported that in a dry climate, dry deposition dominates fallout, with monthly wet deposition contributing only 30%. Annually, they report wet deposition contributes 5% at Shiraz and 7% at Mt. Derak. This aligns with findings by Brahney et al. (2020). They also found during continuous precipitation, there was a progressive decline in the daily number of microplastics recorded, despite variation in the total volume of rain or snow collected. This

might be evidence of a washout effect, where initial precipitation scavenges most of the particles, leaving little in the air to be scavenged by subsequent rain or snow. The authors note that precipitation may also work to dampen the ground and keep microplastics from resuspension. Concerning particle size and season, both locations collected the highest percentage of fine microplastics in late winter and the lowest percentage in late summer and early autumn. Generally, these seasonal results corroborate the findings of Xiong et al.(2022).

The two remaining studies that involve the collection of wet and dry fallout were carried out in non-arid climates. One, (Roblin et al., 2020), sampled in four locations in Ireland, two sampling sites considered rural and the other two classified as remote. The annual rainfall in these locations during sample collection ranged from 841 mm to 1557 mm, which exceeds other studies that separate wet and dry fallout. However, snow accumulation was not observed during the sampling period. This study gathered samples for a year with each sample representing one month. Three sites collected wet deposition only, and one site had a bulk collector that gathered samples during wet and dry deposition. Only fibers were analyzed. The wet only samplers yielded 10.5 #/m²/day while the wet/dry bulk was 15 #/m²/day. Results from the bulk sampler suggests dry deposition makes up 30% of total microfiber deposition in this rainy habitat, which is the inverse of what Abbasi et al.(2021a) found in the dry clime of south-central Iran. However, it is important to point out, this study found no correlation between the volume of rainfall and number of microplastics, which potentially points to the washout effect described by Abbasi et al. (2021a). Finally, the authors estimate a suspended particle would

need 1.3 - 1.6 hours traveling at 8.5 m/s from Derry or Letterkenny to the Malin Head weather station, 40-50 km away, which is only slightly slower than estimated particle movement across the Planetary Boundary Layer over Spain (González-Pleiter et al., 2021).

The last publication involved sampling in the Gulf of Gdansk, Poland, in the Southern Baltic Sea (Szewc et al., 2021). This area is more heavily populated with some 250,000 residents, which is more than all but Shiraz, Iran. Sampling spanned 286 days, with sampling duration lasting 1 week for bulk sampling and 1-8 days for wet/dry sampling. All told there were 15 wet deposition samples, 17 dry deposition samples, and 17 bulk samples containing both wet and dry fallout. Morphologies were similar to Brahney et al. (2020) with 58% of wet deposition particles identified as fibers. For dry deposition and bulk deposition, the proportions of fibers were slightly higher at 65% and 62% respectively. However, compared to other studies, overall fallout was low with a mean of 10 #/m²/day (range: 0 - 30 #/m²/day). Wet deposition was greater than dry deposition, with a wet mean of 14 #/m²/day and dry mean of 7 #/m²/day. This is different than what Abbasi et al. (2021a), Xiong et al. (2022), and Brahney et al. (2020) found. Unlike Roblin et al. (2020) deposition values did increase with increasing precipitation volumes, but some observations fell outside of this trend. For example, five weeks had low precipitation but high deposition rates. Also, similar to what was found by Liu et al. (2019a), the authors reported increased relative humidity may cause particles to deposit more readily. Finally, they found wind speed affects dry deposition flux.

It is difficult to neatly summarize the investigations that involve microplastic fallout from ambient air. Contaminant particles

come in a wide range of sizes, shapes, and densities, which can influence their ability to become suspended in the first place, let alone travel and settle elsewhere. This background aims to provide a glimpse of variations in study design, sampling instruments, and findings. Some very broad trends include a link to human population density, a preponderance of fibers, especially in urban areas, and a link to weather and climate. As far as sources are concerned, based on density, size, and back trajectory modeling, microplastics have the potential to travel long distances, but there is no evidence yet to confirm this.

Review of Literature: Human Exposure Through Ingestion

Evidence for synthetic polymers was found in processed and packaged consumer products as early as 2013. In a paper by Liebezeit and Liebezeit, both fibers and fragments were extracted from commercial honey sourced from four E.U. nations and Mexico (Liebezeit & Liebezeit, 2013). The average across 19 brands of honey was 87 fibers/500g and 4 fragments/500g. Refined, unrefined, and powder sugars were also tested revealing 217 fibers/kg and 32 fragments/kg. To identify particles, the products were sieved, digested with 30% H_2O_2 to remove organics, and then stained with fuchsin and Rose Bengal. These dyes are known to stain 'natural' organic particles, which is why they were used in the study. This initial investigation was soon followed by the same authors, who adapted the protocol slightly as they examined 24 German beers for synthetic particles (Liebezeit & Liebezeit, 2014). Fibers ranged from 2-79/L, fragments from 12-209/L, and granules from 2-66/L, although no clear definition was given to distinguish a granule from a fragment. In reporting their findings, they acknowledge that the

chemical nature of the items found could not be determined without some form of spectroscopic analysis such as FTIR or Raman.

Soon after these publications, the presence of synthetic polymers in consumer products was reported. One study examined 15 brands of commercial table salts purchased from Chinese supermarkets (D. Yang et al., 2015). These salts originated from seas, lakes, and wells. Sample processing consisted of dissolving the salt into water and then treating it with 30% H_2O_2 to digest the organic matter. This team applied FTIR analysis to 152 randomly selected particles and demonstrated that 85% of the particles tested were plastic. The most common variety was the semi-synthetic cellophane at 39.5% followed by PET at 16.3%. In total, 15 different types of plastic were identified. This was the first study of its kind and it was soon followed by many other reports of plastic particles in salt from Spain (Iñiguez et al., 2017), India (Seth & Shriwastav, 2018), Turkey (Gündoğdu, 2018), Taiwan (Lee et al., 2019), Croatia and Italy (Renzi & Blašković, 2018), as well as several publications that examined and reported on salts purchased from multiple nations (Karami et al., 2017; Kim et al., 2018).

After the conclusive detection of synthetic microplastics in beer and salt, studies involving the contamination of seafood emerged. For example, Van Cauwenberghe et al. (2014) examined oysters and mussels reared in the Atlantic Ocean and the North Sea, respectively. They estimated that human exposure to microplastics from consumption of oysters and mussels alone could be as low as 1,800 $year^{-1}$ or as high as 11,000 $year^{-1}$. This figure is dependent on diet. For example, the elderly in Belgium consume 72.1 g day^{-1} while adults in Ireland and

adolescents in France consume much less (11.8 g day^{-1}) (Van Cauwenberghe & Janssen, 2014). In a study from China, 4.3 – 57.2 microplastic were extracted from nine species of bivalves purchased from a market in Shanghai (J. Li et al., 2015). Finally, a study examining 23 species of fish found microplastics in the digestive tract of 28% of fish purchased from markets in Indonesia and in 25% of the fish purchased in California markets (Rochman et al., 2015). It is worth noting a change in the manuscript titles around this time. Papers titled, "Microplastics in commercial bivalves from China," "Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption," and "Microplastics in bivalves cultured for human consumption" (J. Li et al., 2015; Rochman et al., 2015; Van Cauwenberghe & Janssen, 2014)(Van Cauwenberghe et al., 2014), reveal a palpable shift in interest from concern over ecological consequences to a more pointed concern about human exposure and potential negative outcomes that may result from the ingestion.

While some studies indicate the primary source of contamination in human consumables is from raw ingredients, others chose to interrogate food and beverage packaging as a potential culprit. For example, in a study involving 12 brands of beer that brewed their product from Great Lakes water, no correlation was seen between microplastics found in the municipal water supply and those found in the final product (Kosuth et al., 2018). Based on this and a preponderance of fiber recovered, the authors propose the source of contamination was atmospheric fallout during product processing. Evidence supporting contamination from product processing has been suggested by other authors as well. In a 2020 publication, eight brands

of packaged milk were purchased from pharmacies and supermarkets in Mexico (Kutralam-Muniasamy et al., 2020a). Through analysis with SEM-ED, investigators found fibers of the thermoplastics PES and PSU, both of which are often used in ultrafiltration and microfiltration membranes during the processing of milk. The authors asserted that their findings provide a "clear indication of plastic debris originating from the abrasion of membrane filters used during milk processing." Finally, a study examining beers, honey, milk, and soft drinks purchased from eight different provinces in Ecuador sought to investigate correlations between contamination in these products and regional air pollution, weather patterns, human population density, and industry density (Diaz-Basantes et al., 2020). No relationships were found among the listed variables and contamination load, which led authors to conclude the source was in the processing methods and not from environmental pollutants.

Other investigations have unambiguously shown that packaging can be a primary source of food contamination. However, it can also act as a barrier to protect foods from particles suspended in air. For example, one study examined four brands of raw chicken breast in triplicate. This meat had been placed on an EPS tray and then covered with a plastic film wrap (Kedzierski et al., 2020). The authors concluded that the film protected the food from atmospheric fallout since they found ten times more fibers outside the film as compared to space between the raw meat and the inside of the plastic film. However, the authors also found 4 – 18.7 EPS particles/kg of meat.

Other studies looked at the release of particles under conditions involving heat. One study examined two brands of reusable baby food

containers and one brand of reusable food pouches (Hussain et al., 2023). The investigators reported results demonstrating the containers released micro and nano plastic particles under various conditions, such as room temperature, refrigeration, high temperature/heat, both when containing simulated acidic and aqueous foods. The authors reported millions of microplastics and billions of nanoplastics released from a single cm^2 of the containers interior surface after three-minutes of microwave heating. Results similar in magnitude were found when products were subjected to long-term storage at room temperature and refrigeration. In another study, four brands of single-use plastic tea bags revealed a release of 2.3 million micro-sized particles and 14.7 billion submicron particles after submersion and steeping in hot water (Hernandez et al., 2019).

Finally, a few studies show that mechanical stress can cause particles to shed. For example, one team of researchers looked at the release of particles when plastic packaging is used as intended, torn open, cut with scissors, and cut with a knife (Sobhani et al., 2020a). They found a range of 0.46–250 microplastic cm^{-1} released from these daily activities. Another study looked at the shedding of microplastics from mechanical stress when plastic bottles are opened and closed (x1, x10, x100) and when bodies of bottles are rolled and subjected to 5 kg of pressure (1 minute, 10 minutes). While they found shedding from the PET bottle interior to be 148 ± 253 particles/L, the amount of microplastics released from the HDPE bottle cap ranged from 63,400 - 1,225,500 particles/cap (Winkler et al., 2019). Interestingly, the results between brands were very different when looking at cap wear. The authors suggest this may have to do with the structural difference

in bottle design. While the repeated use of plastic may result in shedding, there is also evidence that 'factory new' products may have particles on the surface that were created during manufacturing. For example, one team tested three different types of new PP single-use 'take away' containers, the authors reported shedding of particles in the 0 – 210 nm size range amounting to 3 ± 1.13 mg - 38 ± 5.29 mg per plastic food container package (Fadare et al., 2020a).

There are many papers that focus exclusively on single-use beverage containers. The first was a pilot study examining three bottles of beer and one 3L bottle of mineral water (Wiesheu et al., 2016). Raman spectroscopy was used for chemical analysis. However, contaminant fiber counts were not significantly higher than lab blanks, leading the authors to conclude the cellulose fibers originated from the lab coats and the polyethylene fibers came from the packaging materials that held the cellulose nitrate filters. Water purchased from German grocery stores (Schymanski et al., 2018) and packaged in coated paper cartons, glass bottles, single-use plastic, and returnable plastic bottles were analyzed. The authors reported the highest concentrations in water packaged in the reusable plastic bottles, which they attribute to wear/degradation of the package. Returnable plastic was 8 times higher than single use plastic and 10 times higher than the cartons, which had the least amount (11 – 118 particles/L depending on the brand). The authors suggest the soft plastic insert inside the bottle cap may be contributing to the contamination in the glass bottles, but they do not say what the insert is composed of and whether or not that matches the particles found in the bottle. This publication was followed by a study that also tested packaged commercial drinking

water from German stores (Oßmann et al., 2018). A total of 32 samples from 21 brands that span three different types of containers: glass, single-use PET, and reusable PET (which was further sub categorized as "newish" and "worn"). This study was similar to the work by Schymanski et al. (2018) in that they were able to detect and identify particles on a very small scale by applying μ-Raman spectroscopy, but they could only have the instrument inspect an area of 1 mm^2 on the filter at any time. Given this limitation, they decided to assume an even distribution of particles across the filter and extrapolate. Results show that single use plastic yielded the lowest density (2,649 ± 2857 particles/L) and older multiple-use plastic bottles had the highest density (8,339 ± 7043 particles/L), while "newish" multiple-use plastic bottles were similar to single use bottles (2,689 ± 4371 particle/L). Even though the loading density from these two studies differ by orders of magnitude, they still found single use PET to contain less than the average of the two reusable types (older and newish), 4,889 particles/L.

Both groups of scientists sampled water packaged in glass bottles and found moderate levels of microplastic contaminants. The Oßmann et al. (2018) group believes these contaminants come from washing and processing, and not the bottle caps, as Schymanski et al. (2018) posits. This is based on their finding large quantities of pigmented particles in glass and reusable plastic bottles. These pigments have the same composition as the pigments used on the paper labels. The abundance of pigments in glass (195,047 particles/L) are higher than plastic (23,954 particles/L) which is further evidence of this since the plastic bottle washing cycle is gentler and uses lower temperatures

with a less concentrated washing liquor compared to glass bottles. They also found fewer pigments in the “newish” PET bottles (1,808 particles/L) compared to “worn” ones (35,256 particles/L).

Further work carried out by Mason et al. (2018) had the largest sample size and greatest geographical breadth with 259 bottles representing 11 brands from 9 countries. However, a different detection method was used. Nile Red, which bonds to synthetic polymers and fluoresces under UV light was injected into the bottles before volume reduction. FTIR was used to confirm the chemical composition of MPs greater than 100 µm and fluorescing Nile red stained particles down to 6.5 µm were presumed to be plastic. For a size fraction of particles larger than 100 µm, an average of 10.4 #/L was documented. For a smaller size fraction (6.5 – 100 µm) the average was higher at 325 #/L (range of 3.72 - 2,277 #/L). More recently, a team of scientists detected micro- and nano-plastic particles in bottled water samples using a technique called rapid single-particle chemical imaging using stimulated Raman scattering microscopy. Through this work the authors discovered a microplastic density ranging from 130,000 - 240,000 particles/L across three separate brands of bottled water, 90% of which were in the nano-particle range (Qian et al., 2024).

In addition to water, other beverages have been studied with some new sources of contamination identified. One investigation involves soft drinks, cold tea, and energy drinks (V. C. Shruti et al., 2020). According to this team of researchers, a possible source of contamination would be plastic bottles that were not rinsed before they were filled with the product. They also suggest that routine sanitation practices in the manufacturing plant such as washing the area where

product processing takes place could reduce contaminant loads. Another study looked at beer, mineral water, and tea (Y. Li et al., 2022). The investigators believe raw materials, such as the grains used to make beer, as well as tools and containers used during manufacturing of tea, may be a source of contamination. Finally, a more recent study examined a host of non-alcoholic drinks such as juice, teas, soda waters, soft drinks, as well as sports and energy drinks (Lam et al., 2024). They list possible sources such as worn operational equipment, ventilation, as well as work-place clothing, and even hair nets, as possible sources. They also mention transportation, from the point of manufacture and processing to the point of sale. They cite a study from Australia where imported bottled waters had 4X the contaminant load than locally bottled water (Samandra et al., 2022). It is possible the mechanical agitation during transit could encourage the release of particles. Interestingly, Lam et al. (2024) found no association in beverage carbonation or low pH and contamination density. Instead, they believe that agitation related to transport may be a more important factor in the beverages they tested.

Just as with research concerning the atmospheric fallout of microplastics, care must be taken not to oversimplify or generalize reported findings concerning human exposure to contaminated foods and beverages. Once again, this literature-heavy background seeks to illustrate the expansive array of research questions, and the myriad methodological approaches and techniques that are used to find answers. One important take away is that exposures likely vary depending on an individual’s immediate environment and their diet. For example, those who drink bottled water are likely exposed to more contaminant

particles than those who drink municipal tap water. There is evidence to support the introduction of particles from raw materials, such as honey, product processing, and packaging. Finally, there are very few studies that find no contaminants. Only one of the studies listed here had levels that were below those found in the blanks.

Review of Literature: Human Exposure and Human Health

As previously stated, the very first papers in this discipline involved the discovery of synthetic polymers ingested by biota, such as polystyrene spherules consumed by eight species of fish from Niantic Bay (Carpenter et al., 1972) and rubber bands swallowed by Antarctic fulmars (Crockett & Reed, 1976). However, initial interest in marine biota stemmed from concern for ecological health. By the end of the 'development stage' described by Li et al. (2022a) this area of research shifted to include organisms frequently consumed by humans. These works have already been addressed. One study not previously mentioned is unique as it integrates three environmental reservoirs: air, water, and biota. This investigation involved wild and farmed mussels in Scotland, and on the surface looks like other bivalve studies. However, the authors placed petri dishes to capture indoor fallout during food preparation and consumption and discovered fallout was two orders of magnitude higher than particles recovered directly from the food (Catarino et al., 2018). This study is a reminder that when it comes to human exposure, all possible routes must be considered as they can amplify one another. It illustrates not only the ubiquity of this contaminant, but also the ways that environmental reservoirs are connected and impact human health. The human body is the latest

frontier of microplastics research. It is an area of intense interest even outside of the scientific community.

When considering human exposure to microplastics, the three primary routes are ingestion, inhalation, and dermal contact. Abbasi and Turner surveyed dermal exposure in urban and rural Iran by collecting an impressive 8,000 samples of face rinse, hand rinse, hair rinse, and mouth rinse from 2,000 participants (Abbasi & Turner, 2021b). Women in this study had higher levels of smaller (<100 µm) particles on their face, which is most likely from cosmetics directly applied to the skin. However, the lower limit of detection in this study was 5 µm, and while human skin pore sizes range from 40 – 80 µm (Enyoh et al., 2019), many assert human skin prohibits the absorption of particles greater than 0.1 µm (A. Sun & Wang, 2023). In fact, in an experiment involving pig skin exposed to 20 and 200 nm polystyrene beads, it was found that particles were unable to bypass the stratum corneum and access deeper skin tissue (Yee et al., 2021). Without evidence of synthetic polymer particles entering the human body through the skin, many view it as an area of limited concern. However, recent work has successfully detected nanometer size particles in snow and atmospheric samples, suggesting potential for environmental exposure (Caracci et al., 2023; Materić et al., 2020). In addition, it is an established fact that microplastic particles increase in abundance as they decrease in size (Hale et al., 2020). Finally, particles may be able to enter the skin through cuts, wounds, and other breaks in the skin surface. For these reasons, it may be imprudent to categorically reject the possibility of exposure through dermal contact (A. Xu et al., 2022). Although more research is needed to assess dermal exposure,

far less ambiguity exists in the two remaining routes, ingestion and inhalation.

An initial inquiry of ingested microplastics was a prospective pilot study that involved the examination of human stool (Schwabl et al., 2019). Authors reported a mean concentration of 9.34 fragments and films per gram of fecal matter with nine varieties of plastic detected overall. Participants represented eight nations across the north-eastern hemisphere, spanning the United Kingdom to Japan. This range is important as diets vary geographically however the overall sample size was extremely small, with only a single sample representing each nation (n = 8). Expanding on this work, a slightly larger and more regionally focused study involved young men (n = 24) in Beijing, China. Here, researchers reported 8.88 particles per gram of feces, with up to eight varieties of plastic pulled in a single sample (N. Zhang et al., 2021). In both investigations, the size range of particles fell within 20 – 800 µm and the most prominent type of plastic recovered was polypropylene. A third study compared only PET and PC extracted from the stool of infants (n = 6) adults (n = 10), and the meconium of newborns (n = 3). Using LC-MS/MS for analysis, results were given in mass (ng/g of dry weight) making comparisons with earlier studies difficult. However, the authors were surprised to discover the estimated daily intake for PET was an order of magnitude higher in infants (children <1 year in age) compared to adults, with 83,000 ng/kg of bodyweight/day and 5,800 ng/g of bodyweight/day, respectively (J. Zhang et al., 2021). They posit the abundance of plastic feeding accessories, teething rings, and toys mouthed by infants and their

proximity to carpets constructed from synthetic fibers, offer the best explanation for this difference.

A final biomonitoring method which quantifies synthetic polymers that may follow a pathway to the digestive tract or respiratory system involves the collection of sputum, a mucus from the lower airway that is either swallowed or reabsorbed into bronchial linings. In one such study, patients suffering from lung disease (n = 22) were asked to cough into cleaned sampling vessels. Compared to microplastics recovered from human feces, particles collected from the upper respiratory system were similar in size (20 – 500 µm) but more diverse, with 21 distinct polymers identified (Huang et al., 2022). Perhaps it should be expected that plastic types are more varied at the 'entrance' of a pathways compared to those being eliminated by the human body. These findings may confirm earlier studies where elevated gastrointestinal tract diseases were discovered in workers exposed to synthetic textiles and flock in occupational settings (Wieland et al., 2022a). However, with mean total abundance reported as 39.5 particles/10mL, it is difficult to compare concentrations among individuals, since varying amounts of mucus are likely produced. It is also difficult to understand rates of exposure since no measure of time is recorded. Finally, without knowing the total quantity of contaminants entering the body through direct ingestion or through inhalation/swallowing, it is difficult to quantify the proportion that is effectively eliminated versus those that persist inside the body. Even so, the preceding examples provide evidence of microplastics traveling pathways into and through the digestive system.

A handful of studies have examined microplastics inhaled from indoor and ambient air. The first of these investigations employed a device called a breathing thermal manikin, which is an active air sampler set at a metabolic rate capable of simulating human respiration. In this experiment, the instrument was calibrated to mimic an adult male breathing rate, in both frequency and volume, during light activity. Three separate trials conducted in three furnished apartments in Denmark revealed a maximum quantity of 272 microplastics inhaled in a 24-hour period. Most particles captured were fragments (87%) with median dimensions of 237 by 26 µm. Only 4% of all particles recovered were synthetic in origin, but of those, 81% were polyester (Vianello et al., 2019). Three other studies explored the inhalation of microplastics by human subjects through direct examination of human lung tissue. The first publication gathered samples from cadavers from 20 non-smoking residents of Sao Paulo, Brazil. Here too, fragments dominated (88%) with mean size of 3.92 ± 0.67 µm, and fibers only slightly larger (11.23 ± 1.96 µm). Results, reported as number of particles per gram of human lung tissue, had an overall mean of 0.56 particles/gram of lung tissue. A total of 9 polymers were identified, of which PE and PP were most prominent (Amato-Lourenço et al., 2021).

Next, a study involving 44 living patients from Spain subjected to a procedure known as bronchoalveolar lavage (BALF), which is a rinsing of the lower respiratory system, revealed 68.2% of participants had one or more synthetic particles flushed out. Of these, most (97%) were fibers ranging in size from 9.96mm - 140 µm (mean: 1.73 ± 0.15mm), which is considerably larger than anticipated as it was previously assumed that only particles <1-3 µm could gain access to deep lung

tissues (R. J. Thomas, 2013). Many of the isolated fibers were cellulosic in origin, but the most prominent synthetic polymer was polyester (19%). While the reported mean was 9.75 ± 2.49 particles/100 mL BALF, just as with sputum, the reported units are not easy to use in calculating exposure (Baeza-Martínez et al., 2022). A final study involved 13 samples taken from 11 living patients undergoing thoracic surgery. Roughly half of these were fibers and the other half fragments representing 12 distinct varieties. Compared to Baeza-Martinez et al. (2022), a smaller proportion of the microplastics were fiber, but of those fibers, the lengths were longer than expected, ranging from 12 – 2475 µm and widths from 4 – 88 µm. The authors describe the alveolar duct dimensions as 1410 µm long and 540 µm wide (Jenner et al., 2022). While these dimensions may be smaller than some of the extracted plastic pieces, it would not necessarily prohibit long, gossamer threads from traveling the torturous respiratory passageways to collect in or near the terminal alveoli (Jenner et al., 2022).

The prior publications suggest synthetic polymers enter the human digestive tract through contaminated foods and beverages and enter the respiratory system by evading long-evolved clearance mechanisms, but little is known about the factors that drive elimination from the body versus persistence and possible migration through the body. Microplastics are exceptionally heterogeneous in size, morphology, polymer type (polymer matrix), chemical composition (chemical additives), color, and surface chemistry (surface charge, hydrophobicity, weathering, and adsorbed substances). Therefore, a comprehensive chemical and physical profile will guide our understanding of the systems and tissues accessible to contaminant

particles. For example, the depth at which a particle settles in the lungs is a function of its aerodynamic equivalent diameter (AED), not its geometric size. AED is the diameter of a sphere with the density of water (1 g/cm^3) that has the same settling velocity of the microplastics particle. However, most publications offer geometric size, as AED has yet to be empirically measured. If it is reported at all, it is offered as a rough approximate (Allen et al., 2021; Wright et al., 2020a; Yuan et al., 2023). This metric is important because a particle with an AED > 10 µm will adhere to passages in the upper airway to be swept away by the mucociliary ladder. A particle with an AED < 10 µm, however, is more likely to enter the bronchioles, while a particle < 2.5 µm may even pass through the walls of the alveoli to enter the bloodstream. Alternatively, these particles may be transported by pulmonary macrophages to the lymphatic system (Wieland et al., 2022b). Sneezing and nose blowing may be an effective means of expelling a proportion of particles trapped by nasal mucus just as coughing and beating cilia in the primary bronchus moves particles into the pharynx, but in order to know how effective these barriers are, particle profiles should be fully constructed. Despite the possibility of particles entering alveoli, some estimate a very small proportion of these particles (<1%) are absorbed (Prata, 2023).

Similarly, it has been shown in mammals that particles smaller than 150 µm are physically capable of crossing the gastrointestinal epithelium (Campanale et al., 2020), but it is not yet known what proportion actually do. Synthetic polymers less than 20 µm could access secondary organs and tissues that lack surface areas exposed to the environment (Barboza et al., 2018). Just as with the respiratory

system, clearance mechanisms exist in the digestive tract. These include paracellular and transcellular movement of particles, with the former a possible route to the lymphatic system and the latter is a route to surrounding tissue. Particles can also move via the circulatory system from primary to secondary exposed organs (Volkheimer, 1975). Again, estimates of particles absorbed in this manner are only ≤ 0.3% (Barboza et al., 2018) but more research is needed to quantify the passage of particles from the digestive system.

Despite an anticipated limited absorption of synthetic polymers through ingestion and inhalation, some particles (<2.5 - 5 µm) are still capable of moving beyond anatomical and physiological barriers to access secondary tissues such as the lymph nodes, liver, and spleen (A. Xu et al., 2022). Curiously, one of the earliest studies in the realm of human exposure providing evidence of particle translocation within the human body does not stem from the ingestion or inhalation of contaminant particles. This study involved an examination of postmortem patients who lived with hip or knee metallic/polymer-based prosthetic implants for a mean 69 months and 84 months, respectively. Investigators found micron and submicron (<1-30 µm) granular particles and shredded fibers of polyethylene in 68% of abdominal para-aortic lymph nodes, while only 20% of control group members exhibited "scattered macrophages" in the lymph nodes. Similarly, 14% of liver or spleen tissues from prosthetic patients contained polyethylene fragments, while control group members had none (Urban et al., 2000). While exposures in this research population are limited to those receiving plastic joint implants, these findings are corroborated by numerous animals studies that demonstrate the movement of microplastics

into systems, tissues, and organs that were not directly exposed (Deng et al., 2017).

More recent studies that support the possibility of microplastics migration within the human body involve the examination of an enclosed organ, the placenta, and three biofluids, breastmilk, blood, and urine. The two placental tissue studies differed considerably in subject selection (vaginal birth vs. cesarean), sampling methods (mass of tissue vs. volume of tissue), confirmation tools (Raman vs. FTIR), and reporting (particles/g placenta vs. no concentrations reported). Both had small sample sizes with (n = 6) for vaginal births and (n = 2) for cesarean delivery, and both reported the recovery of PP fragments after applying corrections for negative control (Ragusa et al., 2021; Braun et al., 2021).

The following year, a team analyzed the blood of 22 volunteers and found 77% contained detectable levels of microplastics. The authors focused on five "high production volume polymers," and using pyrolysis – gas chromatography/mass spectrometry, reported a mean concentration of 1.6 µg/ml of blood (Leslie et al., 2022). Similarly, 76.5% of breastmilk samples collected from 34 postpartum patients contained microplastic particles, and using Raman micro-spectroscopy, average concentrations were 0.526 fragments and spheres per gram of milk. The most abundant polymer types were polyethylene, polypropylene, and polyvinyl chloride (Ragusa et al., 2022). Each participant in this study was given a questionnaire to gauge exposure one week before and after delivery, from personal care products and diet, including food and beverage packaging. No statistically significant correlation was found between exposure and microplastic concentrations in milk samples,

indicating a certain level of environmental pervasiveness of this contaminant that cannot be controlled on an individual level.

Finally, in a pilot study involving six healthy volunteers, seven fragments ranging from 4-15 µm in size were recovered from urine samples. These plastics represent four different varieties, polyvinyl chloride, polyethylene vinyl acetate, polyethylene, and polypropylene (Pironti et al., 2022). The authors point out that the latter two plastic types (PE and PP) make up roughly 90% of plastic varieties recovered from drinking water, suggesting beverages could be a potential source (Q. Zhang, Xu, et al., 2020). However, since this contaminant has been isolated from other human biofluids, the ultimate source and point of entry remains unknown. These studies hint at the ubiquity of this contaminant as well as the possibility of exposure in very early stages of human development. Authors of blood, breastmilk, and urine studies cite ingestion and inhalation as possible routes of exposure, but the exact pathways are yet unknown.

Many of these investigations involve the excision of human tissue or the drawing of human biofluids, which provide a valuable snapshot in time, but there is little knowledge about the contaminant's pathway and residence time within the body. For example, there is concern that fibrous microplastics may demonstrate bio-persistence in the lung due to their shape and higher surface area (Enyoh et al., 2019). This may be associated with a particle's aerodynamic properties which allow for longer, finer fibers to move deep into lung tissue where they resists complete phagocytosis (Amato-Lourenço et al., 2020).

For particles that cannot be broken down and removed by the body, they may remain lodged, persistent, and biologically active within the

tissue. A 1990 study examined the dissolution of synthetic fibers in a Gambles solution, or simulated lung fluid, over the course of 180 days. Here, the authors of the publication found the three types of plastics tested to be both durable and persistent with no changes in surface area or surface characteristics (Law et al., 1990).

The body's inability to fully capture and process these foreign bodies is concerning, especially when exposure rates are high. One study described seven cases of bronchopulmonary disease in textile workers with occupational exposure to nylon, acrylic, and/or polyester(Pimentel et al., 1975). Through radiological analysis and surgical biopsies, investigators found trapped fibers within the lung tissues, as well as inflammation, fibrosis, and granulomas. In addition to the particles themselves causing respiratory damage, the surface of microplastics are also known to harbor diverse populations of microorganisms (Enyoh et al., 2019) making suspended synthetic particles possible abiotic vectors for pathogens.

Finally, some microplastics research focuses on the study of particles taken from patients who are being treated for a variety of diseases. This includes the extraction of microplastics from tumoral colon tissue (Cetin et al., 2023), cirrhotic liver (Horvatits et al., 2022a), carotid artery plaque (Marfella et al., 2024) and cerebrospinal fluid taken from patients who were amyloid-positive and amyloid-negative (He et al., 2025), as well as patients with inflammatory bowel disease (Yan et al., 2022). While some of these case control studies show a higher amount of microplastics in individuals with an ailment than those without, it is not known at this time if those relationships are causal.

It should also be noted that many of these studies quantify abundance by particle count and not mass. There are a few that do report mass but based on the techniques they use, they do not report particle size or count. The studies that report mass involve adult and infant stool (J. Zhang et al., 2021), human blood (Leslie et al., 2022), carotid plaque (Marfella et al., 2024), brain tissue (Campen et al., 2024), and testis (Hu et al., 2024). However, one study used both pyrolysis-GC/MS and LDIR to give a full profile and this should be the gold standard moving forward (B. Zhao et al., 2024a)

The Purpose of Present Work

Currently, there is uncertainty about the human and organismal health hazards associated with microplastics exposure. Risk assessment and threshold values cannot yet be calculated, in part because plastic is an exceptionally heterogeneous material. Apart from over 1,000 synthetic polymers currently in production, there is also a vast array of chemical compounds associated with plastics manufacturing. Some of these are used during processing and persist on the product as a residue. Others are added with the intention of being in the final product. Those in the latter category serve a host of practical functions acting as colorants, ultraviolet stabilizers, biocides, fillers, plasticizers, reinforcements, flame retardants, lubricants, or antistatic agents. However, none of them, aside from a specific class of reactive flame retardants, are covalently bonded to the polymer matrix (M. Liu et al., 2021). This means they can leach from the product into their immediate environment.

The exposure and dose of this large suite of chemical compounds is not well known as they are not detected by the most prominent means

of chemical characterization in microplastics research (vibrational spectroscopy). Furthermore, due to proprietary rights, manufacturers are under no obligation to disclose additives. Nonetheless, several teams of scientists have attempted to amass chemical databases for plastic products. A very recent and thorough digital repository reveals 16,325 chemicals in total (Wagner et al., 2024). Of this, 26% (4,219 chemicals) have been studied and are known to be hazardous, while another 66% (10,726 chemicals) have no hazard data (Wagner et al., 2024). In addition to the potential threats from chemical additives, numerous environmental pollutants (PAH, PCBs, DDT, PBDEs, heavy metals) and pathogens (members of a larger microbial community termed the 'plastisphere') demonstrate an affinity to the surface of microplastics (Cole et al., 2011). These sorbed environmental chemicals and microbes leave the potential for desorption once ingested or inhaled.

Furthermore, as evidenced by the work summarized herein, studies vary in terms of how they are designed, and even minor inconsistencies in methods and techniques may result in findings that differ by several orders of magnitude. This was seen in the two German bottled water studies carried out by Oßmann et al. (2018) and Schymanski et al. (2018). All challenges in predicting exposure aside, variations in actual exposure can also be attributed to differences in an individual's immediate environment, diet, and how foods and beverages are packaged and prepared. An understanding of an individual's true body burden at any given time is limited by scant knowledge of microplastic's toxicokinetics. In addition, the effects of chronic exposure to particulates and their chemical additives are not known,

and the complex pharmacodynamics, or interactions between sorbed and endogenous chemicals, remain elusive.

Until now, microplastic abundance in environmental media and biota was expressed solely as a particle count. A method newly applied to this field of research, pyrolysis-GC/MS, which provides mass based on chemical composition could help answer some of these questions (Okoffo et al., 2020; Ribeiro et al., 2020). If this technique were used in tandem with other characterization techniques it would allow for the development of a full contaminant profile. However, some concerns have been raised about the use of this instrument for lipids rich human tissue samples, as fats can interfere with its ability to detect polyethylene and polyvinyl chloride (Rauert et al., 2025). More standardization and quality control and quality assurance measures need to be carried out to validate these new methods.

While there is a palpable urgency to understand the impact that microplastics have on human and organismal health, it is also essential to study, concurrently, all possible routes of exposure. This research will prove critical if a clear link between exposures and negative health outcomes are found, as it will help pinpoint sources that are of high concern, thus expediting the necessary policies and changes in behavior required to protect individuals from harm. Exposure research also provides governments and industries an early opportunity to adopt measures in accordance with the Precautionary Principle by allowing them a chance to transition to, or invest in, alternative materials well in advance of any conclusive findings that link exposure to negative health outcomes. For example, biodegradable erosion control netting has been proven to exhibit superior performance, staying in

place longer, while it is also safer for terrestrial organisms (P. Leete, personal communication, April 23, 2020). This makes it a viable substitute for its synthetic counterpart, of which 31 tons is currently applied annually in the state of Minnesota. Exposure research may encourage municipalities to move toward safer, more sustainable alternatives today, to circumvent possible consequences from microplastic contamination in the future.

Finally, this work is important because of the sheer volume of synthetic polymers in existence. Even if the manufacture of plastics were to suddenly halt, the enormous sum of legacy plastics, some 10.6 billion tonnes, will continue to degrade into microplastics for the foreseeable future. For this reason, it is important to identify 'hot spots,' or areas where the potential for release is highest. It is also important to understand the environmental conditions that provoke large volume particle shedding. The full profile of contaminants, including their chemical make-up (indicating density), their morphology, as well as their size are all important clues to their origins and fate.

There is ample evidence that the rapid growth in plastic production is due in part, not to consumer demand, but industry's encouragement of plastic in general and use of single-use plastic in particular. In fact, the editor of *Modern Packaging* magazine, Lloyd Stouffer, stated to a group of industry insiders at a conference in 1956 'the future of plastic is in the trash can.' (Stouffer, 1963). Certainly, a linear economic model can be very profitable when products are sold to consumers, and the waste from those products is externalized to counties and municipalities. However, the simplicity and ease of this practice is changing as research from the last decade

draws attention to potential ecological and human health threats caused by the profligate use of plastics. In a recent survey involving 1,960 U.S. adults and 882 members of a nation-wide ocean advocacy organization, concern about plastic pollution ranked higher in both populations than eight other categories of concern that were offered, including climate change. Of the adults surveyed, 49% had heard of microplastics, while 91% of the advocacy group members were familiar with the term. These results indicate an almost intrinsic interest in this topic, and more importantly, an incredible opportunity to further educate and engage citizens in the issue of microplastic pollution (Baechler et al., 2024).

Despite this interest, some challenges lay ahead. One stumbling block involves the public's understanding of the scientific method and expectations of scientific findings. According to a Pew Research Center survey that polled 4,464 respondents, a little over half of adults (56%) with a high school education or less, viewed science was an iterative process. In the same demographic, 17% said science 'identifies unchanging core principles and truths.' This implies a proportion of the public believe scientific findings reveal absolutes. Meanwhile, another 25% were not sure how to answer the prompt (Kennedy & Hefferon, 2019). It is noteworthy that among four separate categories of science questions given by the Pew survey, all respondents independent of educational attainment had the lowest scores when it came to understanding the scientific process. A more tenuous grasp of the scientific method may result in an inability or lack of confidence to critically assess research findings. An inability to process and understand results may create vulnerability in individuals to be more

readily influenced or swayed by those with a political agenda. Research conducted with poor study design, lacking experimental controls, loose extrapolations, and/or small or unrepresentative sample sizes are limitations that may be missed by an audience that does not possess a robust understanding of the scientific method.

Another hurdle involves the way that the media portrays science to the public. As stated above, public awareness about microplastic pollution has grown as research findings emerge, but popular media reports often feature oversimplified and inaccurate descriptions of scientific work. Furthermore, limiting factors in study design and nuance in results may be glossed over or completely omitted. For example, results in a publication that used three mathematical models to estimate the mass of plastic ingested by humans were reduced to a single statement: every human ingests a credit card worth of plastic each week (Senathirajah & Palanisami, 2021). This assertion has been challenged and contested by experts in the field (Pletz, 2022), but first they were amplified in countless media outlets and restated by national and international policymakers. Another provocative assertion is that humans with plastic cutting boards ingests 50g from these boards every year (Yadav et al., 2023). A final statement is, every human carries an equivalent weight of a plastic spoons (7 g) of microplastics in their brains (Weise, 2025). Statements like these garners a lot of attention, but the context, limits in study design, and margins of error are underreported, if they are reported at all.

Presenting science as a solitary event instead of a process reinforces an idea that science is not an iterative method, but a collection of absolute and irrefutable facts. When the media fails to

contextualize findings by ignoring the larger body of work and the inherent nuance involved in any study, it runs the risk of insincerely framing a single publication as a full authority. This framing can warp the perception of the public and generate confusion if findings start trending in another direction, as they sometimes do. If the public's perception of science is distorted, or if they have an unreasonable expectation about what scientific results mean and how findings can be used by society to make improvements, it can do more than create confusion, it can seed resentment and mistrust. A separate Pew survey reveals a sixteen percentage points drop in Americans that believe science has a positive effect on society, from 2019 to 2023 (Kennedy & Tyson, 2023). This drop was even more pronounced, twenty-four percentage points, when political affiliation (those who identify as Republican) was factored in. For these reasons, robust scientific literacy and critical thinking skills are essential.

This dissertation seeks to refine microplastic processing techniques to improve accuracy in both particle enumeration and physical characterization through the advancement of quality assurance and quality control measures. Using these enhanced methods and imaging software, this work will fill gaps in knowledge around the fate and transport of microplastics through ambient air and explore direct human exposure to microplastics through the use and care of sports hydration bottles constructed from various plastic components. Finally, these methods are modified and adapted for a citizen science program that seeks to elevate science literacy and promote an understanding about the issue of local microplastic pollution. Each of the following chapters are written as independent manuscripts.

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Chapter 3: Quality Assurance and Quality Control in Microplastics Processing and Enumeration

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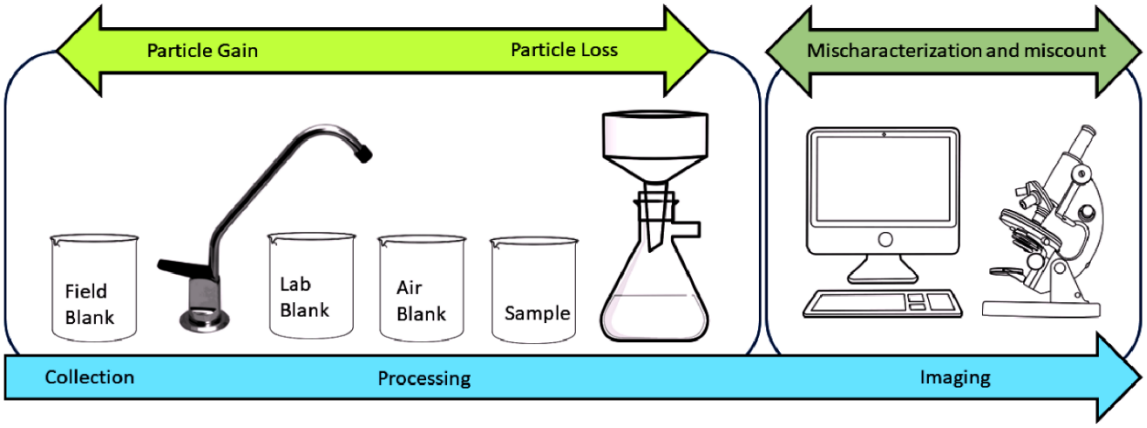

Abstract

Despite six decades of microplastic contamination research, this field struggles to establish clear, universally accepted methods and techniques. Furthermore, scant published work scrutinizes the application, effectiveness, and utility of positive and negative controls. This study examines three common practices involved in microplastic processing, enumeration, and particle characterization. The first component evaluates four filtered water sources that are commonly used to run procedural laboratory blanks and rinse laboratory

glassware and instruments. A statistically significant difference was found between tap water and three sources of filtered water and between two sources of filtered water. This suggests the magnitude of correction applied to samples is dependent on the type of filtered water chosen for blanks. The second component chronicles particle loss, specifically particle adhesion to the filtration apparatus. Water samples spiked with plastic standards representing three distinct morphologies and vacuum filtered through a two-piece borosilicate glass filtration apparatus yielded a notable difference in recovery rates compared to a two-piece filtering apparatus made of stainless steel. The steel filter had significantly higher recovery rates of irregular polyethylene fragments compared to glass, although there was no statistical difference in the recovery of nylon fibers or symmetrical polyester fragments. The final component compares the effectiveness of ImageJ, a popular imaging software program, with Material Image Processing and Automated Reconstruction (MIPAR), a new program with deep learning capabilities. Both systems analyzed identical images captured from a set of polycarbonate filters that contain environmental media spiked with plastic standards. While ImageJ is capable of reporting particle enumeration, as well as basic measurement and categorization, it grossly overestimated fragments and frequently mischaracterized fibers. Particle count summaries from MIPAR, however, were in accord with the known quantity of standard spiked into each sample. These findings underscore the importance of quality controls when developing new methods or when modifying established methods.

Introduction

Scientists have been documenting plastic pollution in our environment for over 60 years, and although plastic pollution has been accumulating in the environment since the 1950s, the scientific interest and research have lagged significantly with microplastics (plastic particles <5mm) gaining traction in the scientific community only for the past two decades (Qin et al., 2020). Despite these early publications, advances in the methodology for detecting microplastics in the environment remain a complex and time-consuming process. Currently, there is no standard operating procedure for sample collection, processing, and analysis/detection of microplastics other than the recommendations from the National Oceanic and Atmospheric Administration published in 2015 (Masura et al., 2015), which only allows the quantification of plastic particles >300 µm.

Numerous methods used to process and identify microplastics are described in the literature. These methods range from basic filtration, sieving, using different digestion solutions to dissolve organic matter (e.g., Fenton's reagent, potassium hydroxide, etc.), and density separations to visual enumeration using standard stereoscopic microscopy, fluorescence microscopy, imaging analysis tools, and spectroscopic/spectrometric quantification (Raman spectroscopy, Fourier transform Infrared spectroscopy, pyrolysis-gas chromatography mass-spectrometry, etc.) used to identify the chemical fingerprint of plastic polymer types (Erni-Cassola et al., 2017; Hendrickson et al., 2018). Each method comprises varying quality assurance and quality control procedures to ensure sample integrity.

Quality control and quality assurance (QA/QC) is imperative in microplastic research, given their ubiquitous nature and high possibility of sample contamination. In addition, the laboratory water used to process microplastic samples varies in purity and is not standardized across different methods. Koelmans et al. (2019) evaluated 50 different microplastic publications in freshwater and drinking water for the QA/QC criteria used in each study. Only 4 out of 50 studies evaluated received a positive score indicating all QA/QC criteria were met, which suggests there is high variability in QA/QC protocols from study to study (Koelmans et al., 2019).

Gwinnett and Miller (2021) investigated procedural microplastic contamination introduced to water samples through laboratory processing methods by using strict QA/QC protocols as well as no QA/QC protocol, and found significantly greater procedural contamination in the samples where no QA/QC protocol was used (Gwinnett & Miller, 2021). Both studies highlight the need for significant improvement in the measures and QA/QC protocols used to maintain sample integrity. The absence of standardized methods to analyze microplastics makes it difficult to compare results across studies and is even more complicated when lacking QA/QC protocols.

The methods used to enumerate microplastics include visual counting, and fluorescence microscopy coupled with imaging analysis tools. Visual counting of microplastics under a stereoscopic microscope is extremely time-consuming and subjective to the individual interpreter and requires skill to differentiate between plastic polymers and organic materials. Visual quantification only allows the detection of larger size fractions of plastics (typically >100 µm) and

may drastically underestimate the amount of microplastics contained within a sample (Erni-Cassola et al., 2017; Prata et al., 2019; Simmerman & Coleman Wasik, 2020).

Fluorescent dyes such as Nile Red have gained popularity in microplastic research methods. Their strong sorption to plastic polymers renders them easy to identify when viewed under a fluorescence microscope (Erni-Cassola et al., 2017; Prata et al., 2019; Shim et al., 2016). In addition, image processing analysis tools can be used in combination with fluorescent dyes to easily enumerate, categorize, and measure plastic particles.

The most commonly used open-source imaging processing software is ImageJ and is widely used in microplastic research. To illustrate the popularity of this free software, a Scopus literature search of library databases using the keywords 'microplastic' and 'ImageJ' revealed 435 documents. ImageJ allows the user to set several thresholds and criteria specific to their individual images to analyze microplastics using the 'Image,' 'Process,' and 'Analyze' tabs within the program. Erni-Cassola et al. (2017) established the methods for the use of Nile Red dye and ImageJ analysis for microplastics and provided an algorithm for manual microplastic identification in ImageJ. No semiautomated or fully automated software plug-ins were available until Prata et al. (2019) built a plug-in/macro for ImageJ (Microplastics Visual Analysis Tool, or MP-VAT) to streamline and semi-automate the threshold setting and image processing of microplastic images.

MP-VAT is capable of quantifying microplastics as small as ~1 µm, which is highly sensitive compared to 300 µm stated in the NOAA 2015 methods. The output data of MP-VAT provide parameters, including the

number, area, shape, and Feret's diameter of the microplastics, and are the only attempts to date to automate microplastic quantification using Nile Red dye and image analysis. Although MP-VAT performed with satisfactory results in the original publication, it is noted that future experiments to validate this method using spikes of different polymer types are necessary to confirm the capabilities of MP-VAT.

Material Image Processing and Automated Reconstruction (MIPAR) is an imaging analysis program that allows code-free feature detection algorithm creation. Using 110 images containing fluorescing plastic standard particles as well as environmental samples representing various media-air, water, and wastewater, a pretrained deep-learning model was used to create a preliminary customized algorithm to identify microplastic fibers >200 µm and particles >30 µm. While potential exists for this algorithm to be further perfected, it worked well for the purpose of comparing the conventional ImageJ by a program with machine learning abilities.

This study highlights the importance of implementing strict QA/QC procedures in microplastic research. The aims of this study include (1) describing the difference in microplastic concentrations detected in five different laboratory water sources, (2) noting the importance of running positive controls by showing sample loss from adherence to labware (in addition to negative controls to quantify sample contamination), and (3) testing the efficacy of two different image analysis tools, ImageJ coupled with MP-VAT;(Prata et al., 2019) and MIPAR, to accurately detect and enumerate fluorescing microplastics.

Materials and Methods

IRB ID: STUDY00008248. The IRB determined that the proposed activity is not research involving human subjects as defined by DHHS and FDA regulations. To determine this, the IRB used WORKSHEET: Human Research (HRP-310).

Filtered laboratory waters

The five laboratory water sources tested include tap water, deionized (DI) water, reverse osmosis (RO) water, high performance liquid chromatography (HPLC) grade water, and nano-pure (NP) >18 MΩ-cm laboratory grade water. The tap water is from a municipal source. The DI water system is municipal tap water with localized secondary filtration. The NP and RO water systems are smaller units confined to the laboratory, both drawing on the DI water system. Finally, the HPLC water is packaged in 4 L volumes, and, according to the manufacturer, filtered to 0.2 µm.

Each type of water was processed in 300 mL volumes in triplicate (n = 15). Processing involved wet peroxide oxidation (WPO), Nile red staining, and contaminant enumeration with customized imaging software, MIPAR. WPO consists of adding 10 mL of a 0.05 M solution of ferrous sulfate and 10 mL of 30% hydrogen peroxide to a sample on a 75°C hotplate with a cleaned magnetic stir bar for 20 min, as described by the NOAA Marine Debris Program (Masura et al., 2015).

Nile red staining consists of adding 0.5 mL of a stock solution to the digested solution to achieve a concentration within the optimal fluorescence range reported by Maes et al., (2017). Neither the WPO reagents nor the Nile red stain were prefiltered. While this lack of

filtering could be a potential contribution of microplastics to our samples, they would also contribute to the laboratory water blanks and therefore be accounted for. After staining, the solution is allowed to cool for 20 min before vacuum filtration through a 5.0 µm polycarbonate membrane filter. A laboratory wash bottle filled with the same water source as the sample was used to thoroughly rinse the beaker that held the sample as well as rinse down the sides of the filtration funnel.

The 47 mm polycarbonate filter, moved from the filtering apparatus with rinsed forceps, was placed in a 47 mm diameter petri dish and covered. By placing the petri dish on a grid under a 450–510 nm blue/green Crime-Lite 2, each filter was imaged in its entirety in 15 separate pictures captured with a Leica EZ4W at 8X magnification. Saved images were then processed with MIPAR, which yields a summary of total contaminant fragments and fibers per sample. Tap water was expected to contain the most contaminants since purification was most distant from the spout. DI water is filtered within the building, so it was expected to contain fewer contaminants than tap water. HPLC water was expected to contain the least since it is filtered to 0.2 µm.

Filtering apparatus

The two filtering apparatuses examined had different material composition, design, and dimension. One was borosilicate glass with a 250 mL volume reservoir secured to a fritted glass base with an aluminum clamp. Another was stainless steel with a 500 mL volume reservoir and mesh screen base secured with a hand-tightened yoke. In this experiment, plastic standards pre-stained with Nile Red, representing three distinct morphologies and plastic types— nylon fiber, irregular polyethylene fragment, and symmetrical polyester

fragment, were sorted into groups of 20 (60 standards per sample) and imaged before they were rinsed into a clean beaker containing 100 mL of RO water. See Chapter 3 Figure 1 in appendix for images and dimensions of standard particles. As described in the filtered laboratory water experiment, these spiked samples were processed with WPO and Nile red staining before they were imaged and analyzed with MIPAR.

During three stages of sample processing, four pieces of laboratory equipment—magnetic stir bar, beaker, filtering cup (funnel), and filtering head (base)—were examined with the Crime-Lite 2 for errant particles. Fibers, irregular fragments, and symmetrical fragments that adhered to labware were tallied. The sum of these 'lost' standard particles was then reconciled with the standard particles 'captured' through vacuum filtration and percent recovery based on the standard morphology type was calculated. Final results were based on 10 samples passed through the glass apparatus and 10 samples passed through the steel apparatus for a sum of 600 standardized particles spiked into each filtering apparatus.

Standard QA/QC

Powder-free nitrile gloves and a 100% cotton laboratory coat were worn during sample handling and processing. All sample processing took place within a biosafety cabinet. All beakers and filtering apparatus reservoirs were covered with aluminum foil and only uncovered during pouring of the sample or addition of reagents. All glassware was washed, wrapped in aluminum foil, and baked in an oven for 6 h at 450°C to reduce cross-contamination (Prata et al., 2021).

Imaging software

A set of environmental samples consisting of tap water collected from 20 homes was used to compare the conventional imaging software program, ImageJ, with the contemporary program, MIPAR. Residents from 20 separate domiciles collected two 400 mL volume samples of tap water (n = 40) with a set of instructions for the sake of standardizing collection procedures. One tap water sample was immediately capped, and the other was left out on a bedstand overnight and capped in the morning. All samples were processed with WPO and Nile red staining as described in the filtered laboratory water experiment. Images of sample filters were captured before each filter was spiked with 15 Nile red stained plastic standards (5 nylon fibers, 5 symmetrical polyester fragments, and 5 irregular polyethylene fragments).

After spiking, each filter was immediately re-imaged. To evaluate each program's ability to accurately enumerate and characterize fluorescing contaminants, a summary of fiber and fragments gathered from images taken before and after spiking with standards was analyzed with both ImageJ and MIPAR. For example, a total count of fibers in an original sample processed with MIPAR was subtracted from the total count of fibers in the same filter after it had been spiked and re-imaged.

An overall average of the difference in fiber count for all filters imaged with MIPAR can then be compared with a difference in fibers count for all filters imaged with ImageJ. Similarly, a total count of fragments (symmetrical and irregular) in an original sample processed with MIPAR was subtracted from a total count of fragments in the filter after it had been spiked and re-imaged. The average of these differences was also compared with ImageJ to compare the two programs.

Comparing the difference between spiked and un-spiked totals will indicate each program's ability to accurately detect the standards, since the quantity of standard spiked on each filter is known.

Results

Filtered laboratory waters

Comparing the average of each of the five laboratory water samples reveals that tap water exhibited the highest concentration at 17.67 ± 1.7 particles/300 mL (mean ± standard deviation), followed by DI water at 12.3 ± 3.4 particles/ 300 mL, RO water at 6.67 ± 2.6 particles/300 mL, HPLC at 2.67 ± 0.94 particles/300 mL, and NP water at 2 ± 1.6 particles/300 mL (Figure 3.1). The laboratory water data set was normally distributed, so parametric comparisons were used (t-test).

There was no statistically significant difference between RO, NP, and HPLC waters. Significant differences were observed between the microplastic concentrations in RO and TAP (p-value <0.05), NP and TAP (p-value <0.001), and HPLC and TAP (p-value <0.01). In addition, significant differences were observed between the microplastic concentrations in DI and NP (p-value <0.05). There was no significant difference in the fiber to fragment ratio between the five different laboratory waters. See Chapter 3 Table 1 in appendix, for morphology and size of contaminants in lab waters. Similarly, there was no difference in the sizes of particles and fibers recovered from the various laboratory waters, although both morphologies were smallest in the NP water.

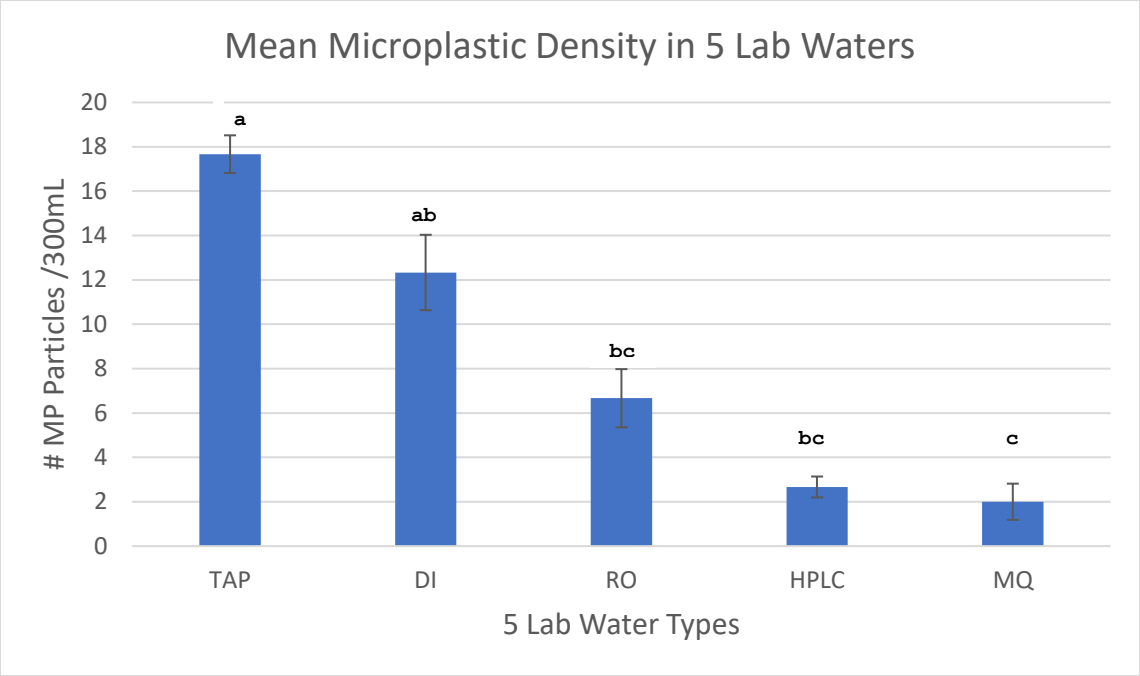

Figure 3.1 Mean microplastic concentration in five different laboratory waters. The letters a, b, and c indicate statistically significant differences in the means among five laboratory waters at level $p < 0.05$ as determined by t-tests. The error bars represent the standard deviation.

Filtering apparatus

Summarizing the results of the 20 trials in this experiment reveals that most lost particles are lost on the filtering apparatus (Figure 3.2). Only 0.73% of particles (1 symmetrical fragment and 1 irregular fragment) adhered to the magnetic stir bar, 3.67% of particles (5 symmetrical fragment and 5 irregular fragments) adhered to the rinsed beaker, 6.59% of particles (2 symmetrical fragment, 14 irregular fragment, and 2 fiber) adhered to the filtering head, and 89.01% particles (33 symmetrical fragment, 95 irregular fragment, and 115 fiber) adhered to the filtering cup. Based on morphology, the particles lost overall to labware adherence were 15% symmetrical fragments, 42.1% irregular fragments, and 42.9% fiber.

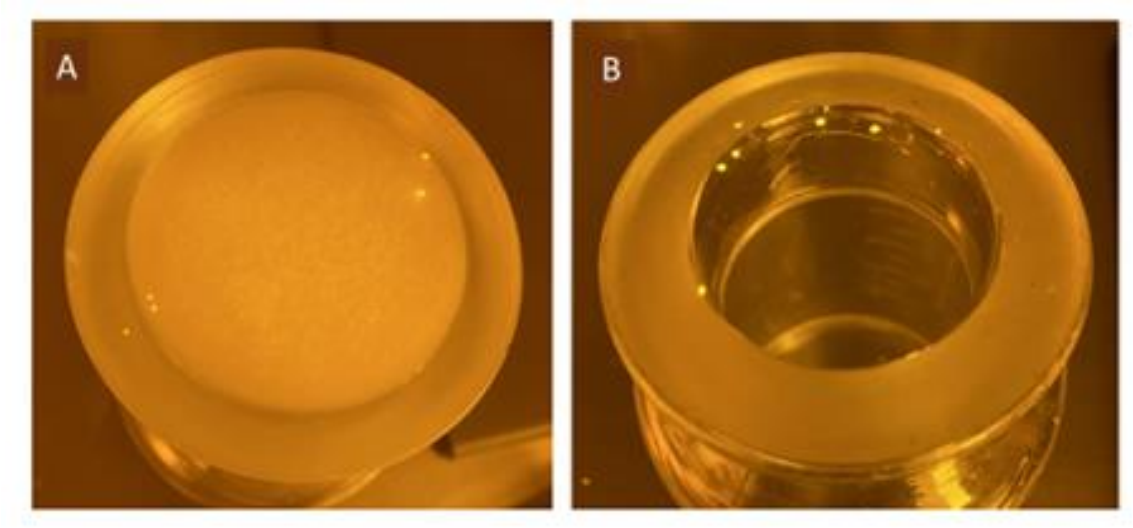

Figure 3.2 Stained standard particles adhered to the cup (A) and head (B) of a borosilicate filtering apparatus, revealing the potential for missing particles in a sample.

When comparing performance of the borosilicate glass against the stainless-steel filtering apparatus using a Wilcoxon Rank Sum test, a significant difference is observed for microplastics of the irregular fragment morphology (p-value < 0.01), suggesting the effectiveness of the two filtering apparatuses are not equal. However, there was no significant difference in the symmetrical fragment or fiber morphology (Figure 3.3).

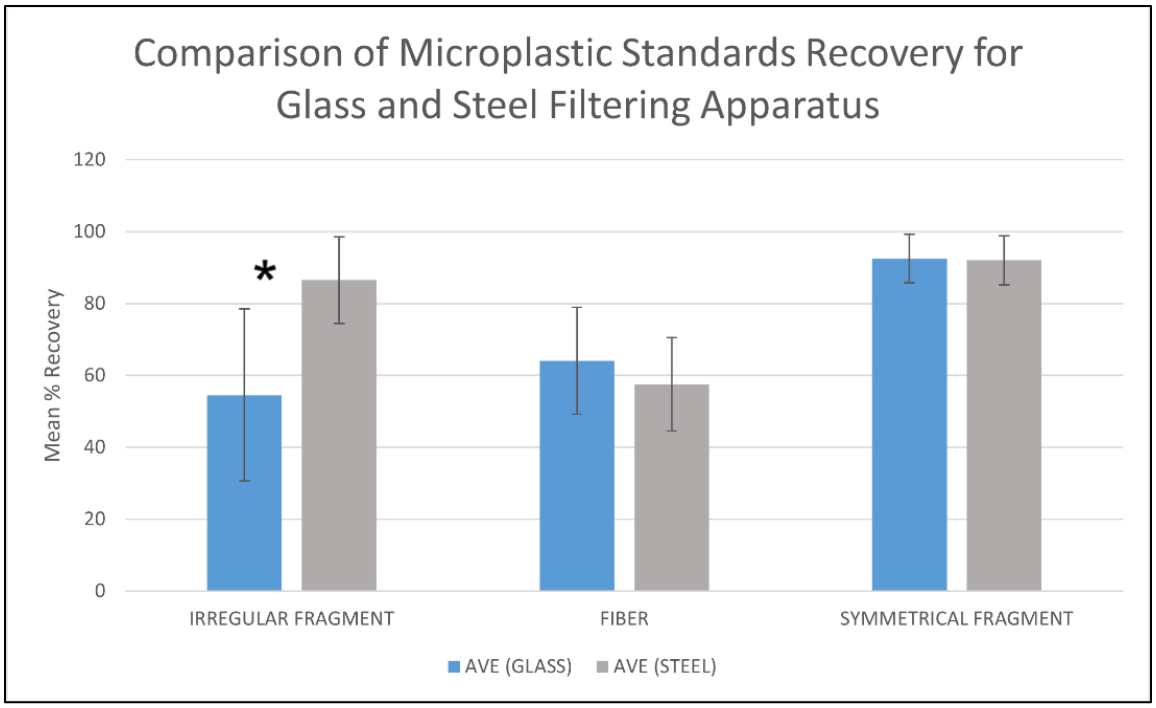

Figure 3.3 Comparison of recovered plastic standards representing three morphologies, filtered through a glass apparatus versus a steel apparatus.

Imaging software

Since each filter was spiked with 15 standard particles, an expected quantity (5 fibers, 5 symmetrical fragments, and 5 irregular fragments) can be compared to what was detected by each of the two imaging software programs, ImageJ and MIPAR. When the initial customized algorithm for MIPAR was developed, it was only capable of identifying two morphologies, fiber and fragment. ImageJ with MP-VAT, however, was capable of identifying three morphologies: fibers, fragments, and particles. Fragments are defined as irregular and particles are defined as circular. Due to this difference in the number

of morphological categories in each imaging system, there were two groups analyzed with both ImageJ and MIPAR.

One analyzed fragments (combining symmetrical and irregular) and another analyzed fibers. The expected number of fibers per filter after subtracting spiked images from un-spiked filters was five. Of the 40 samples collected and processed, the average number of fibers counted by ImageJ was 9.025, while the average number of fibers counted by MIPAR was 5.300. Next, the expected number of symmetrical and irregular fragments after subtracting spiked images from un-spiked images was 10. Again, of the 40 samples collected and processed, the average number of fragments counted by ImageJ was 83.55 while the average number of fragments counted by MIPAR was 14.4. Overcounting was seen for both imaging programs, but the degree of overcounting in ImageJ exceeded that of MIPAR (Figure 3.4).

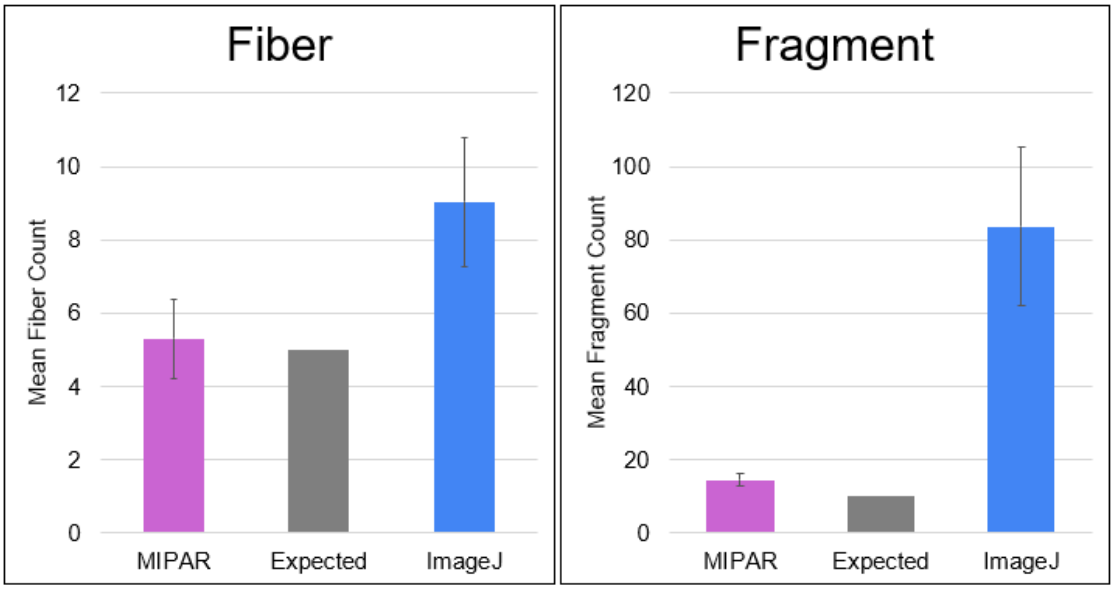

Figure 3.4 Expected fibers compared to actual fibers counted by MIPAR and ImageJ (left) and expected fragments compared to actual fragments counted by MIPAR and ImageJ (right).

To compare the performance of two separate imaging programs, ImageJ and MIPAR, in their ability to accurately quantify microplastics in a total of 40 tap water samples collected from 20 domestic homes, a Wilcoxon Rank Sum test was used. Significant differences were observed between counts of MIPAR and ImageJ fibers (p-value < 0.0001) and between counts of MIPAR and ImageJ fragments (p-value < 0.0001). This suggests the output of the two imaging software programs are not equal.

Discussion

Filtered laboratory waters

High-quality environmental testing requires laboratory water systems that provide an abundant supply of pure, filtered water. In microplastic research, a reliable water system is essential for washing and rinsing glassware and other laboratory implements. It is also used to run negative controls. Negative controls quantify contaminant particles that have been inadvertently introduced during sample collection and processing. These controls may consist of collection blanks, air blanks, and processing blanks (also known as laboratory, method, or procedural blanks).

In the case of a processing blank, a control may consist of a volume of filtered water run alongside samples, while a collection or air blank may consist of an empty beaker or petri dish set out, while an environmental sample is gathered or processed. This empty glassware is then rinsed with filtered water and run alongside samples. When sample processing is complete, the abundance of particles in negative controls is often subtracted from the abundance or particles in the

environmental samples as a means to correct the unintentional introduction of particles (Kosuth et al., 2018).

However, Dawson et al. (2023) analyzed six different methods to correct environmental concentrations of microplastics from negative controls in 51 publications and determined that subtraction and mean subtraction is the least accurate way of correction, and establishing a limit of detection, or limit of quantitation (LOD/LOQ) is the most accurate way to correct environmental samples from negative controls. This work is important in all environmental contamination research, but it is especially important in microplastic research because the plastic is extremely pervasive. Particles can be introduced by chemical reagents, such as WPO, as well as through glassware and laboratory implements, such as stir bars and gloves.

They can also be introduced by the person collecting and processing the samples from the clothing they are wearing, while processing samples. Finally, they can be present in laboratory air. Additional measures are taken to guard against the unwitting introduction of microplastic particles to environmental samples. For example, processing samples in a fume hood, wearing laboratory coats made from 100% cotton over clothing made of natural fibers, and keeping samples covered during processing and transport are all common practices. Despite these precautions, contaminant particles that were not part of the original sample can sometimes find their way into a sample, and they must be accounted for. If they are not, results will be inaccurate and inflated.

According to the critical review published by Koelman et al. (2019), less than half of the studies examined conducted adequate

negative controls. In addition, in these studies, it was assumed that the filtered water used to carry out negative controls was itself completely void of microplastic contaminants. In some publications, this assumption is explicitly stated (Simon et al., 2018). No study to date has compared filtered laboratory water to see if there are differences in contaminant levels between filtered water systems.

While a variety of filtering water systems exist, each unique in their components, engineering, and volume output, this investigation was designed to measure possible variations in the five laboratory water sources available in a single laboratory. As stated in the results, tap water had the highest concentration of contaminants, which was expected. The lowest concentration was found in NP water, but no statistically significant difference was found between NP, RO, and HPLC filtered waters.

However, significant differences were observed between these three filtered waters and tap water, as well as between DI water and NP water. These variations in contaminant level may have to do with the plastic component parts that make up the filtering system. For example, a study of processed cow's milk found that the two types of plastic extracted from samples, polyethersulfone and polysulfone, most likely originated from the ultrafiltration and microfiltration membranes (Kutralam-Muniasamy et al., 2020b). All five water systems in this investigation contained plastic components.

The results suggest that the laboratory waters with higher contaminant concentrations may result in larger subtracted sum/average from sample concentrations, depending on the correction applied. This will result in under-enumeration of contaminant particles reported in

samples (Dawson et al., 2023). A full inventory of plastic components in a laboratory water filtering system coupled with chemical analysis of particles would reveal contaminant origins.

The age of the filter as well as pressure and volume of flow may also contribute to a greater number of contaminant particles. Based on these results, the authors strongly advise preliminary testing of filtered laboratory water before sample processing to ensure the lowest contaminant load. If microplastics are found in the filtered laboratory water, a LOD/LOQ must be established (Dawson et al., 2023). Results reported in this study should be considered to ensure negative controls serve their intended purpose: to quantify particles that are introduced during sample collection and processing.

An unfortunate limit to this test is the absence of chemical characterization of recovered particles. A list of plastic varieties isolated from a particular source reconciled with a complete inventory of plastic components in each system would have confirmed each contaminant's origins. Synthetic polymer fragments could originate from the filtering cartridge, tubing, or source water. However, the age of the system, the total volume of water filtered, and the pressure of piped water are variables that should be considered when measuring particle shedding. This study brings attention to the issue of potential contamination from filtered water, but identifying the absolute source is outside the scope of this investigation.

Filtering apparatus

Another important aspect of accurate reporting lies in running positive controls, which quantify the number of particles lost during sample collection and processing. Again, the critical review published

by Koelman et al. (2019) found only 6.3% of microplastic research conducted adequate positive controls. According to this review, positive controls are limited in that they often isolate a single step in the analysis or only focus on a particular size range. Another shortcoming of positive controls is that they are run with an insufficient number of replicates, meaning a correction cannot be made. When they are effectively carried out, they examine recovery rates of a reference material that in size, morphology, and chemical composition represent the types of plastic expected in the sample. Typically, if a recovery rate is reported, it is rarely used to correct for lost particles.

This study examines the possible loss of contaminant particles by way of adhesion to labware during sample processing. Results show that very few particles are lost to the stir bar (0.73% of total recovered) and glass beaker (3.67% of total recovered) used during WPO and Nile red staining. A larger proportion (95.6%) was lost on the filtering apparatus as a whole, and the vast majority of that was in the filtering cup, or funnel, (89.01% of total recovered) as opposed to the filtering head, or base (6.59% of the total recovered). These results indicate that rinsing the stir bar and beaker with filtered water from a squeeze bottle is an effective technique to move contaminant particles.

Since a stir bar has a small surface area and can be held with forceps and rinsed completely, it is not surprising that it has the lowest particle adherence. The beaker, while it has a larger surface area, can be held upside down over the funnel of the filtering apparatus and completely irrigated with a pressurized stream of

filtered water. However, when it comes to the filtration apparatus, despite the vacuum force applied, it is common for particles to get trapped at the base of the filtering cup where it articulates with the polycarbonate filter. Excess rinsing with a vacuum seems to do little to keep some particles from preferring the vertical surface of the filtering apparatus to the polycarbonate filter once the lower and upper portions are disengaged.

A siloxane coating was applied to the inside of the reservoir top of the glass apparatus, but no change in particle adhesion was observed. It is possible that ethanol, with hydrophilic and lipophilic properties, may serve as an effective rinsing solution, although Yang et al. (2023) tested ethanol against four other types of filtered waters and it had the highest load of contaminants (J. Yang et al., 2023). The authors suggest that the bottom of the filtering cup be examined for wayward particles. If particles are found, they should be transferred to the filter if possible. If this is not possible, they should be counted and added to the total.

Based on morphology, results show that symmetrical fragments were least likely to be lost to adherence, making up only 15% of total particles lost. The adherence of the other two morphologies, irregular fragments and fibers, was evenly split at 42.1% and 42.9% of total particles recovered, respectively.

This difference is possibly linked to the smaller size and shape of all fibers, which are uniformly 19.3 µm in diameter, and some irregular fragments, which varied in size and dimension. See Chapter 3 Figure 1 in appendix for images and dimensions of standard particles. There were also differences between the three standard morphologies and

their affinities with certain laboratory equipment. No fiber was found adhered to the stir bar and the beaker. Irregular fragments were 7 times more likely than symmetrical fragments and fiber to adhere to the filtering head. However, fibers were the most likely to adhere to the filtering cup, 1.2 times more likely than irregular fragments and 3.5 times more likely than symmetrical fragments. The dimensions of the surface area and possibly the rigidity of the fiber standards used in this study may contribute to their being trapped at the base of the filtering cup.

This study also included a comparison between two filtering apparatuses, one glass and one steel. The mechanism that holds the cup and head of the steel apparatus together is tighter and more secure than the glass, which is held together with an aluminum spring clamp. Glass filters may also have chips or imperfections in the surface where the two parts articulate, creating small gaps or cracks, giving particles the opportunity to migrate beyond the polycarbonate filter. As stated in the results, there was no significant difference found between the two filtering setups for the fiber and symmetrical fragment morphology, but a higher recovery was seen in the steel apparatus compared to glass when it involved the irregular fragment morphology. Of the three morphologies tested, fragments were the least uniform in both size and shape.

While the standard particles in this study represent three distinct morphologies, this testing is limited in that they do not represent a wide range of sizes. The purpose of this investigation is to show that particle adherence to labware occurs and that frequency may be dependent on particle morphology and the type of laboratory

implement. Before investigating a new media, it is important to become familiar with the size, shape, and types of synthetic polymers encountered through preliminary tests. Once this is known, standard particles that best represent those found in environmental samples can be used to run positive controls. If a significant number of particles are lost during these positive control tests and effective improvements cannot be made, then losses should be quantified and accounted for in the final reporting.

Finally, it should be recognized that certain chemical treatments intended to reduce biofouling, such as the WPO, may potentially degrade or warp microplastic particles. The three plastic standards used in this study were imaged before and after spiking and no significant change was observed. However, Munno et al. (2018) found low recovery rates of two varieties of microbeads extracted from personal care products after WPO treatment. These findings indicate caution should be exercised when choosing a method to reduce natural organic matter and the application of positive controls ought to be extended to include chemical digestion.

Imaging software

The third arm of this study examines the effectiveness of ImageJ, a popular imaging software program in microplastic research. It is commonly used for enumeration and characterization of fluorescent microplastics that have been stained with Nile red. Using a total of 600 standardized particles (40 sample filters and 15 plastic standard microplastics per filter) of three distinct morphologies, results indicate both software programs over-enumerate in the fiber and combined fragment categories. However, the extent of overcounting by

ImageJ far exceeds that of MIPAR. In the morphological category of fibers, MIPAR counts in excess of 6%, while with ImageJ, the average is more than 80% of what is expected. This difference is particularly extreme in the combined fragment category with an increase of 44% by MIPAR and an increase of 736% by ImageJ. One factor contributing to the inaccuracy in ImageJ is its inability to recognize the border of a particle and identify it as a uniform whole.

This is especially problematic when there is a gradation of fluorescing intensity in a single particle (Figure 3.5) or when the fluorescent light reflecting off the filter creates a soft edge around a fragment. ImageJ may view this as multiple fragments or a central particle surrounded by a spattering of tiny particles, all of which are counted as individual pieces. This not only compromises the accuracy of the overall count but also skews particle size and possibly other dimensional metrics, such as aspect ratio. Another drawback to ImageJ run with the MP-VAT plug-in is its inability to consistently identify fibers. Often, a segment of fiber fluorescing with intensity is identified as a discrete point instead of being cataloged as one segment of a continuous thread.

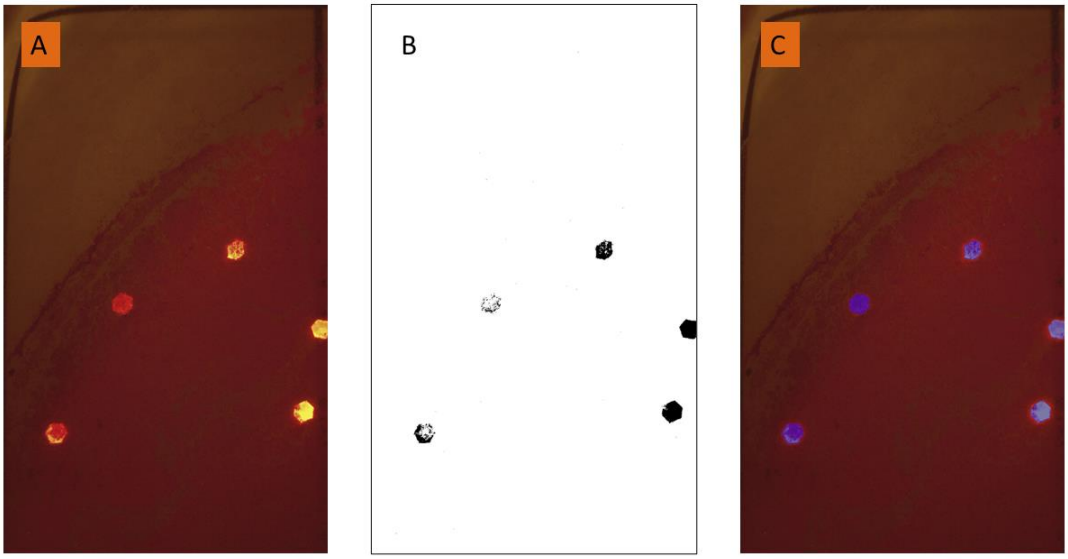

Figure 3.5 Symmetrical polyester fragment standards, original image (A), ImageJ (B), and MIPAR (C).

While fibers in environmental samples may have a cylindrical shape, they can also show up as fine, gossamer threads that are undetected by ImageJ or tallied as multiple, unconnected points (Figure 3.6). Furthermore, fibers that bend and curl over themselves are measured by area and not length by ImageJ. However, fiber length is a critical metric when it comes to understanding the fate and transport of synthetic polymers. It is also necessary in research that involves exposure through inhalation. MIPAR is able to provide a true fiber length even if the thread is not laid out in a straight line.

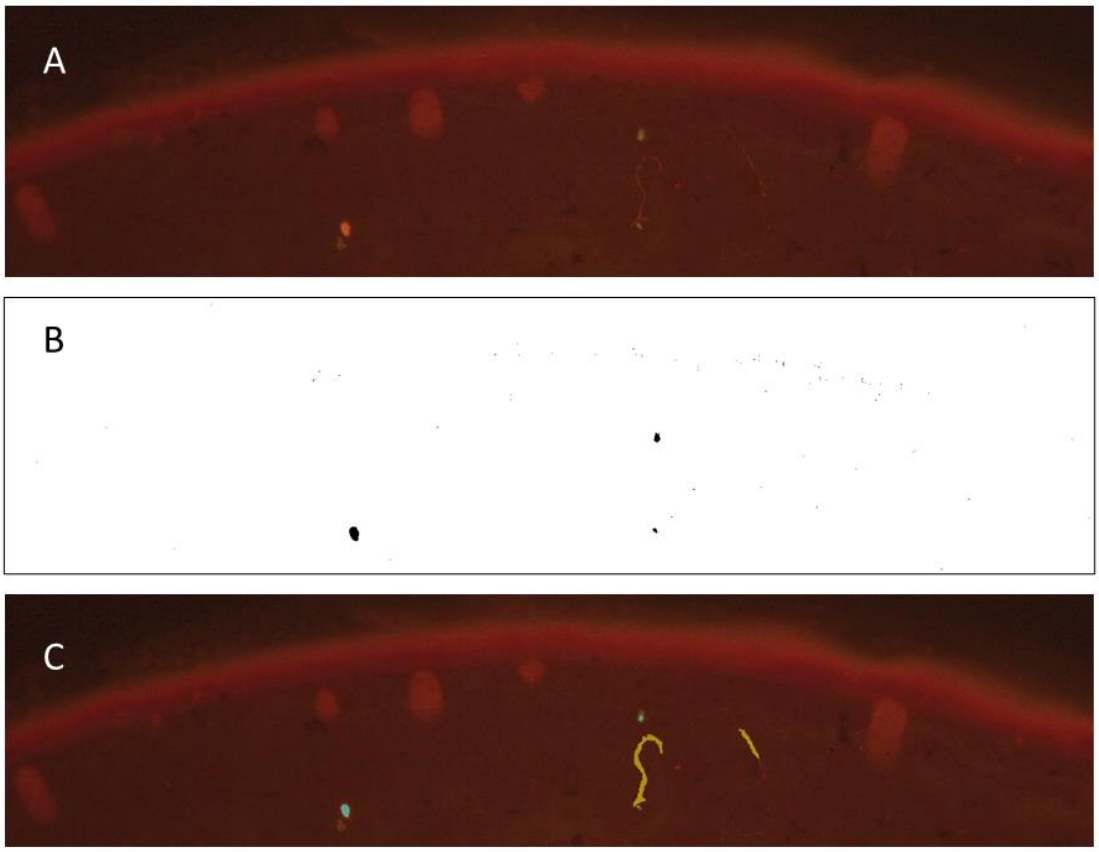

Figure 3.6 Original image of filter containing both particles and fibers (A), ImageJ reporting only particles (B), and MIPAR capturing both particles and two fine fibers (C).

One limitation of MIPAR is its larger size detection threshold, set at 30 µm for particles and 200 µm for fibers. While this parameter can be shifted to include smaller fragments and fibers, an overadjustment may result in the inclusion of background noise. Care must be taken when setting limits to prevent both overcounting and undercounting. Another limitation of MIPAR is that the algorithm was designed to identify only two morphologies, particles and fibers, while ImageJ with the MP-VAT plug-in puts fluorescing contaminants into three categories: fibers, fragments, and particles.

Fibers are those with a larger aspect ratio, fragments have irregular edges, and particles are symmetrical/circular. However, this could be changed by modifying the MIPAR algorithm. In summary, ImageJ was designed for a broad application and then modified (with MP-VAT) to fit the needs of microplastic research. While both programs can be customized, ImageJ, with a macro/plug-in, must be customized by editing the programming language of the plug-in such as JavaScript.

While this can be done and users can edit the quantification constraints within the MP-VAT script to be more conservative/accurate, this may not be intuitive for those who lack coding experience. To generate a high-quality, customized algorithm for this study, over 110 images with examples of fibers and fragments of various size and circularity were used, but this process can be continually fine-tuned to improve accuracy as the program is capable of deep learning. Once an algorithm has been constructed, it can be shared by multiple users; however, a subscription to the program is necessary. While not open source, the program is inexpensive for research institutions.

The authors acknowledge that this work is continually evolving. Another factor influencing the accurate quantification and enumeration of microplastics using image analysis tools is the quality/resolution of images taken, which is controlled by a number of factors such as microscope configuration, magnification, exposure, lens quality, and camera quality. Although the authors did not investigate different microscope configurations, magnifications, exposure times, lenses, or cameras, it is important to mention that image quality also influences the accuracy of computer-driven quantification. The goal in this arm of the study is not to make recommendations or promote specific programs

or equipment. Instead, the aim is to bring to light the current shortcomings of current imaging software and start an important conversation about what new technology has to offer this discipline.

Conclusions

While recent interest in microplastics initiated a surge of international investigations providing evidence of this pollutant's expansive global reach, cross-study comparisons and overall utility of published results are constrained by a dearth of QA/QC measures. This is detrimental to the discipline of microplastic research as experiments designed to examine potential links between microplastic contaminant exposure and injury to whole organisms or cell cultures hinge on reliable, real-world contaminant levels.

Filtered water is sourced from a variety of systems and no study to date has examined the potential for variability between systems. In addition, few studies in this field carry out positive controls and none assessed the performance of filtered water systems based on material or design. Finally, many studies use imaging software for total particle quantification and classification based on morphology, but it has not been properly vetted. While this article does not offer solutions to all the problems uncovered, the intention is to bring awareness and generate conversation around these and other related QA/QC issues. If these problems are not addressed, inaccurate reporting will persist in this discipline, wasting time and resources.

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Chapter 4: Characterizing Microplastic Fallout in an Urban, Humid Continental Climate for 52 Weeks

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Abstract

Microplastics are globally distributed and have been recovered from a wide array of environmental media across the planet, including ambient air. For a decade, research was focused on identifying their presence and tracking their movement. One point of interest is the interplay of physical and chemical characteristics of contaminant particles and weather conditions, such as precipitation and temperature. The present study examines passive fallout of microplastics in an urban, humid continental climate over the course of a year. Using a newly modified method for image processing, it tracks particle abundance and includes precise measurements of exposure time for wet and dry fallout, which allows for a granular observation of particle washout from ambient air. Mean dry flux is 25.6 ± 74.8 particles and fibers/m^2/hour, mean wet flux is 720 ± 2620 particles and fibers/m^2/hour and combined. Seasonal variations were found in both wet and dry deposition, with the heaviest flux in early May through June. Over the span of a year, dry deposition made up 75.7% of total contaminant fallout while wet deposition made a smaller contribution (24.3%). Particles were the dominant morphology (82.8%) over fibers (17.2%). No significant difference was seen in the size of contaminants when comparing wet and dry fallout in above and below freezing temperatures. This study adds to a growing body of

knowledge that links the physical and chemical characteristic and abundance of microplastics to climate.

Introduction

Over the past two decades, a rapidly accumulating body of evidence has given way to an indisputable fact: Microplastic contamination is pervasive and globally distributed. These particles, defined as fossil fuel derived synthetic polymers up to 5mm in size, may also contain endogenous chemical additives and sorbed environmental pollutants. Initially discovered in biota (Crockett & Reed, 1976), they have since been found in various abiotic media including marine waters (Andrady, 2011), fresh waters (Eriksen et al., 2013), and soil (Rillig, 2012). In a 2015 seminal study (Dris et al., 2015), they were captured for the first time in atmospheric fallout. Here, microplastics were collected through passive air sampling in Paris, France, an urban center with 2.2 million residents. These early findings prompted researchers to apply similar methods in municipalities with more residents, such as the heavily populated city of London, England, home to 8.9 million (Wright et al., 2020b) and Dongguan, China, home to 8.3 million (Cai et al., 2017). It also inspired passive sampling in regions with very few permanent human residents, such as a meteorological station in the French Pyrenees (Allen et al., 2019) and icefloes west of Svalbard Norway (Bergmann et al., 2019). Active air sampling, a method where entrained particles are drawn directly from the atmosphere, were also applied in densely populated areas (K. Liu, Wang, et al., 2019; X. Wang et al., 2021) and remote locations (K. Liu, Wu, et al., 2019b; Trainic et al., 2020). Finally, indoor air was studied through both passive (Dris et al., 2017; Q. Zhang, Zhao, et

al., 2020) and active (Vianello et al., 2019) means. Although variation was found in abundance and variety, microplastics were present in every location.

The omnipresence of microplastic pollution is concerning, as there is now emerging evidence of contaminant particles inside human bodies. Research intentionally focused on recovering microplastics from human biofluids and tissues started in earnest in 2019, and over a relatively short span of time, a substantive collection of publications raise pressing questions about the potential health impacts. The primary route of exposures is believed to be ingestion (Prata et al., 2020), but plastic fragments and fibers have also been found in the upper and lower respiratory tract (Huang et al., 2022; Jenner et al., 2022). Furthermore, inhaled particles can still be routed to the digestive system when mucus is swallowed from the sinuses and upper airways, or when mucus is expelled from the trachea or bronchi. While the toxicokinetics are not well known, there are some mechanisms whereby particles may gain access through the respiratory system. In the upper airways, the mucus lining is relatively thick and mucociliary clearance is likely. However, particles with a small aerodynamic diameter (<1μm) may penetrate deep into the lung where the mucus lining is thinner. Here, they can access the epithelium and if sufficiently small (< 1μm), particles can translocate into the bloodstream through diffusion. It is also possible for particles < 5 μm to be engulfed by alveolar macrophages (Wright & Kelly, 2017).

While there is intense interest in human health outcomes, it is difficult to link exposure to specific health endpoints given the vast heterogeneous nature of this material as well as the uncertainty in

dose, bio-accessibility, and bioavailability of particles and their associated chemical compounds. Investigations by physicians, immunologists, cell biologists, and toxicologists are ongoing, but conclusive findings will take time. Despite a lack of concrete threshold values and risk assessment, studies that examine the fate and transport of these anthropogenic contaminants are essential as it provides a better understanding of particle sources, degradation, and movement, which is needed when determining how to effectively constrain its flow and potentially reduce exposure.

Studies involving atmospheric entrainment and fallout of microplastics reveal some trends. First, higher concentrations tend to be associated with more densely populated areas. Second, fibers tend to dominate, but not all studies are concordant around morphology. Some differences can be attributed to variations in collection methods. In a 2022 review of analytical methods and models in atmospheric sampling of microplastics, 36 passive and active air sampling studies were examined (Luo et al., 2022). The authors include 14 active sampling studies, where particles were collected directly from the air, and 22 passive sampling studies, where particles were collected after they were driven out by precipitation (wet deposition) or settled out on their own (dry deposition). Of the 22 passive air sample studies, 21 combined wet and dry deposition through a method known as bulk sampling. Only one study (Brahney et al., 2020) separated wet and dry fallout, using an automated device that opens and closes in direct response to the beginning and end of a precipitation event. The device was employed in 11 remote and protected areas in the Southwestern U.S.

Since the publication of the analytical methods review, four other studies attempted to approximate wet and dry deposition. One gathered wet and bulk samples and used results to estimate the proportion of dry fallout. Two other studies depended on the manual opening and closing of sampling vessels when precipitation was predicted, but this left uncertainty around the start and end of a rain or snow event that lasted several hours to a day. Only one of the four studies used an automated collection instrument that was similar to the one used by Brahney et al. (2020), and here, the time the sampler was open or closed is unknown. In this study, Xiong et al., (2022) collected 20 1-week samples, of which 10 contained measurable levels of rain or snow. Results for dry deposition were reported in terms of flux (particles/m^2/day) while wet deposition was reported in terms of density (particles/mL). The wet samples representing the entire season of winter consisted of two samples collected in January (Xiong et al., 2022).

Another study involved two air sampling locations in Iran, representing yet another arid/semi-arid climate. This investigation lacked an automated device, requiring the manual opening and closing of dry and wet samples. This introduces more opportunities for the introduction of contamination particles during sample collection. A total of 29 wet samples were taken, but according to the authors, it was several hours to a day after a precipitation event before the wet deposition vessel was collected and the dry deposition vessel covered. The last two studies took place in non-arid climates adjacent to large bodies of water. One had four locations in Ireland, three of which were directly on the coast (Roblin et al., 2020). Three sites collected wet

deposition and the fourth collected a bulk sample. No dry deposition samples were collected. The final study was in northern Poland, on the banks of the Baltic Sea (Szewc et al., 2021). Here, a total of 49 samples were collected manually (15 wet, 17 dry, and 17 bulk). First, only dry and bulk samples were taken between January and August of 2017, with a seven-day sample duration in all but one. Then, there was a shift in collection methods from December of 2017 through December of 2018, where only wet and dry samples were taken, with sample duration periods anywhere from 0.8 to 8 days in length. These wet and dry samples were not taken with an automated, wet and dry sampling instrument.

The above findings shed light on the relationship between the climate, weather, and proximity to human populations and the abundance of microplastic fallout from ambient air. However, the difference in sampling methods, the irregularity in sample duration and total sample size, makes it difficult to compare results. Furthermore, a lack of granular data, with wet and dry sample units spanning one day to several weeks, as opposed to a fraction of an hour, makes it difficult to understand the relationship between weather conditions (temperature and precipitation) and particle size, morphology, and chemistry. To date, only two known studies employed an automated device that continuously separates wet and dry deposition. However, Xiong et al. (2022) did not report wet flux and Brahney et al.(2020) assumed the wet sampler was open for 30 minutes for each precipitation event, which is an estimate that results in some imprecision. Also, both studies examined rural areas in relatively arid climates.

Automated sampling has yet to take place in an urban area where precipitation falls as both rain and snow at a moderate rate throughout the year. The work presented here seeks to understand how particle abundance, morphology, size, and chemistry is linked to 1) the presence or absence of precipitation and 2) the type of precipitation. This will pinpoint specific characteristics that may drive particle settling as opposed to continued entrainment. This study not only considers precipitation type (rain or snow), but also associations related to precipitation volume. In addition, by consistently and continuously collecting wet and dry fallout over the course of a year, seasonal variations can be observed. The present study involves weekly interval sampling of both wet (n = 52) and dry (n = 52) deposition, with exposure time recorded to a tenth of an hour over the course of a year in an urban, humid continental climate.

Materials & Methods

Sampling Location

A passive air sampler (Aerochem Metrics wet/dry collector, Model 31) was positioned on the rooftop of the Mayo Memorial Building on the University of Minnesota's Minneapolis campus, East Bank (Figure 4.1). The collection site was on the 8th floor rooftop of a two-tiered building roughly 34 meters above the ground (29.7 – 33 meters). The wet/dry collector was 13.2-meters from a building doorway to the east that connects the rooftop to another segment of the building that extends to floor fourteen (Figure 4.1). This means the wet and dry collector was somewhat protected from direct easterly wind and/or precipitation, but there were no built obstructions to the west, north,

and south. The remaining buildings are of varying heights. There were no obvious heating ventilation and air condition (HVAC) vents coming from the buildings near the sampler.

Figure 4.1 A map showing the sampling site on the University of Minnesota Twin Cities campus (left). A photo showing the area around the sampler on 8th floor rooftop with downtown Minneapolis 2-miles distant (right).

The sampling site is two miles from downtown Minneapolis, a city with 429,954 residents (*United States Census Bureau*, 2020a) and seven miles from downtown St. Paul, a city with 311,527 residents (*United States Census Bureau*, 2020b). Together, these Minnesota 'Twin Cities' are home to 741,481 residents, or 3.7 million residents if the 10-county metropolitan area is included (*US Census Bureau*, 2023). The area within a mile radius of the sampling site contains a mixture of institutional buildings, residential housing, commercial buildings, light-industrial (train yards), and green spaces (landscaped lawns) around campus and along the Mississippi River. There are also several major transportation corridors including highways 94, 35, 55, and 280 (Figure 4.2).

Figure 4.2 A map of the sampling site in Minneapolis, Minnesota with a 17-mile radius around the sampling site (red arrow).

Sampling Weather Conditions

The climate in the Twin Cities is best described as humid continental, where mean annual precipitation from years 1871 – 2024 was 713.7 mm combined rain and snow, with snow as rainfall equivalent (Minnesota Department of Natural Resources) During the sampling year (2022-23), precipitation was (662.1 mm) which is about 7.2% less than the annual average (NOAA). A summary of precipitation during the sampling year categorized by month can be seen in Table 4.1. According to the daily characterization of precipitation for the sampling region by NOAA, there were 23 samples overall where snow (both trance and measurable amounts) was reported to have fallen between October 10, 2022, and April 24, 2023. In addition, there were 47 samples where rain (both trace and measurable amounts) was reported between July 2022 and July 2023. Therefore, out of 52 samples collected in total, 44% had

some quantity of precipitation fall in the form of snow, and 87% of the samples had some amount of precipitation fall in the form of rain. For a total of five samples (9.61%), NOAA reported no snow or rain for the full sampling period (8/31 – 9/7, 10/17 – 10/24, 11/21 – 11/28, 1/29 – 2/5, 5/22 – 5/29). These dates correspond with the respective time (in units of hours) that the sampler was opened for the sample period (0.1, 0.6, 0.1, 0.1, 0.1). Every sample where snow was reported, some rain was reported as well, which is plausible in all sampling weeks except for 12/19 – 12/26 where temperatures for the sampling period were well below freezing (ranging from -24 to -10°C). Since all 23 snow samples also had some quantity of rain, that leaves 15 total samples that exclusively collected rain, representing 29% of total samples.

There were two occasions when the full sample period was below freezing (0°C). These were 12/19 – 12/26 (where temperatures ranged from -24 to -10°C) and 1/29 – 2/5 (where temperatures ranged from -25 to -2°C). Independent of precipitation falling, there were a total of 17 sampling weeks where temperatures over a sampling period at least dipped below freezing, even if they did not remain below 0°C for a full week. There was also one week during which sampling was interrupted. This took place from 9-27-2022 to 10-3-2022, but it was the only instance that the sampler malfunctioned.

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
2022							1.35	4.2	0.26	0.42	2.2	1.85
2023	2.15	2.71	2.62	4.06	1.21	2.17	3.08					

Table 4.1 Actual precipitation in 2022-2023.
Monthly totals in the green had totals between 30th and 70th percentile. Monthly totals in red were in the lowest 30 percentile of the period of record distribution. Monthly totals in blue were in the highest 30th percentile of the period of record distribution.

Sample Collection

The sampler holds two metal buckets of identical dimensions (Figure 4.3). An automated arm is connected to a sensory grid and plate system. The arm covers the wet bucket (left) until the sensor plate and sensor grid are shorted by rain or snow. An electrical short between the plate and the grid causes the arm to "open" or move to cover the dry bucket (right). The arm remains in this open position until the circuit in the sensor plate, which is heated to hasten evaporation and melt snow, breaks. At this point, the arm shifts again to close the wet bucket and leave the dry bucket fully exposed until the next precipitation event. It should be noted these images were collected during a pilot phase of this experiment. No bungee cords were used during the actual sampling period as they were found to degrade from weather and UV light, thus contaminating the samples. In place of synthetic bungee cords, a cellulosic twine was used to keep the buckets tied to the sampler. This binding was necessary as high winds on the rooftop were strong enough to carry buckets from the sampler to lower rooftops below.

Figure 4.3 Close up of wet and dry deposition sampler collecting during winter (right) and summer (left).

An important modification to this collection apparatus that sets it apart from any wet and dry sampling used in previous studies, is a counter fixed to the sampler that records the number of 'open hours' in a sampling period, that is, the number of hours that the wet deposition bucket was exposed. The sampling periods were anywhere between 140 and 200 hours. The range of 'wet' hours was 0.1 to 38.8 hours, with an overall mean of 8.87 hours per sampling period. The range of 'dry' hours was 125.6 to 198.4, with an overall mean of 157.6 hours over a sampling period. A graph depicting wet and dry fallout proportions per sample are given in the appendix Chapter 4 Figure 1. Total precipitation volumes range between 0 and 2.62 liters. However, some weeks when trace precipitation was reported by NOAA, no measurable precipitation was recorded. This is not surprising as small volumes of water can evaporate between collection and slight discrepancies are expected between the sampler and the NOAA sensor due to the distance between the two.

Sample Processing

Sample processing for this work consisted of collecting both wet and dry metal buckets from the rooftop sampling device and covering them with new or used (inwardly folded) sheets of aluminum foil. This was done to protect samples from the introduction of contamination during sample transport from the 8th floor rooftop to an 11th floor wet chemistry lab within the building. Once in the lab, the full contents of the wet deposition bucket were poured into a pre-ashed graduated cylinder and the volume recorded. This graduated cylinder was then immediately covered with aluminum foil to prevent any contaminants settling out of indoor air into the sample. Occasionally during the

winter months when temperatures were below freezing, the buckets were left in the lab until the contents thawed and could be poured into a graduated cylinder. After measuring the volume of snowmelt or rain, the wet deposition bucket was thoroughly rinsed 3X with a total of 700 – 800 mL filtered reverse osmosis (RO) lab water. This rinse water was then poured into a pre-ashed glass Pyrex media bottle. Rinsing the bucket consisted of washing down the sides with filtered water directly from the metal RO spigot and then swirling the contents around several times before emptying it into a glass bottle. This was repeated 3X until the media bottle was full. The dry deposition bucket was always empty therefore it was simply rinsed with 700 – 800 mL of RO water in the same manner as the wet deposition bucket. After rinsing, the sample was stored in a similar 1L pre-ashed glass Pyrex media bottle. The graduated cylinder and two bottles were then covered with aluminum foil and/or capped and then placed in a fume hood for further processing. In all but two weeks, samples were processed within 24 hours of sample collection.

After successfully transferring each sample into a pre-ashed temporary glass container, both metal buckets were washed with Alconox and scoured with a horsehair brush with a wooden handle. After thoroughly rinsing the cleaned buckets with tap water, they were then rinsed 3X with deionized water and finally rinsed 3X with RO water. After washing and rinsing was complete, each bucket was filled with 700-800 mL of RO water. Then the water was immediately transferred to two separate pre-ashed glass Pyrex media bottles right before the empty buckets were covered with aluminum foil and returned to the air sampler. These two bottles, referred to as ‘bucket blanks,’ were then

labeled accordingly as ‘dry blank’ and ‘wet blank.’ They were processed along with the samples collected the following week. All told, the steps involved in sample transport, volume measurement, transfer to temporary glass containers, and bucket washing and rinsing took roughly 20 - 30 minutes to complete. This process was carried out for each sample collected for 52 weeks.

Next, the rain or snowmelt from the wet deposition bucket combined with 700 – 800 mL bucket RO rinse water was filtered on a stainless-steel vacuum filtration apparatus using a 5 µm pore polycarbonate filter (47mm diameter, Isopore from MiliporeSigma). The dry deposition bucket RO rinse water was treated in the same way. Care was taken to rinse out the graduated cylinders and the glass Pyrex media bottles serving as temporary containment vessels for the samples and bucket blanks. Rinsing was done with RO water in a plastic squeeze bottle. After filtration, the polycarbonate filters were transferred to a 250- or 600-mL pre-ashed glass beaker using forceps. Then, the filtering funnel was removed from the vacuum filtering apparatus and placed over the beaker and using 100 mL of RO water, the sides were rinsed down into the beaker. Next, the 100 mL of RO rinse water and the filter were treated with wet peroxide oxidation (WPO), which involved adding 10 mL Fenton’s reagent followed by 10 mL 30% hydrogen peroxide to the sample. A cleaned and rinsed PTFE coated magnetic stir bar was also added to the solution before it was placed on a hotplate at 75°C for 20 minutes. Finally, 0.5 mL of Nile red solution at a concentration within a range recommended by Maes et al. (2017) (1 - 1000 µg/mL or 0.001 - 1 mg/mL) was added to the digested sample and left to sit for 20 minutes before the solution was filtered a second time with a new

filter. The first filter was immersed in the sample during digestion and staining. It was rinsed with RO water over the sample before the second filtration. Then it was removed and inspected for glowing particles under the stereo microscope. During this final filtering stage, the beaker containing the sample was rinsed 3X into the filtering funnel and the sides of the funnel were also rinsed down 3X. Finally, the filters were placed in labeled petri dishes (polycarbonate) for storage.

Sampling Imaging and Physical Analysis

After digestion and staining, suspected plastic fibers and particles captured by the 47mm polycarbonate filter were imaged and digitally processed with MIPAR software designed to count and physically analyze them (Kosuth et al., 2023). Filters were imaged with a Leica EZ4 W stereo microscope at a magnification of 8X. Each filter, without removing it from the petri dish, was laid on a 3 X 5 grid so that the entirety of the filter could be captured in 15 images. The images were collected under a CrimeLite 2 (450 – 510 nm blue/green) positioned next to the microscope on a stand fitted with a clamp as this frequency of light causes synthetic polymers stained with Nile red to fluoresce. The 15 images were then batch-processed with the imaging software MIPAR, using a custom-made recipe. The program bins fluorescing debris into two morphological categories: particle and fiber. Examples of captured ‘particles’ and ‘fibers’ can be found in

Figure 4.4. The lower size limit for particles is 30 µm and for fibers it is 200 µm.

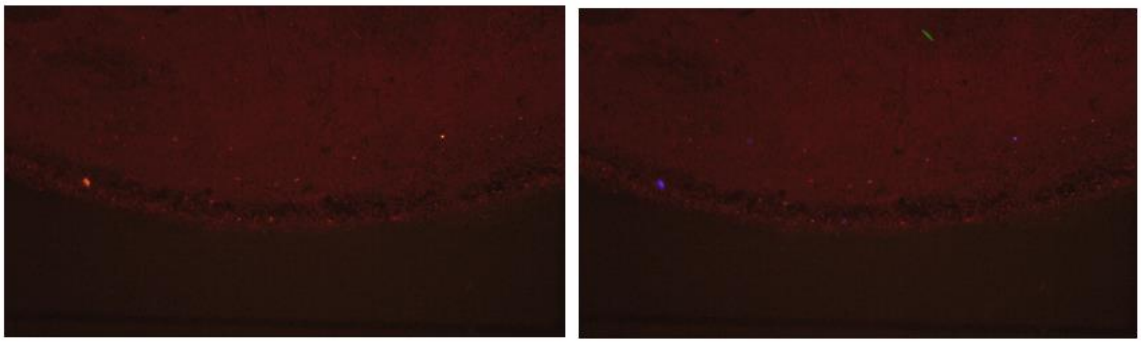


Figure 4.4 Image 14/15 from dry sample November 21 – 28. Raw image (left) shows Nile red fluorescence. Image processed with MIPAR (right) shows fiber (green) and particles (blue) identification.

Sample Chemical Analysis

Once sample images were processed and particles counted, measured, and classified by morphology, Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR – FTIR) was performed using a Thermo Scientific is50 FT-IR with a Nicolet Continuum Infrared Microscope. First, two samples, one wet and one dry, were selected randomly from four sampling periods representing the four seasons. The first was an autumn sample from October 10, 2022 – October 17, 2022. This was followed by a winter sample from January 2, 2023 – January 9, 2023. Next, the spring sample was from April 17, 2023 - April 24, 2023. Finally, the summer sample was from June 26, 2023 - July 4, 2023. Two blanks were also selected for a total of ten samples for chemical analysis. This subsample represents 7.7% of all samples and 1.9% of all the blanks taken. Preparation involved rinsing particles from the polycarbonate filters using RO water from a pressurized squeeze bottle through a miniature vacuum filtration system to a 0.2 µm pore, 25mm

diameter Anodisc filter (Whatman, Buckinghamshire, UK). After this step, filters were placed in a smaller petri dish to protect and contain particles of interest during transport.

The method used for chemical analysis was initially developed by Fox et al. (2022) and then further refined by Thomas et al. (2024). The FTIR spectra were collected on a diamond crystal ATR cell. Collection parameters included a scan range of 4000 - 400 cm-1, 64 scans per sample with a resolution of 4 cm-1. Transmission mode was used with a custom-made Anodisc filter holder constructed as a microscope slide with an 18 mm diameter hole. Two glass slides were used to pin the Anodisc filter to the holder. In a 2 mm by 2 mm grid, 1600 spectra were collected automatically. On each filter, 10 randomly spaced but not overlapping 2 X 2 mm grids were collected. This results in an area that made up 19.5% of the total filter. Later, the data was extrapolated to estimate the particles on the full filter. Since the instrument requires 6.6 hours to scan a single grid, this method allowed for a reasonable subsampling of the particles recovered from air samples for chemical analysis. However, since it is a fraction of the total area, it should be recognized that more uncommon polymer types may be under or over reported (Löder et al., 2015; Mintenig et al., 2020). A few corrections were applied to the spectra to make reading easier. This includes converting the percent transmission to absorbance, using atmospheric suppression followed with baseline correction. A background was collected using a blank Anodisc filter from the same manufacturing lot as the Anodisc holding the sample. This background spectrum was then subtracted from the µ-FTIR spectra collected in each sample.

The instrument generates a C-H sensitive heatmap that is based on the 2850 cm^{-1} peak. This allows for synthetic polymers to be easily discovered and accounted for. Each pixel in the spectral heat map represents a position on the filter. However, the whole region (1700 – 1300 cm-1) was used by the Hummel Polymer Library to identify synthetic polymers by matching them with reference spectra. Particles with a match >50% were recorded. This process was repeated for each 2 X 2 mm field of view, of which there were 10 per sample. The total counts per field of view were tallied and results were extrapolated for the full count per air sample.

Quality Assurance/Quality Control

Aluminum foil was fitted over beakers, glass containers, the filtration apparatus, and sampling buckets during transport to reduce the contamination from the air during sample handing and processing. A cotton lab coat and latex free nitrile gloves were worn during sample processing and all work took place under a fume hood. All laboratory glassware was cleaned with Alconox and scrubbed with a natural fiber wooden brush to minimize contaminants. After washing, laboratory glassware was covered in aluminum foil and baked in an oven for 6 h at 450°C to reduce cross-contamination (Prata et al., 2021). Laboratory RO water was found to be one of the least contaminated of the lab waters available (Kosuth et al., 2023), so this source of filtered water was used for all glassware rinsing and procedural blanks.

Each time a wet and dry sample was collected, a blank was run for both wet and dry buckets. Bucket blanks were processed alongside the samples and subtracted from each sample based on morphology (i.e. fibers from the wet deposition blank were subtracted from fibers in the

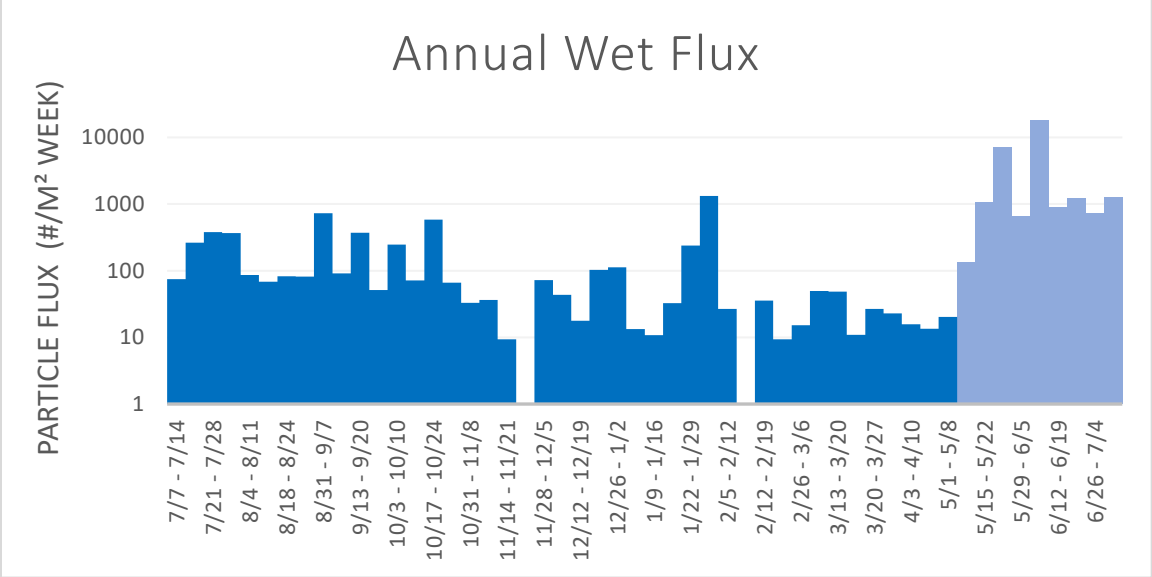
corresponding wet deposition sample, while particles from the wet deposition blank were subtracted from particles in the corresponding wet deposition sample). After making blank corrections, five out of 52 samples were considered 'non-detect' defined as a value less than 3X the corresponding blank value. These were all wet samples that occurred on the following dates: 8/31-9/7, 10/31-11/8, 11/21-28, 2/5-12, 4/3-10.

Results

A total of 104 samples were collected over the course of a full year representing both wet (n = 52) and dry (n = 52) deposition. After applying blank corrections to each sample, the mean for dry particle flux is 21.7 ± 17.5 particles/m^2/hour (mean ± SD), while the mean for dry fiber flux is 3.86 ± 4.79 fibers/m^2/hour. Combining morphologies, the total mean dry flux is 25.6 ± 74.8 particles and fibers/m^2/hour. As far as precipitation is concerned, the mean for wet particle flux is 555 ± 2360 particles/m^2/hour, while the mean for wet fiber flux is 173 ± 364 fibers/m^2/hour. Together these morphologies yield a combined total mean wet flux of 720 ± 2620 particles and fibers/m^2/hour. Finally, the average of all wet and dry fibers and particles is 746 ± 2680 particles and fibers/m^2/hour. Because the standard deviations are larger than the averages, the distribution of fluxes is not normally distributed. Therefore, we also report the geometric mean and standard deviation as 110 ± 5.73 particles and fibers/m^2/hour and the median as 78.0. It is conventional in studies involving the atmospheric fallout of microplastics to express flux in terms of days instead of hours. These averages are for a full year of sampling and the variability among the samples, which are approximately one week each, is captured by the high

standard deviations. The raw data can be found in the appendix (Chapter 4 Table 1).

Looking at the full year, wet flux was highest during the early weeks of summer with 83.8% washing out between May 8 - July 10. This time of high wet flux is not associated with an increase in precipitation. See Chapter 4 Figure 2 in the appendix for a comparison of wet flux and precipitation volume. This leaves the remaining 16.2% to wash out with some consistency for the remaining nine months. Dry flux was also highest in early summer with 84.3% falling out between May 8 - July 10, which leaves 15.7% to deposit, again with uniformity for the rest of the year. These results are presented in Figure 4.5 on a logarithmic scale to capture the full range of fallout, capture the full range of fallout, while maintaining of magnitude in early spring through early summer.



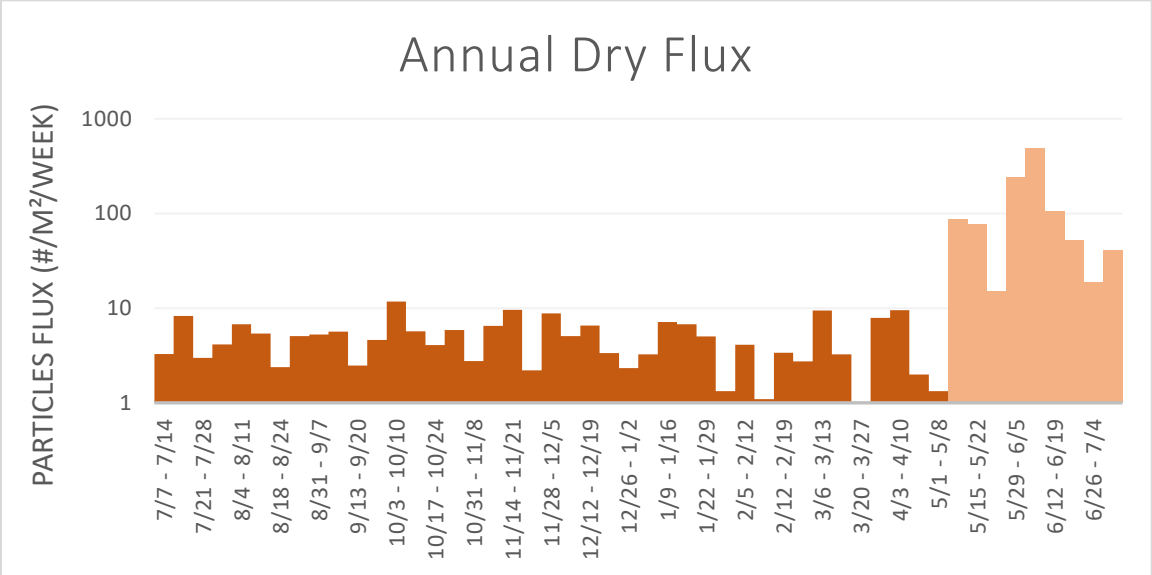


Figure 4.5 Annual wet (above) and dry (below) flux in an urban environment on a logarithmic scale with May 8 – July 10 highlighted for emphasis.

No difference was seen between wet and dry fallout when it comes to the size of the contaminants. Mean fiber length was 359 µm ± 62.4 (Range: 211 – 562 µm) while mean particle length, across the longest dimension, was 151 µm ± 40.7 (Range: 70.0 – 295 µm). Parametric comparisons (t-test) were used to examine the size of contaminants in dry versus wet samples. No statistically significant difference was seen between mean wet fiber length (359.7 µm) and mean dry fiber length (358.8 µm) ($p = 0.0733$). Also, no significant difference was seen between mean wet particle length (149.4 µm) and mean dry particle length (152.9 µm) ($p = 0.438$) (Figure 4.6).

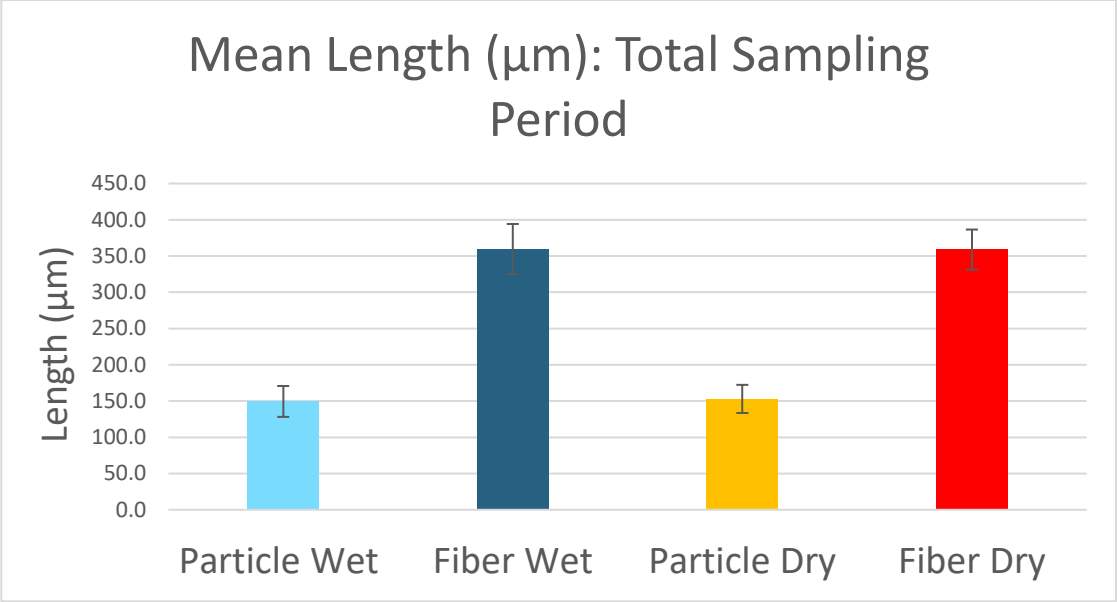


Figure 4.6 Graph of mean fiber length and mean particle length in both wet and dry deposition from 52 weeks of sampling.

When comparing samples that were collected above and below freezing temperatures, no significant differences were seen between the mean length of dry fibers ($p = 0.99$), dry particles ($p = 0.58$), and wet fibers ($p = 0.199$). However, a significant difference was seen in the lengths of wet particles ($p = 0.030$) with an 18.4% increase in the size of particles falling during above freezing temperatures (Figure 4.7).

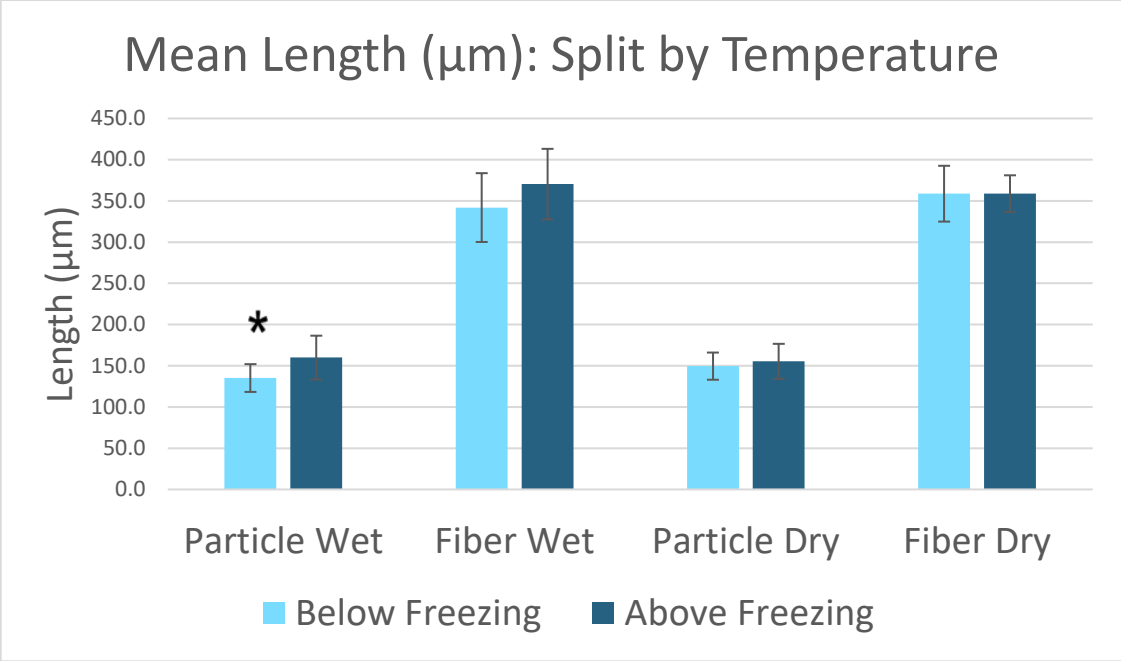


Figure 4.7 Graph of mean fiber length and mean particle lengths in both wet and dry deposition from 52 weeks of sampling, split into temperatures above and below freezing.

To compare fallout (wet vs. dry) and morphology (particles vs. fibers), proportions from total annual flux were used. Wet deposition contributed to 24.3% of total fallout while dry deposition made up the remaining 75.7%. Also, particles made up 82.8% of total fallout while fibers contributed to the remaining 17.2% (Figure 4.8).

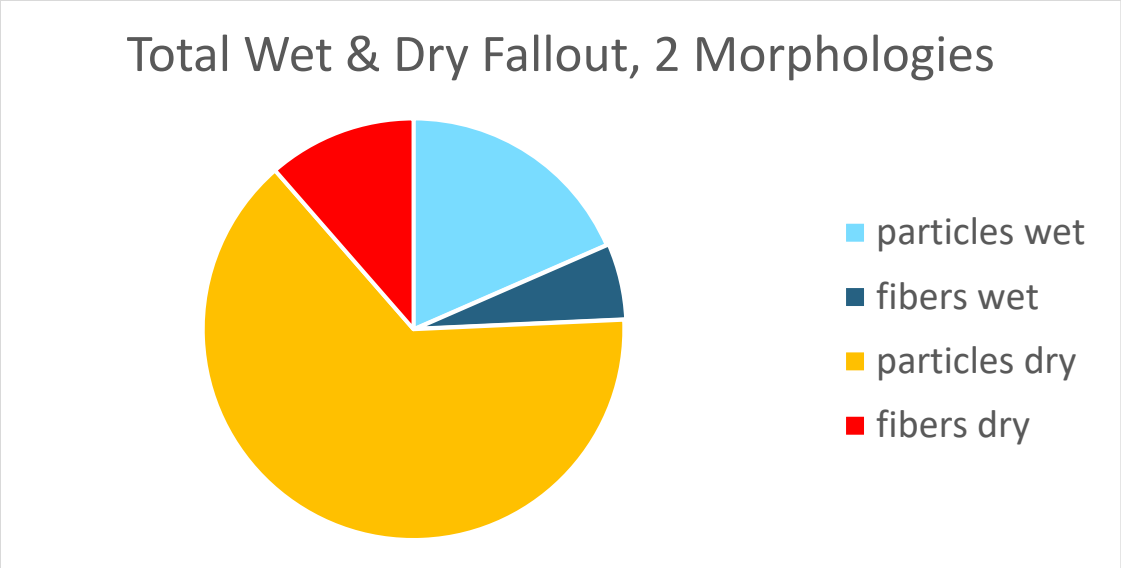
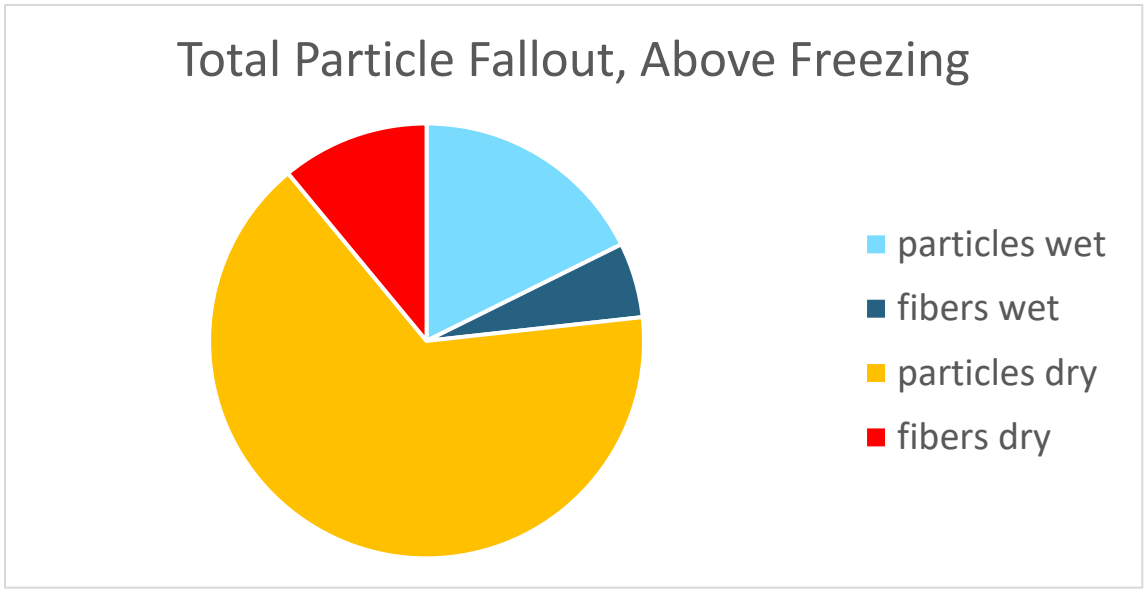


Figure 4.8 Proportion of fallout based on morphology (particle vs. fiber) and deposition type (wet vs. dry), derived from data spanning 52 weeks.

There were some differences in morphology when comparing fallout in above and below freezing temperatures. In both above and below freezing, dry particles dominated, but their presence was lower in below freezing samples (48.9%) compared to above freezing samples (65.8%). Wet particles were the second most abundant contaminant morphology in above and below freezing, but their presence was opposite dry particles with a higher proportion below freezing (26.7%) compared to above freezing (17.7%). Shifts in proportions were less pronounced in wet and dry fibers. Due to season length in the climate tested, there were more samples representing above freezing temperatures (n = 30) than below freezing temperatures (n = 22) (Figure 4.9).



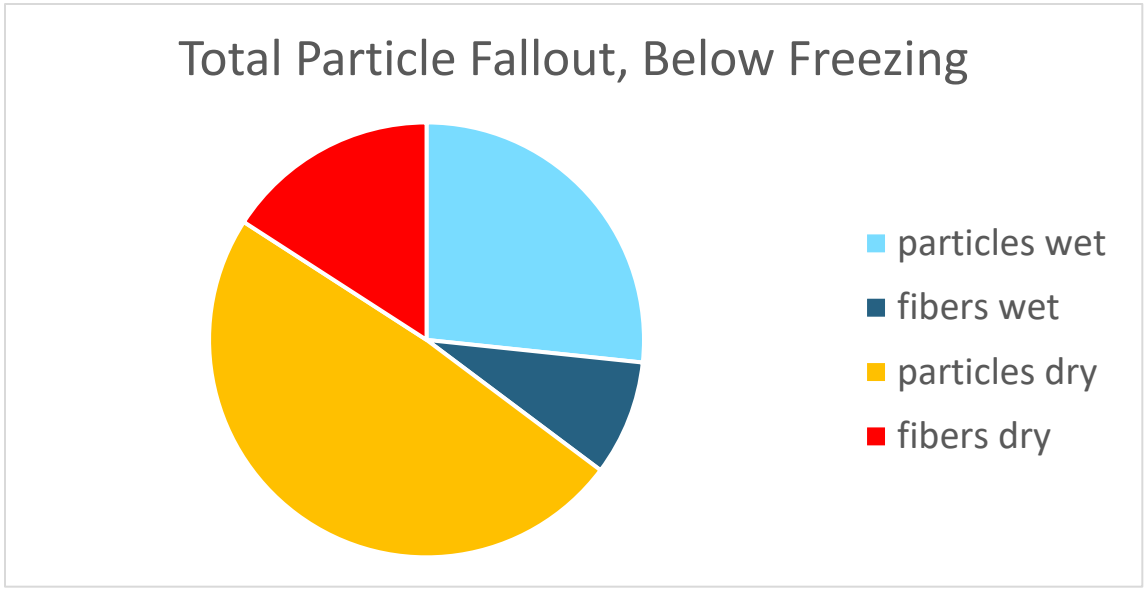


Figure 4.9 Morphology proportions in above freezing temperatures (above) and below freezing temperatures (below) for two morphologies in both wet and dry deposition for 52 weeks of sampling.

Ten filters were analyzed with ATR – FTIR. Eight represented four seasons, four wet and four dry. Two bucket blanks were also subjected to chemical characterization, one wet and one dry. The season with the highest count in a sample was summer dry (3,850 microplastics),

followed by summer wet (1,430 Microplastics), autumn dry (523 microplastics), and spring dry (291 microplastics). In terms of atmospheric flux, summer wet yields an abundance two orders of magnitude higher (13,100 microplastics/m^2/hour) than autumn wet (299 microplastics/m^2/hour), summer dry (284 microplastics/m^2/hour), and spring wet (176 particles/m^2/hour).

Across the eight samples and two blanks analyzed, a total of 37 synthetic polymers were identified. The season with the highest variety of plastics was summer dry (24 polymer types), followed by spring dry (15 polymer types). Summer wet and autumn dry had the same number (12 polymer types, each). Aside from the blanks, winter wet (9 polymer types) and winter dry (8 polymer types) had the least variety. A complete list of the synthetic polymers that were identified, and their representative proportions based on deposition type and season, can be found in Figure 4.10.

Across all categories of season and deposition type, the most frequently occurring polymer was poly(ethylene:propylene:diene) (29.5%) followed by poly(ethylene:vinyl chloride) (15.8%), urethane alkyd, lindseed oil-rich (14.5%), poly(butadiene) + naphthenic oil (9.6%), alkyd resin (9.4%), cellophane (7.1%), and poly(vinyltoluene:butadiene) (2.7%). Looking at individual seasons, both autumn wet and dry were dominated by cellophane, alkyd resin, and poly(ethylene:propylene:diene). Both winter wet and dry were dominated by polyamide 6 + polyamide 6,6, poly(ethylene:propylene:diene), alkyd resin and poly(aryl ether). Spring wet and dry were dominated by the same three polymers as autumn wet and dry. Finally, summer wet and dry differed from one another. Both had poly(ethylene:propylene:diene), but

in summer wet it was 4X greater. Both had poly(ethylene:vinyl chloride) and poly(butadiene) + naphthenic oil, but these were 6X and 3X more prominent, respectively, in summer dry as opposed to summer wet.

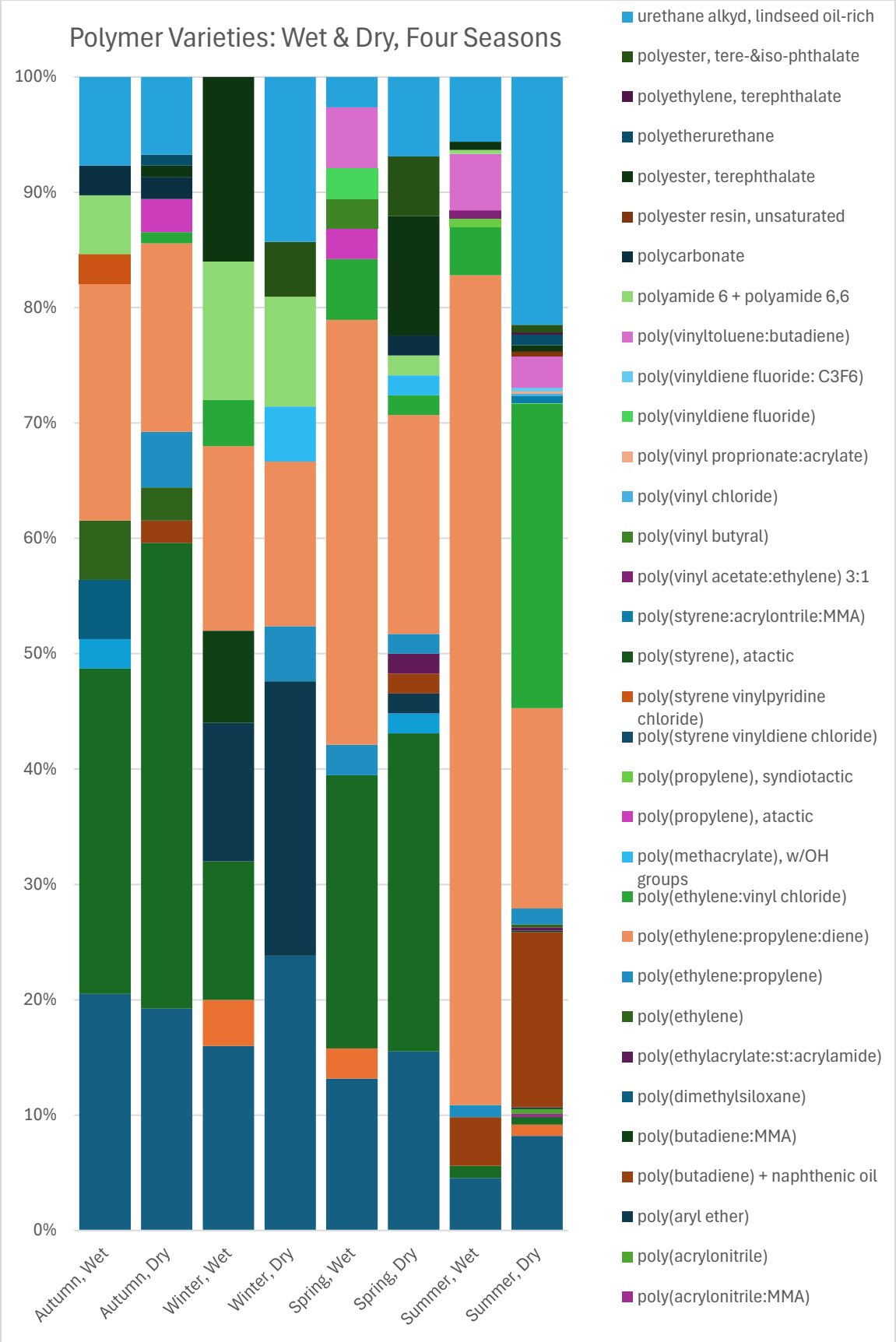

Polymer Varieties: Wet & Dry, Four Seasons
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Autumn, Wet
Autumn, Dry
Winter, Wet
Winter, Dry
Spring, Wet
Spring, Dry
Summer, Wet
Summer, Dry
urethane alkyd, lindseed oil-rich
polyester, tere-&iso-phthalate
polyethylene, terephthalate
polyetherurethane
polyester, terephthalate
polyester resin, unsaturated
polycarbonate
polyamide 6 + polyamide 6,6
poly(vinyltoluene:butadiene)
poly(vinyldiene fluoride: C3F6)
poly(vinyldiene fluoride)
poly(vinyl proprionate:acrylate)
poly(vinyl chloride)
poly(vinyl butyral)
poly(vinyl acetate:ethylene) 3:1
poly(styrene:acrylontrile:MMA)
poly(styrene), atactic
poly(styrene vinylpyridine chloride)
poly(styrene vinyldiene chloride)
poly(propylene), syndiotactic
poly(propylene), atactic
poly(methacrylate), w/OH groups
poly(ethylene:vinyl chloride)
poly(ethylene:propylene:diene)
poly(ethylene:propylene)
poly(ethylene)
poly(ethylacrylate:st:acrylamide)
poly(dimethylsiloxane)
poly(butadiene:MMA)
poly(butadiene) + naphthenic oil
poly(aryl ether)
poly(acrylonitrile)
poly(acrylonitrile:MMA)

Figure 4.10 (from previous page). Variety and proportion of synthetic polymers recovered from four seasons for both wet and dry fallout over 52 weeks of urban sampling.

Using FTIR to examine abundance, the season with the most fallout was summer dry, representing 56.2% of all the fallout across four seasons. This was followed by summer wet (21.9%) and autumn dry (8%). The lowest proportion of fallout occurred in winter, with winter wet and winter dry representing 1.9% and 1.6% of all fallout across the four seasons, respectively. Summary of fallout as determined by ATR-FTIR is in Figure 4.11. For polymer variety across four seasons, see Chapter 4 Figure 3 in the appendix.

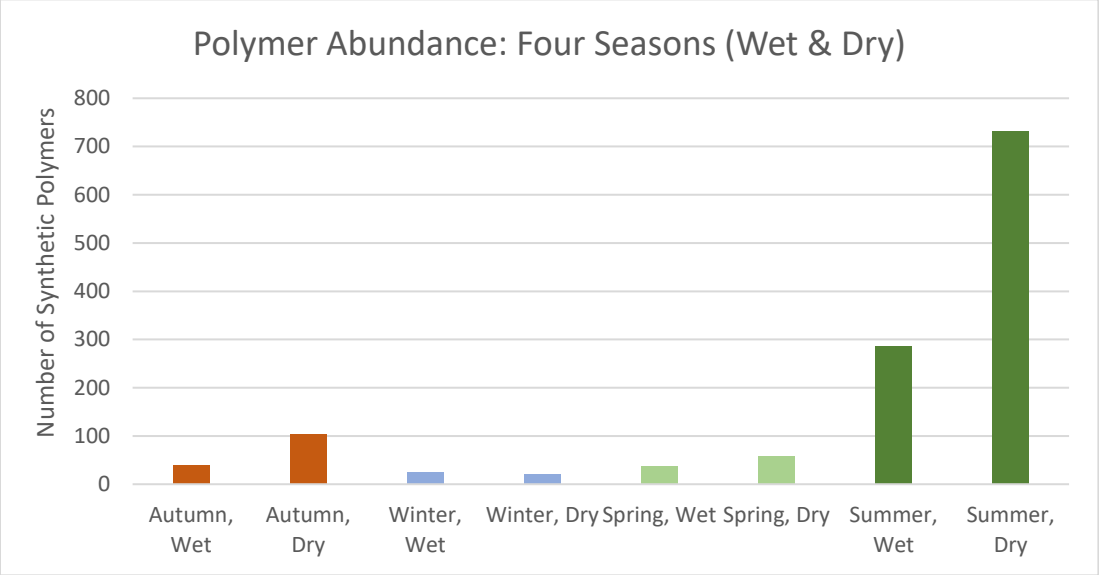


Figure 4.11 Polymers abundance across four seasons, split into wet and dry fallout.

Discussion

Conventionally, flux is reported as contaminant particles/m^2/day. This presents no problem when bulk samples are collected because fallout has the potential to occur during both wet and dry deposition. Therefore, constant fallout does not compromise the accuracy of the

sample. However, collection of wet and dry fallout creates variations in exposure times for each type of deposition that depends on the frequency and duration of precipitation events over a sampling period. To circumvent this problem some authors simply did not provide a wet flux. For example, Xiong et al. used an automated sampler and reported dry flux as particles/m^2/day and wet fallout as density (number of particles per mL of precipitation collected). Others estimated the time of total precipitation. Brahney et al. (2020), also using an automated sampler, assumed each rain or snow event was 30 minutes in length. Still others who gathered samples manually could provide a length of time that is directly connected to their wet and dry samples. However, some authors (Abbasi & Turner, 2021a) admit that the manual transition before and after a rain event took several hours to part of a day. In the work by Szewc et al. (2020), some wet samples ran 7 or 8 days, and it is not clear if rain fell steadily over that period of time. In the present study, an automatic sampler was used, and a counter was affixed to the instrument allowing for a precise count (within a tenth of an hour) of time that the sampler for each sampling period. Having recorded the total hours of each sample, the number of open hours can be used to determine the number of closed hours. To the authors knowledge, this method allowed for the most accurate means of collecting wet and dry air samples to date.

While the hours of exposure were known in a sampling period, the discrete number and duration of precipitation events was not known. This was estimated using precipitation data collected by NOAA from a meteorological station 13 miles south of the sampling site. For this reason, in the current study, flux over a sampling period is reported

in terms of hours and not days (Figure 4.5). This is also the reason annual flux, and not daily flux, were used to report the proportion of wet and dry deposition, and the proportion of fibers and particles. Figures with a conventional flux (particles/m^2/day) may include distortions that tend to inflate wet fallout. For example, if a small quantity of contaminant particles deposited in the sampler over a brief period of time, a conventional flux calculation would yield a higher figure than the same number of particles deposited during a long exposure time. The shorter exposure time may not accurately represent the wet fallout expressed per day, for the sampling period.

Despite the difference in units, sampling methods, and devices, results from the present study can be compared to those from other urban, passive ambient air studies. The mean bulk flux in the city of Minneapolis for a full year when converted to daily deposition is 782 ± 2290 particles and fibers/m^2/day (range 25.1 to 15,600 particles and fibers/m^2/day). Again, since the standard deviation is larger than the average, the distribution of fluxes is not normally distributed so the geometric mean and standard deviation is 232 ± 3.51 particles and fibers/m^2/hour, and the median is 212. These results are the same order of magnitude as mean bulk sampling in other cities. For example, the mean total flux (both wet and dry) in Paris, France was 118 particles/m^2/day (Dris et al., 2015), in Dongguan, China it was 228 particles/m^2/day (Cai et al., 2017), in London, England it was highest at 771 particles/m^2/day (Wright et al., 2019), in Guangzhou, China it was 114 particles/m^2/day (Huang et al., 2021), in Sao Paulo, Brazil it was 123 particle/m^2/day (Amato-Lourenço et al., 2022), and finally in Newark, United States it was 327 particles/m^2/day (Yao et al., 2022).

Another important difference in comparing findings from previous urban studies is particle morphology. Particles recovered in urban areas from other studies tend to be fibers. For example, the morphology of microplastic contaminants collected from Paris, London, and Dongguan, were all > 90% fibers. Fibers were also the dominant morphology in Guangzhou, (78% fiber) and Sao Paulo (87% fiber). However, 82.8% of the total fallout recovered in Minneapolis were particles, while the remaining 17.2% were fibers. This agrees more with the findings of Klein and Fischer (2019) who sampled Hamburg, Germany. Here, passive bulk air samples were collected from a university campus where 95% of contaminant particles were identified as fragments and the remaining 5% were characterized as fibers. Another study involving bulk sampling identified only 12.1% of their recovered particles as fibers. However, this study took place near a meteorological station in a remote area in the French Pyrenees (Allen et al., 2019). It is already established that the drag forces on fibers, due to their higher surface to volume ratios, reduces settling velocity and allows them to remain aloft to travel long distances (Bullard et al., 2021), but this does not explain why fibers settled so readily in most other urban passive sampling collectors. This may be due to the immediate environment around the Minneapolis sampling device. First, the top six stories of a fourteen-story building likely blocks air traveling from the east. Second, the area directly north, west, and south of the sampling site is comprised of lower rooftops. If contaminant sources are splintered, weathered, UV degraded fragments of aging rooftop material, the morphology would make sense, and the bulk of the contamination source could very well be local. A thorough inventory of the synthetic

polymers on external faces of buildings and SEM analysis of contaminant particle surfaces to see evidence of weathering could bring clarity to the question of source. Some of the synthetic polymers that dominate the samples collected are used in construction, but further testing of construction materials is needed before such a link can be established. According to Plastic Europe, building and construction made up 23% of global synthetic polymers produced globally in 2023 (Plastic Europe, 2023).

When comparing results from this study to other studies that separated wet and dry deposition, there were also noteworthy similarities. First, the study by Brahney et al. (2020) looked at 11 remote conservation areas in the Southwestern United States. They found dry deposition was responsible for more than 75% of particles deposited by mass. While the Minneapolis study did not estimate mass, based on the annual count dry deposition contributed 75.7% and wet deposition made up the remaining 24.3%. Abassi and Turner (2021b) and Xiong et al. (2022) also sampled wet and dry deposition in Southwestern Iran and Western Montana, respectively. From two locations in Iran, the authors found dry deposition contributes more to microplastics fallout than wet deposition, which provided a mere 5% of fallout in an urban site and 7% in the rural site. Xiong et al. (2022) used the annual precipitation data from the nearby town of Polson (409 mm) which lay 23 km from the air sampling site, to estimate the annual wet deposition. They report a range of 0.006 - 0.050 particles/L, and with this approximation, they found dry deposition contributed two and a half times that of wet deposition in the sampling area of Flathead Lake, Montana.

Xiong et al. (2022) also point out that snow was collected in the wet sampler and suspect snow is more effective at capturing microplastics from the atmosphere as they found a higher amount in wet deposition during the winter and the lowest amount during summer. This is similar to findings in Iran, where Abbasi & Turner, (2021b) found higher levels of microplastics at two sampling sites in late winter and lower levels in late summer and early autumn. This makes sense as snow has been shown in previous work to scavenge aerosols 50X more efficiently than rain (S. Zhao et al., 2015). However, the sample size for Xiong et al. in winter consists of two samples collected in the month of January, while the study in Minneapolis has a more robust sample size (n = 23) with snow gathered from early October through late April. If the Minneapolis samples are analyzed in the same manner as Xiong et al., some differences are seen. In Minneapolis, fall saw the highest wet deposition volumes followed by summer and winter. Spring was the lowest (Figure 4.12).

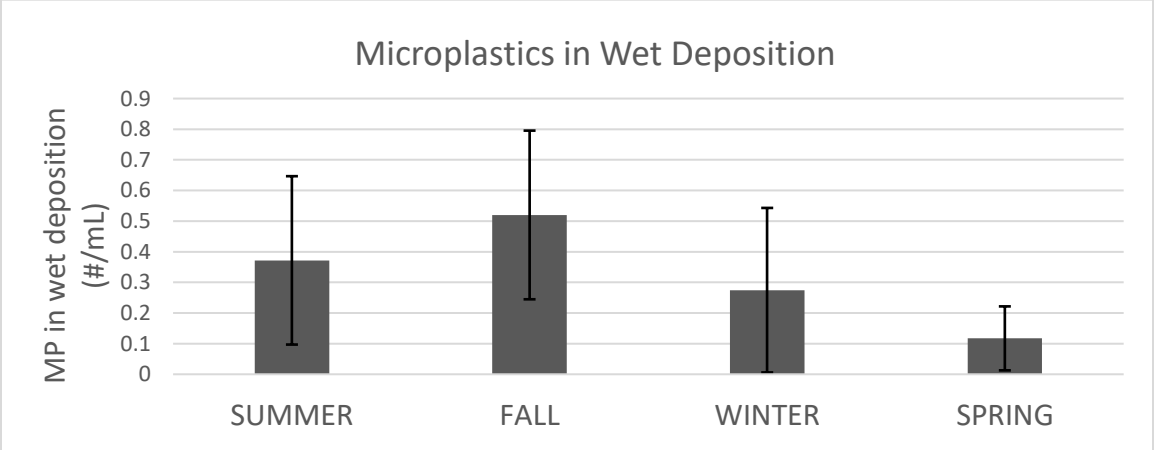


Figure 4.12 Wet deposition expressed as density (particles/mL) across four seasons in Minneapolis, MN.

The size of particles captured in this study were relatively large, with a mean fiber length of 359 µm ± 62.4, while mean particle length was 151 µm ± 40.7. According to Brahney et al. (2020), these sizes are too large for long-range global transport, which must remain below 25 µm. However, Van Der Does et al. (2018) found "giant" quartz dust particles up to 450 µm that had traveled 2,400 – 3,500 km across the Atlantic Ocean from the coast of West Africa. Therefore, if synthetic particles are found to be local, there is the possibility that they could travel far beyond the urban source under the right weather conditions, especially since their density, ranging from 900 – 2100 kg/m^3 (Scientific Polymers, 2025) is less than quartz (2,648 kg/m^3).

A large variety of synthetic polymers were identified across the eight air samples that were chemically analyzed in this study. In comparing results from Minneapolis to other urban studies, the synthetic polymers that are manufactured in the highest quantities, globally, are conspicuously few or absent in these findings. For example, according to Plastic Europe, the plastic varieties that are mass produced are in descending order: PP (19%), LDPE (14%), PVC (12.8%), HDPE (12.2%), PET (6.2%), PUR (5.3%), and finally PS (5.2%)(Plastic Europe, 2024). However, in the samples collected from ambient air on a Minneapolis rooftop, PP makes up only 0.7% of the total particles discovered. Poly(ethylene), which can consist of low or high density, makes up 0.6%, poly(vinyl chloride) makes up 0.1%, polyester, terephthalate makes up 1.3%, polyetherurethane makes up 0.6%, and poly(styrene), atactic makes up 0.1%. Only poly(ethylene: vinyl chloride) co-polymer PE/PVC was found in large quantities (15.9%). In many urban air sampling studies, fully natural or semi-

synthetics are typically found in abundance. For example, cellulose made up 73% of the particles captured in Dongguan, China, Rayon made up 50% of particles found in Guangzhou, and wool made up 50% of particles from Paris. The most frequent synthetic polymers found in the urban air studies are PE, PET, PAN, PA, and PVC (Munyaneza et al., 2022a).

As far as the particles that were found in abundance across seasons, Urethane alkyd, linseed, is found in all but winter wet. This is a semi-synthetic polymer used in paints and coatings. Another prominent polymer is alkyd resin. This is fully synthetic thermoplastic polymer used for the same application. Another one prominent polymer is poly(ethylene: propylene: diene), which is a type of synthetic rubber. It is possible that some of these polymers are shedding from the exterior of the building. It is also possible that nearby inconspicuous exhaust vents are conveying particles from the inside of the building, as it has already been suggested that indoor air is a possible source of ambient air pollution (Dris et al., 2017; Yao et al., 2022). It is possible that some are arriving from a distance, but modeling like HYSPLIT may not be effective in an urban environment with potential contaminant sources emanating from all possible directions.

There are several possible reasons for a higher seasonal flux in late spring and early summer over the 52-week sampling period. One is the spring freeze-thaw cycle in the Twin Cities causes infrastructure such as roads and buildings, to expand and contract. This may increase opportunities for the shedding of paint and fragmenting of construction materials. In addition, for the duration of the winter, snow scavenged particles on the rooftops surrounding the sampler and likely pinned once airborne particulates to the ground until this snow melted in

spring. Once melted, these accumulated particles could be released into the air in higher quantities until they are dispersed. Another possible reason is linked to the dispersal of biological material such as cottonwood seeds, which are prominent in the late spring and early summer. These seeds float some distance before settling and they could scavenge atmospherically entrained particles as they move. This kind of material was found in both wet and dry samples during months of high flux. Furthermore, spiderwebs can be effective passive air samplers, trapping microplastics from the atmosphere in the German city of Oldenburg (Goßmann et al., 2022).

The strengths of this study are in consistency of sampling during the course of a year and the granular data captured through a meter that was able to record the time the wet and dry buckets were exposed for each sample down to a tenth of an hour. There were also strong QA/QC methods with blanks to match each of the 104 samples. Some limits of this study include the small sub-sample pulled for chemical characterization to represent wet and dry fallout from four seasons. The time constraints on the FTIR were the reason as each sample needed 66 hours to run. However, this small sample could not represent the full spectrum of particles that fell throughout the year. Furthermore, the method for chemical characterization involves extrapolation with <20% of the anodisc filter analyzed which leaves room for uncertainty, although it has been validated and used in several other works involving microplastic pollution (Fox et al., 2022; A. Thomas et al., 2024). The imaging analysis program MIPAR was also limited in what particles and fibers it could see, 30 µm and 200 µm respectively. The lower limit of the particles captured by the 5 µm pore filter, were not

fully represented in the results. However, even modifying the threshold for imaging would not change the fact that µ-FTIR was only able to view particles as small as 50 µm. In addition, the laser had to land right on a particle for it to be analyzed, and with the pre-determined grid, this was not always the case. Finally, the Hummel Polymer Library was used for spectral matching, but this library database was built off of virgin plastics and many environmental plastics have experienced some degree of weathering, which makes their identification difficult to match. This could result in identification errors.

It should also be noted that Nile red is a lipophilic stain which readily binds with most synthetic and non-synthetic polymers, such as chitin. This is why WPO is an important step in processing since it digests the bulk of the non-synthetic polymers. This is also why chemical characterization is needed to verify this 'first pass' with Nile red, as it is possible that arthropods, which have exoskeletons made from Chitin, may be present in the sample (Erni-Cassola et al., 2017; Prata et al., 2019). It is possible that some components of insects may not be fully digested during the WPO process.

Conclusions

This work is the first of its kind to collect both dry and wet fallout for a full year using an automated sampler that was capable of recording exposure time with great precision. This work closely examined the influence that specific environmental factors had on particle size, morphology, and abundance. This work corroborates many other findings that collectively indicate dry fallout is an important pathway for the settling of particles. It also revealed some seasonal variations in abundance that are less influential when it comes to

particle size and morphology. Continued work in this area will help to pinpoint sources possibly predict the flow and settling of particles as it relates to climate. This will be especially important moving forward as climate patterns are expected to shift, globally.

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Chapter 5: Microplastic Quantification, Sports Hydration: An Examination of Reusable Sports Water Bottles

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Abstract

Microplastics originate from a plethora of sources, which include durable products that humans interact with daily. This study examines the abundance of microplastics in reusable sports water bottles while they are being used and cared for by a local cycling team from June - September 2024. Variation among brands ranged from 1 – 33 particles/L to 291 - 3,770 particles/L. This difference may be linked to bottle design and material composition. Over the course of the sampling period, particles became more abundant as the fibers decreased in abundance. Size also changed over the sampling period, with both fibers and particles decreasing in size. Finally, a significant relationship was seen between particle abundance and bottle washing/rinsing. Since this is the first study of its kind, findings may raise many more questions about the habits of handling and use that may contribute to particle shedding, as well as questions around bottle construction and design. Also, reusable bottles are gaining popularity in realms outside of cycling, and even outside of sports, bringing saliency to questions of microplastic exposure through direct consumption.

Introduction

Microplastics are anthropogenic contaminants that shed during the entirety of a product's lifecycle, from pre-processing (Hossain et al., 2025) to transportation (Karlsson et al., 2018) to fabrication (Shahsavaripour et al., 2023) to use, and finally, to disposal and recycling (He et al., 2019; Stapleton et al., 2023; Su et al., 2025). These particulates are defined as solid, fossil fuels derived synthetic polymers less than 5 mm, as well as all associated endogenous chemical additives and sorbed environmental pollutants. Their global presence is well documented, having been recovered from some of the most remote corners of the planet (Mishra et al., 2021; Padha et al., 2022). Since the products shedding microplastics were designed with direct human contact in mind, it is not surprising that these particles are common in physical spaces frequented by humans. This includes indoor air (Perera et al., 2023) and municipal tap water (Kosuth et al., 2018; V. Shruti et al., 2020). They are also found in commercial products for human use and consumption, such as raw (Azeem et al., 2021) and processed human foods and beverages (Lin et al., 2022).

The ubiquity of microplastics in human environments is concerning as it presents persistent and unremitting opportunities for exposure. At this time, microplastics have been found in nine out of eleven human systems, excluding only the skeletal and muscular systems. While synthetic polymers have been recovered from human lung tissue (Amato-Lourenço et al., 2021) and the upper respiratory tract (Huang et al., 2022), ingestion is known as a primary route of exposure (Prata, 2023). A few studies show that microplastics pass through the digestive tract as they have been detected in human feces and urine. This provides

clear evidence that 1) contaminant particles do gain access to human bodies, and 2) human bodies have an ability to purge some proportion of this material. There are two regions in the human gastrointestinal tract that are vulnerable to particle movement across the GI barrier (Wright & Kelly, 2017). One is the Peyer's Patches in the lumen, an area of immune surveillance where 0.1 - 10 µm sized particles can conceivably move into lymphoid tissue with the aid of specialized microfold 'M' cells. Another route is paracellular persorption where particles up to 130 µm move through loose junctions between cells (Wright & Kelly, 2017).

Because the lower detection limit for the first two studies involving human feces were 50 µm (Schwabl et al., 2019) and 20 µm (N. Zhang et al., 2021), it is not yet known if a smaller size fraction of microplastics were effectively excreted. The amount of contaminant <10 µm cleared by the body, as well as the quantity of this particular size fraction that is ingested is critical to understanding the number of particles re-routed *into*, as opposed to *through*, the human body. Furthermore, if particles <10 µm, are detected in feces, as 4-15 µm particles have already been detected in human urine, it is possible their pathway was routed through the spleen or liver and not directly through the GI tract (Prata, 2023). Although there are some mechanisms by which particles can access human tissues and biofluids that are not open to the environment, the specific routes and quantities are not known as the toxicokinetics around microplastics are presently not well understood. However, there is ample evidence that these particles are present in the lymph nodes (Urban et al., 2000), placenta (Ragusa et al., 2021), brain (Amato-Lourenço et al., 2024; Nihart et al., 2025),

spleen (Urban et al., 2000), liver (Horvatits et al., 2022a), testes (Q. Zhao et al., 2023), and the vitreous humor of the eye (Zhong et al., 2024), among others.

Another important unknown is the total amount of microplastics that humans are exposed to. An early attempt to quantify ingestion reveals an annual range of 39,000 – 52,000 particles for four demographic categories based on diet as determined by sex and age (Cox et al., 2019). Including inhalation only boosts this range to 74,000 – 121,000 particles, annually (Cox et al., 2019). There are significant limits to these initial estimates, however, as the figures for ingestion were based on the results from 26 publications that cover only 15% of an average American diet. Furthermore, inhalation studies that accurately capture real life exposures, such as the Breathing Terman Manikin that was employed by Vianello et al. (2019), were not included in this estimate. In addition to uncertainties surrounding the physical particles, the exposure, dose, bioaccessibility, and bioavailability of endogenous chemical additives and sorbed pollutants are also unknown and they make up an incredibly vast list of chemical compounds. The most recent and comprehensive list of chemical additives to date put this figure at 16,325, of which 10,726 have no hazard data (Wagner et al., 2024). However challenging to study, these compounds cannot be discounted as some in vitro and in vivo research links exposures to tissue inflammation, cytotoxicity, oxidative stress, immune responses, and genotoxicity (Llorca & Farré, 2021; B. Zhao et al., 2024b), but there is uncertainty about true to life doses.

The human body is the latest frontier in microplastics research. There is keen interest in the public health aspect of this discipline,

both within the scientific community and from consumers who buy and use plastic products. However, given the heterogeneous nature of this contaminant, there are many challenges involved in linking exposure to specific negative health outcomes. Given the complexity of this problem, it may take many years for results to converge. Moreover, if findings compel lawmakers to change policies, as with the 2015 Federal Microbead Free Waters Act (H.R.1321 - Microbead-Free Waters Act of 2015, 2015), the public will need to be educated about the risks and industry will require time to adapt. Therefore, while research around human health impacts are ongoing, it is essential to simultaneously maintain the study of contaminant sources and exposures. Even in the absence of causal relationships connecting exposure to specific health endpoints, there are plenty of opportunities to apply the precautionary principle and seek viable alternatives. However, it is important that alternatives do not present more hazards or risks than the original product.

Food and beverage packaging is an area of interest that has been explored from multiple angles (Muhib et al., 2023). Many people, driven by environmental concerns about plastic waste are making a preemptive move towards reusable bottles (Pandey & Shivarkar, 2025). In addition, several high-profile studies that involved single-use plastic water bottles (Mason et al. 2018; Qian et al., 2024) may have influenced members of the public to choose reusable bottles for health reasons. However, very little is known about the release of microplastics from reusable water bottles that are constructed with all, some, or few plastic components. This work focuses on sports water bottles, which are used by professional and amateur athletes alike, but are also

marketed to a broader audience. This study seeks to quantify the release of microplastics in five varieties of sports water bottles assigned to members of a cycling team over the course of a summer. Using true-to-life washing/rinsing habits and bottle use, community partners took care of bottles as they would their own, while the products were tested intermittently for the release of microplastics over a total of 92 days.

Materials & Methods

Five bottles representing four brands were selected for this study and tested in triplicate (n = 15). All bottles were purchased online, straight from the manufacturers rather than from brick-and-mortar stores in order to minimize pre-testing handling. Two of the bottles had a metal body, both with a hard plastic screw top lid and built in silicone straws. One of the metal bottles had a coating of black paint on the outside (Metal A) and the other had a black rubber coating on the outside (Metal B). Both had caps that were PP and silicone straws. The other three bottles were made entirely of plastic. The first (Plastic A) was made of LDPE, HDPE, PP, and TPU. It had a light-colored opaque plastic body with a dark plastic lid and red nozzle. The second plastic bottle (Plastic B) was made of PP, PC, and silicone. It had a dark-colored body, black lid and clear plastic nozzle. This bottle was made from the same company as Metal A. Finally, the third plastic bottle (Plastic C) was made LDPE and internally coated with silicon dioxide. It is a translucent light-colored plastic body with a light-gray lid and black nozzle. The three plastic bottles are designed to be squeezed to release water while the two metal bottles were designed with an aeration aperture at the top which allows

water to readily flow from the bottle when inverted. The brand names of these bottles will not be released.

The fifteen test bottles were not used before the experiment began. Apart from these, another ten bottles were donated to the study by cyclists, and they estimate that those bottles had been used for 5 – 10 years. Of the aged bottles, six were made exclusively of plastic; three were translucent, one was light-opaque, one was green, and one was red. The four remaining aged bottles were made of metal, and three of these were from the same company as the test bottle, Metal B.

Community Partners

Each of the 15 test bottles were given a three-digit code number for internal record-keeping as well as a gender-neutral name for ease in identification with community partners. The name was written on the outside of the bottle in indelible ink. These bottles were then deployed to members of a cycling team based in Minneapolis, Minnesota. Each team member had possession of a bottle for a total of 13 weeks during the summer of 2024. They were instructed to use and care for the test bottles just as they would their own water bottles. They were also asked to document bottle use and care each completing a weekly online survey (see Figure 5.1). The full survey can be found in the appendix (Chapter 5, Figure 1). Through the survey, participants were asked to give the bottle's name and the number of hours the bottle was used (during cycling) for the duration of the week. Values were rounded to the nearest integer. Next, participants were asked about their rides, with options of 1) pavement, 2) gravel, 3) trail, 4) trainer (stationary bike), and 5) other. They were also asked about environmental conditions during their rides. They could select several

from the following options. 1) Night ride, 2) Sunny, 3) Rainy/cloudy, 4) Temperature (40-55˚F), 5) Temperature (55-70˚F), 6) Temperature (70-80˚F), 7) Temperature (80-90˚F), and 8) Temperature (+ 90˚F). Finally, community collaborators were asked to describe their bottle washing habits for the week. The following options were provided. 1) The bottle was hand washed, 2) The bottle was washed in a dishwasher, 3) The bottle was ONLY rinsed with tap water, 4) The bottle was neither washed nor rinsed, and 5) other.

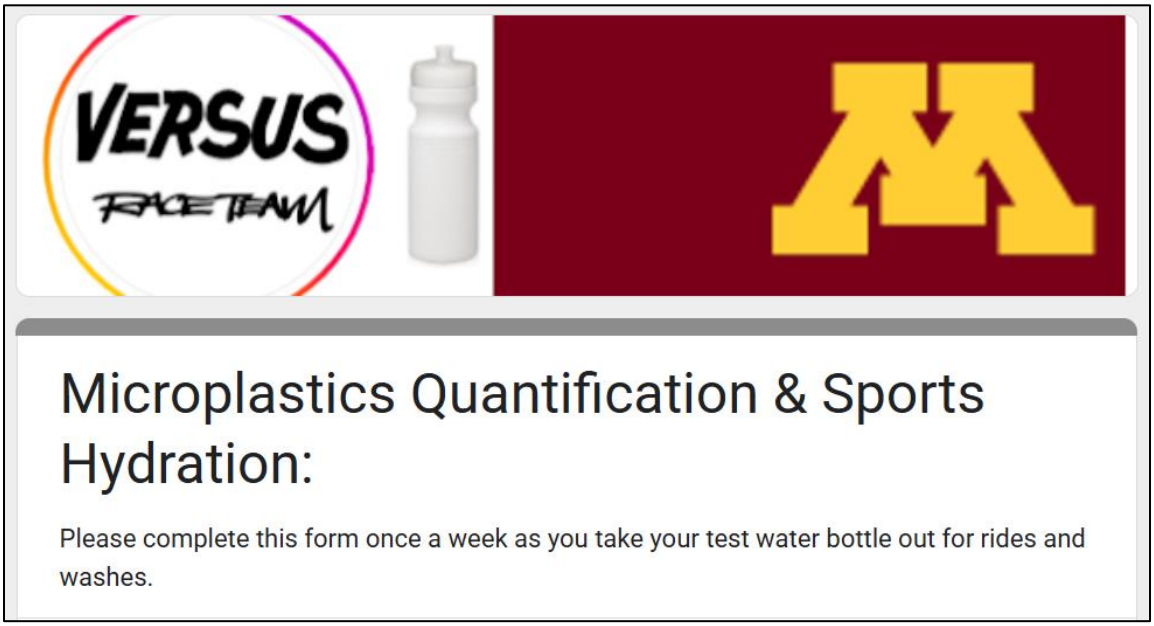

Figure 5.1 Community partners were asked to complete weekly surveys to document bottle use and care.

Sample Collection

Four sets of samples were collected and processed during the span of this research project. The first set of samples were collected after the new bottles were stripped of their auxiliary packaging. This included plastic and paper labels that were attached to the necks and bodies of the bottles. Metal A bottles were unique in that they

contained cardboard inserts holding a silicone straw cleaner and paper envelope containing bolts for a water bottle cage (Figure 5.2). All the other bottles arrived empty with information/marketing labels on the outside. It should be noted, the brand Plastic C had visible plastic threads left over from the manufacturing process, tenuously adhered to the bottle lid (Figure 5.2).

Figure 5.2 Image of cardboard inserts that were inside the bottles Metal A (left) and a cursory visual inspection of a lid from the Plastic C bottle group. Light ribbons of plastic can be seen left over from the manufacturing process.

Once packaging materials were cleared from the new test bottles, the first of four sample sets (M_0) were collected. Sample processing is described in the following segment. After processing M_0, the bottles were filled with 500 mL of reverse osmosis (RO) water and taken out on 6/24/24 for an hour-long, urban, majority paved, bike ride by members of a local racing team. This ride was meant to *simulate* bottle use and cyclists were explicitly instructed not to drink from the test bottles during the ride. After the ride, the bottles were collected and the RO water contained in the bottles were processed and measured in the same manner as M_0. This sample set is M_1 and represents Day 1 of bottle use. Next, the bottles were returned to the cyclists for 7 weeks, during

which time the cyclists used the bottles as they would normally. The bottles were collected on 8/12/2024 and filled with 500 mL RO water before going out with the racing team members on another hour-long urban bike ride. After the ride, the RO water was collected, measured, and processed as with M_0 and M_1. This sample set was designated M_2 and represents Day 42 of bottle use. Next, the bottles were returned to the cyclists for 6 weeks, during which time the cyclists used the bottles as they would normally. They were then collected for a final time on 9/23/2024, filled with 500 mL RO water and taken out on an urban evening ride. This sample set (M_3) which represents Day 92 of bottle use, was processed in the same manner as M_0, M_1, and M_2. See Figure 5.3 for an illustration of bottle deployment dates, use (M_{1-3}), and test rides.

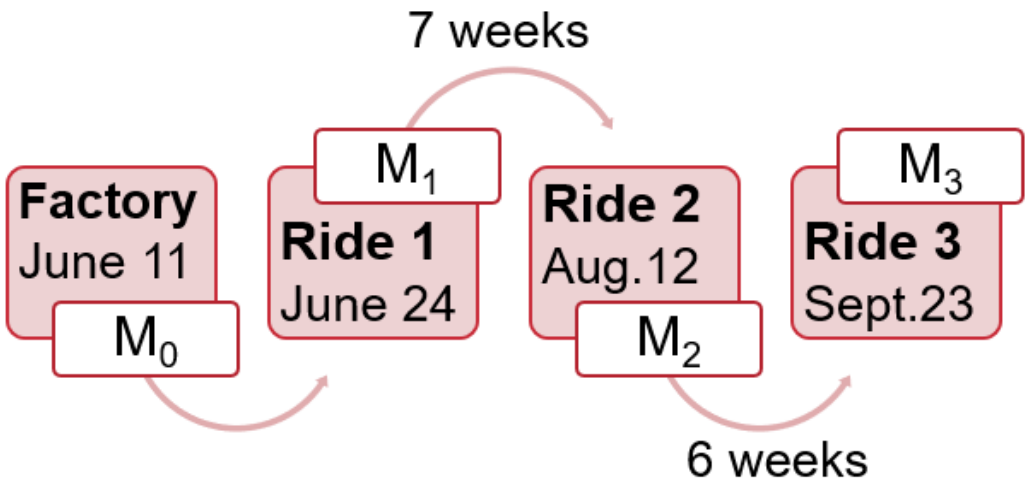

Figure 5.3 Flow chart schematic for sports water bottle testing over the summer of 2024.

Sample Processing

Sample processing for each of the four sample sets and the ten aged bottles, which were each tested once, was identical. The procedure involved pouring each sample, which is comprised of a 500 mL volume of RO water, into a stainless-steel vacuum filtration apparatus fitted with a polycarbonate filter (47mm diameter, Isopore from MiliporeSigma). For sample set M_0, 5 µm pore filters were used, but then it was decided that 0.4 µm pore filters would be used for samples M_1, M_2, and M_3 in the hopes that smaller particles could be viewed with SEM. When transferring the samples through the vacuum filtration funnel, care was taken to rinse out the bottles with RO water from a plastic squeeze bottle. Often, raw water samples undergo a digestion step using acids, bases, enzymes, or wet peroxide oxidation. However, since these samples consisted of filtered lab water where no biological contaminants such as algae, arthropod exoskeletons, decomposed leaves, and other organic detritus were anticipated, this step was omitted. Instead, after initial filtration, the polycarbonate filters were transferred to a 250- or 600-mL glass beaker using forceps. Then, the filtering funnel was removed from the apparatus and placed over the beaker. Using 100 mL of RO water from the squeeze bottle, the sides of the funnel were rinsed down into the beaker. Next, a 0.5 mL quantity of Nile red solution at a concentration within a range recommended by Maes et al (2017). (1 - 1000 µg/mL or 0.001 - 1 mg/mL) was added to the water bottle sample and left to sit for 20 minutes. Finally, this solution was passed through the filtration apparatus a second time using the same filter that was used for the first filtration. During this final filtering stage, the beaker containing the sample was rinsed

3X into the filtering funnel and the sides of the filtering cup were rinsed down 3X. Finally, the filters were placed in labeled (polycarbonate) petri dishes.

Sample Imaging and Physical Analysis

After sample processing, the suspected plastic fibers and particles captured by the 47mm polycarbonate filter were imaged and digitally processed with MIPAR, a software with AI capabilities customized to count, measure, and categorize particles based on morphology (Kosuth et al., 2023). Filters were imaged with a Leica EZ4 W stereo microscope at a magnification of 8X. Each filter, without removing it from the petri dish, was laid on a 3 X 5 grid so that the entirety of it could be captured in 15 images. The images were not collected with the incandescent built into the microscope, but with a CrimeLite 2 (450 – 510 nm blue/green) positioned next to the microscope on a stand fitted with a clamp. The frequency of this light causes synthetic polymers stained with Nile red to fluoresce. It should be noted, Nile red is a lipophilic stain which readily binds with most synthetic and some non-synthetic polymers, such as Chitin (Maes et al., 2017; Shim et al., 2016; Tamminga, 2017). This is also why chemical characterization is essential to verifying this 'first pass' with Nile red. The 15 images are then batch-processed with the imaging software MIPAR, using a custom-made recipe. The program bins fluorescing debris into two morphological categories: particles and fibers. Examples of captured particles and fibers can be found in Figure 5.4. The lower size limit for particles is 30 µm and 200 µm for fibers.

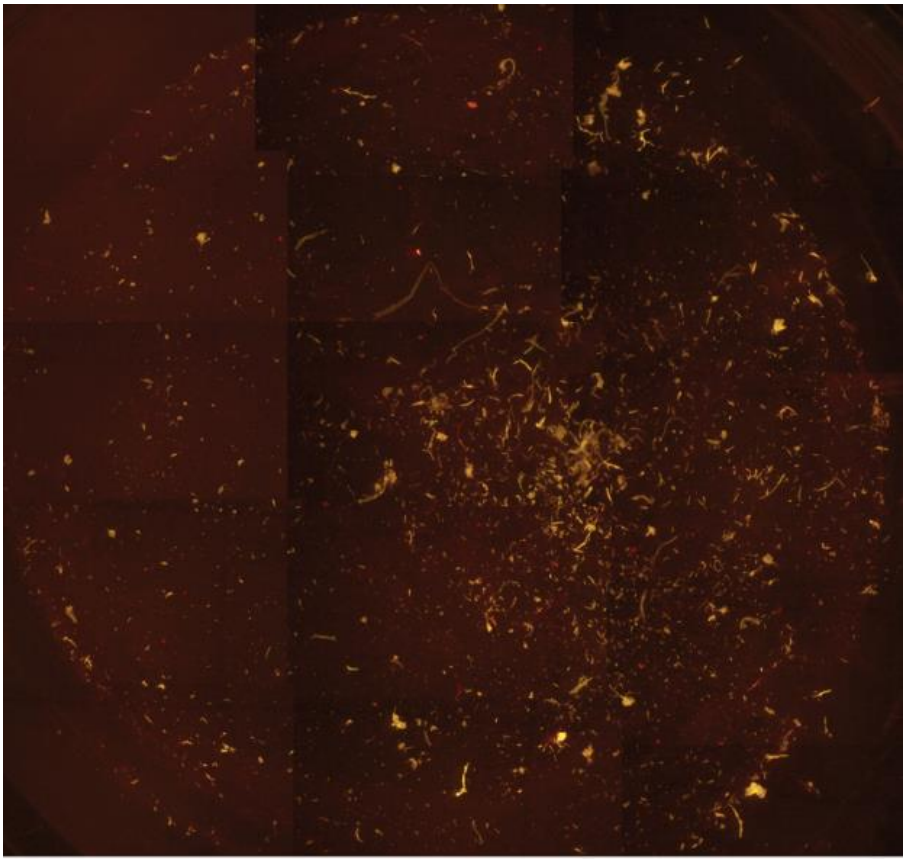

Figure 5.4 A total of 15 images were taken of each 47mm filter and batched processed with MIPAR. This image is a mosaic of one filter from one of the three samples from Plastic C in the M_0 sample set.

Sample Chemical Analysis

After completing sample enumeration, a Fourier-Transform Infrared Spectrometer (Thermofisher Nicolett iS50 FTIR) was used for chemical characterization. Particles were selected at random from each of the sample bottles for chemical analysis. Three to four fluorescing fibers and particles were picked off each filter with a sewing needle and placed on a NaCl cell window for a total of 10 - 13 particles for each bottle type tested. In addition to subjecting recovered particles for analysis, samples taken directly from the bottle were tested to see if they matched with particles collected from the filtered RO water. Finally, particles from seven of the aged bottles were tested.

FTIR spectra were collected in transmission mode with an aperture size of 50 µm x 50 µm using a Thermo Scientific continuum microscope coupled with an iS50 main bench and equipped with a liquid-nitrogen-cooled MCT-A detector. Samples were placed on NaCl windows and data were collected with 256 scans at resolution 4 over a spectral range of 650 to 4000 cm^{-1}. Data collection and spectral analysis were performed using Open Specy, an open source, web-based polymer library that allows users to tap into a collection of reference spectra. This reading tool, reference library, and online community is necessary because microplastics represent an exceedingly heterogeneous suite of contaminants (Cowger et al., 2021). The polymers emerging from industry are chemically diverse, but when they are then subjected to myriad environmental conditions which alter the surface chemistry, identification can be even more challenging.

Quality Assurance/Quality Control

Aluminum foil was fitted over beakers, graduated cylinders, and the filtration apparatus, to reduce the contamination from the air during sample transport and processing. A cotton lab coat and latex free nitrile gloves were worn during sample processing and all work took place inside a biosafety cabinet. All laboratory glassware was cleaned with Alconox and scrubbed with a natural fiber brush to minimize contaminants. After washing, laboratory glassware was covered in aluminum foil and baked in an oven for 6 h at 450°C to reduce cross-contamination (Prata et al., 2021). Because laboratory RO water was found to be one of the least contaminated of the lab waters available (Kosuth et al., 2023), this source of filtered water was used for all glassware rinsing and procedural blanks. Lab blanks were run each day

that samples were processed. Since samples were processed in batches of 7 or 8 out of a total sample set of 15, there were two blanks per sampling set. Blanks consisted of a 64 mm diameter watch glass set out in the biosafety cabinet during the processing of one sample. Then, this watch glass was rinsed into a cleaned beaker using RO water from the same plastic squeeze bottle that was used to process samples. It was then processed in the same manner as the test samples. To blank correct for background levels, the average of the two blanks were subtracted from the mean from each of the five bottle types. For the aged bottles, all 10 samples were processed in a single day. One blank was run during that processing and was used for blank corrections.

Results

Results for each of the five reusable bottles tested are presented as mean values for each of the five bottle types tested in triplicate (Figure 5.5). Across all varieties of bottles tested, the highest quantities were seen in M_0, which were samples collected from bottles sent from the factory, with no prior rinsing or use. For the bottles Metal A and Metal B, detected particles decreased gradually through sampling sets from M_0 to M_3, with the lowest particle counts on the last sampling day. However, it should be noted that the Metal A count for M_0 was 133 particles/L, while for Metal B it was 479 particles/L. This difference is addressed in the discussion. For Plastic A, the highest count was M_0 and the lowest count was the next sample set, M_1. This was followed by a marginal rebound in M_2 and then a more pronounced increase in sample M_3. The mean values for Plastic B also saw a decline from M_0 to M_1, and a continued decline to M_2, followed by a slight rebound in M_3. For Plastic C, M_0 was the most abundant and M_1

the least, a trend similar to Plastic A. However, this was followed by a significant rebound in M_2 and then a slight taper in M_3. In short, the two metal bottles shared the same trend across four sample sets, but the plastic bottles did not. Overall, the magnitude for Plastic C was higher than the other test bottles. In terms of contaminant density (particles/L), the range for Plastic C (291 – 3770 particles/L) was one to three orders of magnitude greater than the range for Plastic A (49.3 - 745 particles/L), Plastic B (6.33 - 114 particles/L), Metal A (1.33 – 133 particles/L), and Metal B (15.3 - 479 particles/L).

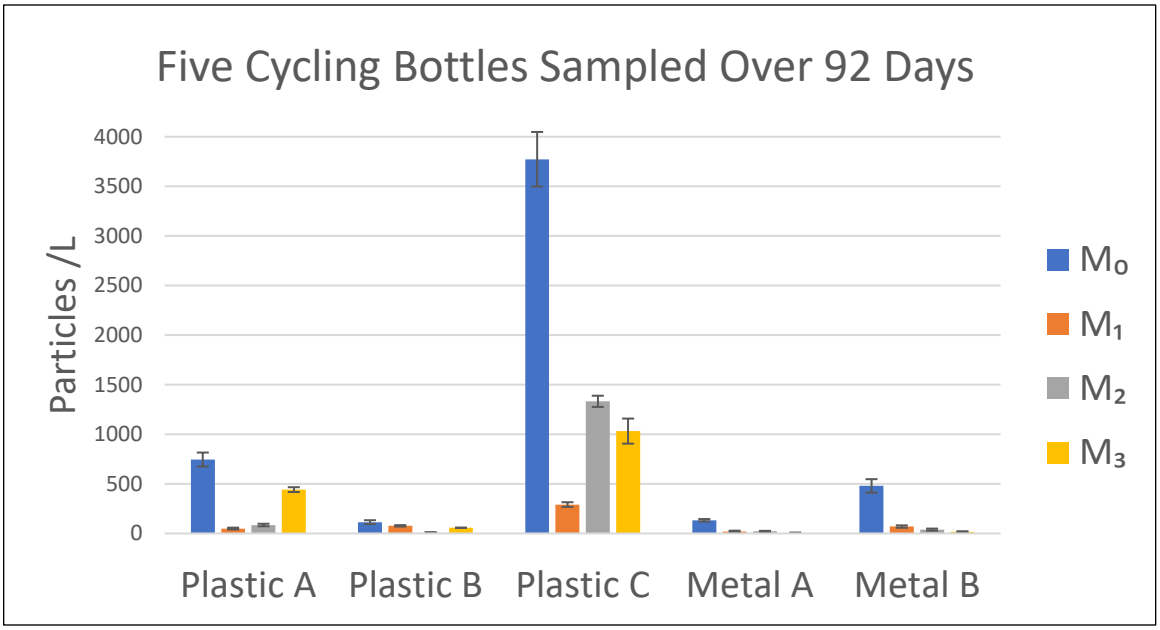

Figure 5.5 Five types of reusable bottles that were tested four times over the course of a summer after exposed to 'regular' use and care. The legend shows M_0 to M_3 processing.

The total particle abundance recovered in four metal and six plastic aged bottles exhibit noticeable differences between brands (Figure 5.6). The range of particles recovered from the four metal bottles was 62 – 184 particles/L, while the range from six plastic bottles was 90 – 2200 particles/L. Similar to the test bottles, one brand exhibits a

particle load that far exceeds the rest. The bottle PY is > 6X than the other nine bottles tested.

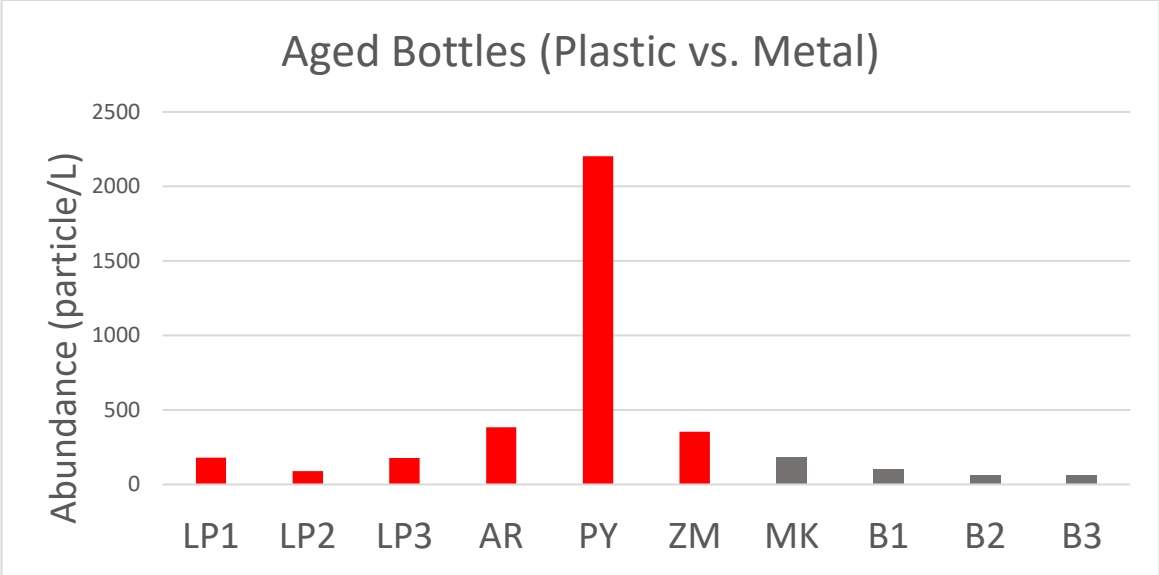

Figure 5.6 Totals from ten aged bottles that are labeled with initials, four metal (gray color, right) and six plastic (red color, left).

A small subset of particles recovered from new bottles were tested with FTIR. Of the two metal bottles, Metal A and Metal B, none of the particles analyzed matched the materials subsampled from the straw or components from the lids of the bottle. Metal A had no matches to synthetic polymers at all while Metal B had two particles that were identified as synthetic polymers. Of the eleven particles analyzed from Plastic A, five matched as synthetic, but only one out of five matched the bottle (polyethylene). Plastic B had four synthetic particles, two matching the bottle components (polypropylene and polycarbonate). The remaining two were not from the bottle. Finally, Plastic C was unique in that ten of the particles were synthetic polymers and of those, eight matched the plastic that the bottle was made from (six PE and two LDPE). The three remaining particles of the thirteen particles tested

in total were synthetic but they did not match the bottle material. A summary of this can be found in Table 5.1, and a comprehensive account of chemical analysis for both recovered particles and component parts from test bottles can be found in the appendix (Chapter 5 Table 1.).

Bottle Types	**Component Parts Tested**	**# Recovered Particles Tested**	**# Synthetic Polymers ID**	**# Matched Bottle Components**
Plastic A	PE, LDPE	11	5	1
Plastic B	PP, PC	10	4	2
Plastic C	PE, LDPE	13	10	8
Metal A	Silicone, PP	12	0	0
Metal B	Silicone, PP	10	2	0

Table 5.1 A summary of five bottles tested (from M_0), the synthetic polymers that make up each bottle, the number of recovered particles chemically analyzed, the number of particles identified as synthetic, and the number of synthetic particles that matched the bottle's plastic components.

A small selection of particles from the aged bottles were also analyzed with FTIR. One of the metal bottles contained three particles that were made from polyamide (nylon). Some other synthetic polymers identified with a > 60% match were low density polyethylene, high density polyethylene, polyethylene terephthalate, polyvinyl chloride, polypropylene, polyvinyl acetate, and polyvinyl esters. There were 19 particles analyzed in total from the older bottles and of these, 13 were synthetic. A selection of images for a fiber and two fragments can be seen in Figure 5.7.

Figure 5.7 Left: polycarbonate fragment (79% match). Center: polypropylene fragment (81% match). Right: low-density polyethylene fiber (66% match).

Particle morphology proportions shifted throughout the 92-day sampling period. Starting with M_0 there was some parity between particles and fibers at 53.3% and 46.7% respectively. Then, in the M_1 sample set, particles became more prominent at 65% compared to fibers at 36%. In the M_2 sample set, proportions split further still with particles at 75.3% and fibers at 24.7%. Finally, for the M_3 sample set, they were more in line with M_1 with 68.7% particle to 31.3% fiber (Figure 5.8).

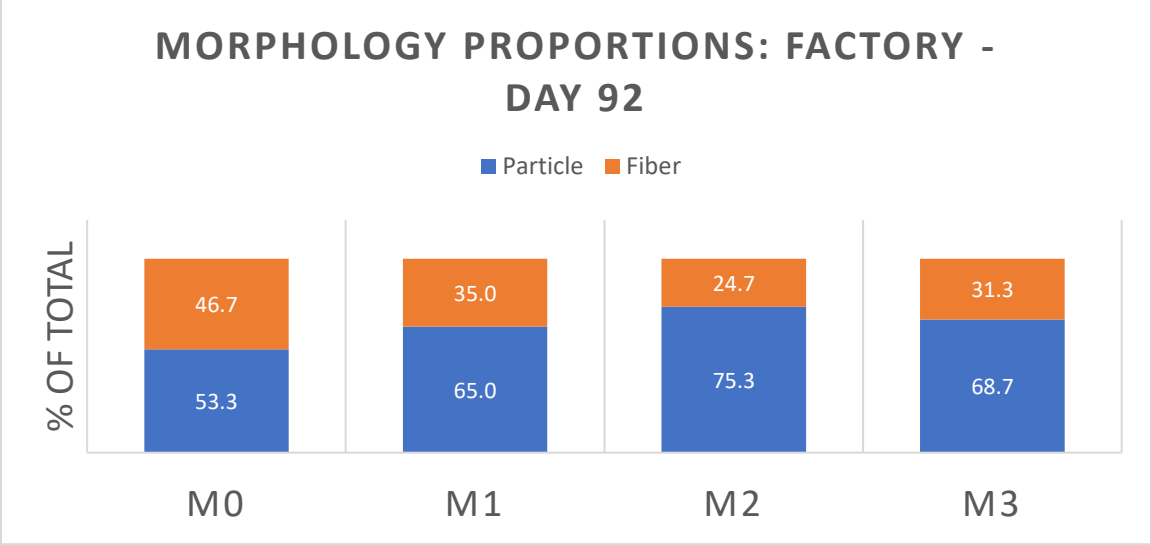

Figure 5.8 Fiber and fragment proportions from sample set M_0 through M_3.

Particle sizes also changed throughout the 92-day sampling period. Fiber length decreased from 482 µm to 436 µm to 407 µm from M_0 to M_2 and then for M_3 increased back to 436 µm, the same as M_1. Using a t-test, fiber length from M_0 to M_2 was significantly different (p-value = 1.473). Similarly, particle length, as determined by the longest dimension, decreased from 193 µm, to 155 µm, to 136 µm from M_0 to M_2 and just as with fibers, M_3 increased slightly to 141 µm. Here again, particle length from M_0 to M_2 was significantly different (p-value = 2.207) (Figure 5.9).

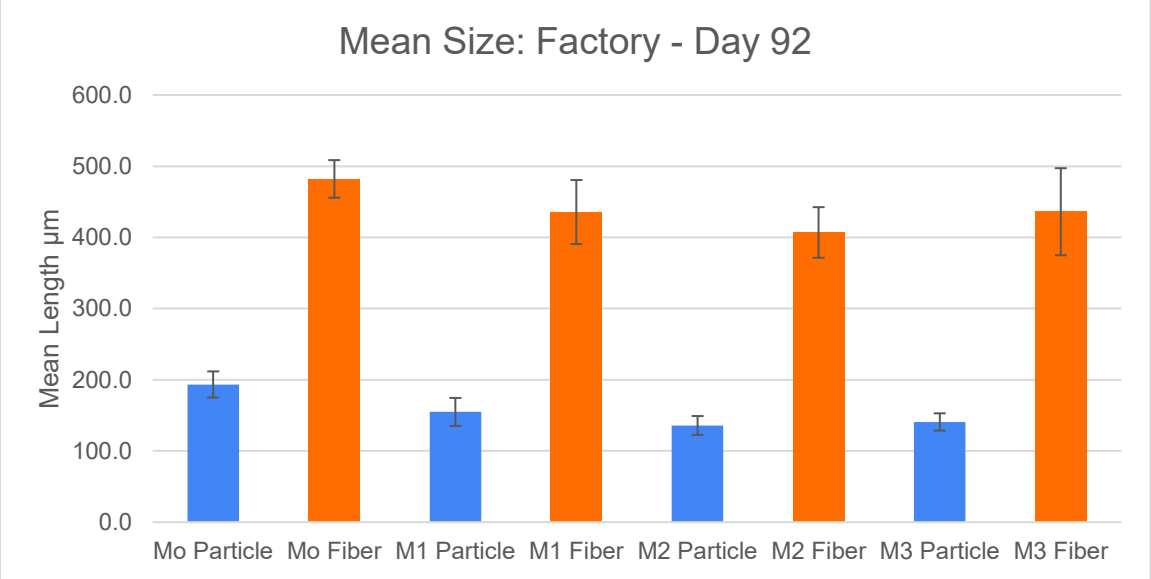

Figure 5.9 Mean length of fibers and fragments from sample set M_0 through M_3.

Finally, examining the survey results from the community partner, bottles were used anywhere from 5 - 157 hours, with an overall mean of 53.9 ± 41.4 hours. Of all the hours, Metal A had the highest mean (79 hours) followed by Metal B (61 hours), Plastic A (58 hours), Plastic C (38 hours), and Plastic B (34 hours).

A weak negative correlation (r = -0.22) was found between the hours of bottle use and the number of particles recovered. The relationship

between these two variables in the first seven weeks of summer (6/24 - 8/12) reveals a weaker link, while in the latter six weeks of summer (8/12 - 9/23), the link is stronger. The relationship between the number of washes and particles recovered is a non-significant negative correlation ($r = -0.46$, $p > 0.05$) but if washes and rinses are combined, the relationship is significant ($r = -0.51$, $p < 0.05$G). These relationships are depicted in Figure 5.10. When these two sets of variables are split into two summer sessions (6/24 - 8/12 and 8/12 - 9/23), no strong associations were seen.

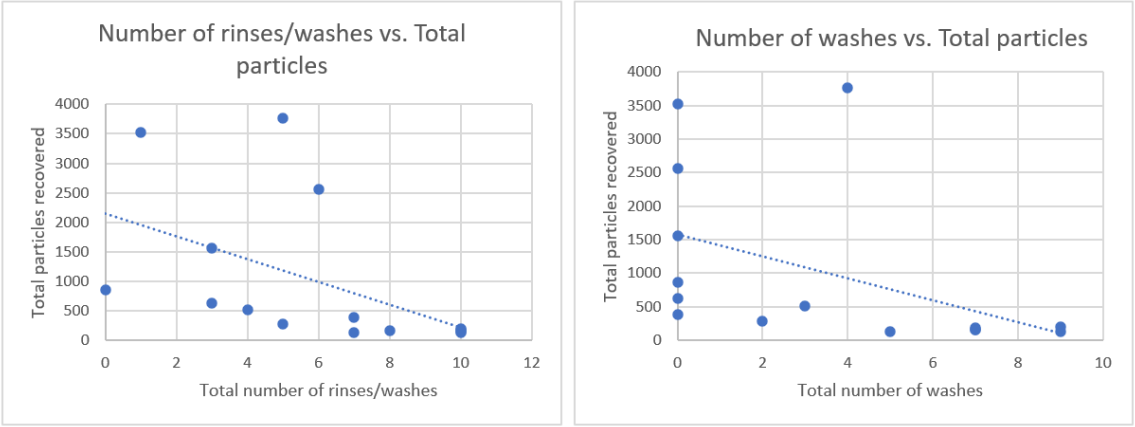

Figure 5.10 A moderate negative correlation seen between particle abundance and number of washes (right) and a moderate negative correlation between particle abundance and number of washes and rinses combined (left).

Discussion

Many studies have shown the presence of microplastics in human foods and beverages, but the proportion of contaminants that come from packaging, processing, or through raw ingredients by way of environmental exposure, is not yet known. While some studies have examined plastic packaging for food and single-use beverage bottles, no study to date has looked at the prolonged use of non-disposable bottles constructed from a variety of components that include plastic. Since

this study is the first of its kind, and since there is so much variation among bottle types, it is difficult to compare these results to prior work. However, it is still important to put this work into context.

Originally, Schymanski et al. (2018) looked at water packaged in a variety of materials including returnable and one-way plastic bottles both made from PET. The study took place in Germany where consumers can return specially marked reusable bottles to stores to be washed, refilled, and restocked. While there was variation among brands, the returnable bottles had the highest contamination load with means that were eight times the amount found in single-use bottles. Using Raman Spectroscopy, 84% of the particles recovered from returnable bottles matched the plastic packaging, causing the authors to link contamination to bottle wear (washing and use). Plastic particles found in single-use bottles ranged from 2 - 44 particles/L, which is similar in range to the contaminant abundance in Metal A in the present study (1.33 – 133 particles/L). On the other hand, particles in reusable bottles purchased in Germany ranged from 28 – 241 particles/L, which is similar in range to the number of contaminant particles found in Plastic B in the current study (57 – 114 particles/L).

In another German bottled water study conducted by Oßmann et al. (2018) single-use and reusable PET bottles (split into the two subgroups ‘older’ and ‘newer’ based on their appearance) were examined. Results were in accord with the findings of Schymanski et al. (2018) in that the reusable bottles had a higher contaminant load compared to single-use plastic bottles. An important difference between these two studies was in the overall amount, as Oßmann et al. (2018) reported

mean values that were an order of magnitude higher. The single-use bottles had a mean of 2,648 ± 2,857 particles/L, while reusable bottles had a mean of 4,889 ± 5,432 particles/L. Both studies used Raman, but Oßmann et al. (2018) could only examined a small part of the filter and extrapolated to get the total amount. This best matches the current study's range for Plastic C (291 – 3770 particles/L). Finally, another study that involved an investigation of 259 internationally sourced, single-use plastic water bottles, reported a contaminant load ranging from 3.72 - 2,277 particle/L, which has an upper limit that is similar to that of Plastic C (Mason et al., 2018).

While it is important to contextualize current findings with other work involving single-use and reusable bottled water, all prior research provides only a snapshot of a product's lifecycle. The present study sets itself apart in that it tracks the relationship between particle abundance and bottle use over a span of time. The data graphed in Figure 5.5 show that products from the manufacturer prior to use have the highest load. This is not surprising as single-use polypropylene containers purchased directly from manufacturers have already been shown to shed nano-plastics (Fadare et al., 2020b). The way that plastic products are fabricated and if/how they are washed after production and before sale may contribute to residual particles lingering on surfaces. Figure 5.5 also shows a downward trend in particle shedding, although this is clearly not linear across all bottles tested as there is an increase in particles in Plastic C from M_1 to M_2 and in Plastic A and Plastic B from M_2 to M_2. However, this may indicate that particles that persist on the surface of products when

they are first purchased and rinsed, eventually detach and slough off through use and repeated washing.

The initial high level in Plastic C, with an M_0 mean value of 3,770 particles/L is easy to understand. An image of the cap from one of the three bottles tested can be seen in Figure 5.2. In this image, visible ribbons of plastic are readily being shed from the inside of the cap. It is likely this excess material was left over from the manufacturing process. Furthermore, in Figure 5.4, the processed filter from the M_0 sampling set shows an abundance of rinsed particles that were within one of the Plastic C bottles. What is more difficult to explain is the persistent elevated levels in Plastic C as well as the high magnitude of particles found in the aged bottle 'PY' (see Figure 5.6) which shed 2,200 particles/sample. Water bottles can be constructed from an assortment of materials. Some brands tout features intended to maintain the quality of the water in both taste and odor. For example, the makers of Plastic C, which had the highest abundance among all the brands, claim to have a silicon dioxide coating that lines the inside of the bottle. This is meant to create a barrier between the water and the low-density polyethylene body of the bottle. Other brands may simply be made from a material that is more prone to degradation, especially if it is exposed to heat and light. Alternatively, it maybe be poorly constructed or made from a material that has low structural integrity. This is speculation as bottle design and construction is well out of the scope of this paper.

Results from the chemical analysis indicate four possible sources for the contaminant particles, 1) components shed from the bottle's interior, 2) deposition from the atmosphere when the bottle is open, 3)

water introducing contaminants from the water source, and 4) tools used for dishwashing. For Metal A and Metal B, none of the contaminants matched the materials collected from the plastic straw or lid components, which means the two synthetic polymers collected from Metal B most likely originated from outside the bottle. However, it is possible that the synthetic components found in Metal B through FTIR analysis and the elevated particle count in the M_0 sample set was inadvertently introduced with the cardboard insert depicted in Figure 5.2. Plastic A and Plastic B only had one and two particles, respectively that matched the bottle components. However, Plastic C was unique in that 80% of the particles tested matched with the bottle components. Again, Plastic C also had the most contaminants and visible shedding of particles from the manufacturing process which makes the detection of particles from the bottle more likely. Finally, the three nylon particles recovered from the aged metal bottle 'MK' (Figure 5.6) may come from a nylon scrub brush, which was used to wash the inside of bottle.

There are a few noteworthy trends in both particle morphology and size over the 92-day testing period. Initially, during the M_0 sample set, there was parity between fibers and particles, but through two sample sets, particles increased in abundance while fibers proportionately declined. Since fiber morphology is associated with atmospheric deposition, especially in indoor environments (Yao et al., 2022), bottles may be exposed to indoor deposition during the manufacturing process. Then, through routine use and maintenance, the opportunity for exposure to indoor air may decline while new pathways leading to the entry of particles, either through water or scrubbing

tools, may increase. Additionally, some of the visible contaminants in Plastic C were thin strands, many of which were larger and easily washed away after the M_0 sample set. These threads are recognized as fibers by MIPAR, the imaging software used in this study. The size of particles also trended downwards over the course of three sampling sets. From M_0 to M_2, the length of fibers decreased by 15.6% while the length of particles decreased by 29.8%. Once again this may have to do with a shift in the source of the contaminants as particles straight from the factory are likely left over from the plastic extrusion blow mold process, and as a result, they might be larger. Smaller particles that were recovered in successive sample sets may have come from the abrasion of the plastic material or introduced through use and washing.

Community partners were invaluable in this study, providing authentic use and care of test bottles for the duration of the study. According to surveys filled out by the cycling team, bottles were taken out for as few as five hours to as many as 157 hours. The mean hours of use across all test bottles were 53.9 ± 41.4 hours, with Metal A accruing the most at 79 ± 55.8 hours and Plastic B, accruing the least at 33.7 ± 19.6 hours. Variations in hours of use, both among and between bottle types, makes it hard to compare shedding with bottle design. The bottles with more use had fewer particles. Metal A and B were the two bottles with the highest mean hours of use. They also had the lowest particle counts. Still, the differences in bottle handling are an asset as they reflect a true-to-life range that is most likely experienced by bottles purchased for use by consumers.

There are some notable limits to this study. One is a lower threshold of 30 µm for particles and 200 µm for fibers is not small

enough to capture contaminants that would pose a significant threat to human health. As Zhang et al. (2021) states, microplastics that are larger than 150 µm are purged from the human body through feces. While a small fraction of particles in this study might be small enough to move beyond the gastrointestinal tract, most would likely pass through. Another limit involves the possibility that Plastic B had an artificially low count because heavily pigmented plastic particles do not readily take up Nile red stain rendering them invisible to the imaging software used to count suspected particles. The bottle Plastic B was black in color. Therefore, if a proportion of the particles from that sample originated from the bottle, they would be uncounted and undocumented for physical characterization.

A third limitation is also linked to detection and characterization. Typically, a sub-sample for chemical characterization would involve at minimum, 10% of all suspected particles found visually, with the help of a stain, through a hot needle test, or some other form of 'first pass' vetting. Unfortunately, this paper was not able to test the minimum number of particles needed to know the proportion of plastics varieties in each sample. The random spot checking was still able to provide some link between bottle components and recovered contaminants. A final limitation involves a lack of knowledge around bottle design and manufacturing. Some producers list the materials that bottles are made from, but others are less transparent. To the authors knowledge, no producers list the plasticizers used both during manufacturing and in the final product. However, insights into manufacturing may help explain why some brands shed more than others and why some bottles maintain continuous elevated levels of particle shedding while others

appear to decrease over time. All in all, this is an opportunity to improve product design, where cost, performance, and microplastic shedding over the course of a product's lifetime are considered by the makers.

Future research in this area

Future study should involve a more comprehensive chemical analysis of particles. It should also include particles that are smaller (> 1µm). Also, because cyclists are not the only population that use reusable sports water bottles, more brands should be considered. Future studies should include bottles that are marketed to those who play other sports. It should also include brands that are sold to people living with disabilities, the elderly, and children. The present study provides some tenuous evidence that some form of washing and rinsing reduces microplastic abundance, but a more rigorous study should be done to confirm this. It would also be interesting to see if dishwashing increases or decreases particle shedding. Perhaps an investigation that focuses on this could happen in a more controlled environment where bottles are manually agitated in a lab setting.

Conclusions

This investigation is the first of its kind to examine reusable water bottles over a span of time. This initial investigation reveals variation both between brands, as well as within brands as time passes. The results produced in this study fall within the boundaries of prior investigations involving single-use and refillable or reusable bottled water studies. Future work could examine different brands and possibly do more to control for the time the product is in use and the kind of

washing the bottle is subjected to, in order to see if these actions actually reduce particle shedding. The results produced in this study raise questions about the manner in which plastic bottles are made. It also presents an opportunity for manufacturers to design bottles with health of end users in mind.

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Chapter 6: Microplastic Analysis Through Citizen Science: An Authentic Experience for Students and Community Members

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Abstract

Microplastics are particulate contaminants of concern and have been found in soils, sediments, oceans, lakes, rivers, ambient air, rainwater, snow, and biota. While the global reach of this pollutant is well documented, contaminant sources described in the literature are often broad and speculative with little known about pollution gradients, rates of accumulation, and residence time within an environmental reservoir. A robust spatial temporal dataset would advance understanding of the fate and transport of microplastics, but collecting large sums of empirical data over time is both expensive and time-consuming. A citizen science approach facilitates collection of data and expands awareness of microplastic pollution at both local and global scales. The inquiry-based lab activity described herein is targeted towards grades eight through twelve and provides opportunities to gather, process, and analyze environmental samples for a scientifically rigorous, regional survey. In addition, the curricular materials frame the issue of microplastic contamination as a complex

problem with many stakeholders. Questionnaires administered before and after classroom, lab, and field activities track changes in knowledge and attitudes around the scientific method and microplastic pollution. This authentic research experience directly addresses how scientific results are produced and reported and encourages students to consider how findings shape behavior, attitudes, and policies in their local communities.

Introduction

Global plastic manufacturing has been growing steadily and is expected to continue. Nearly 80% of the world's plastic waste is managed through landfilling and open dumping practices (Geyer et al., 2017). This means that worldwide plastic production contributes to a manifest accumulation of plastic trash in the environment. Furthermore, plastics subjected to mechanical and chemical degradation splinter into increasingly finer particles called microplastics when smaller than 5 mm. These particles shed during the entire life cycle of plastic products, from synthesis (Change, 2018) to transport (Corcoran et al., 2020), fabrication (X. Xu et al., 2018), use (De Falco et al., 2019; Sobhani et al., 2020), recycling (E. Brown et al., 2023; Guo et al., 2022), and disposal (Kabir et al., 2023). Since plastics do not mineralize, microplastic particles will continuously accumulate in, and flow between, environmental compartments (Brahney et al., 2021). Studies have demonstrated the presence of these pollutants in remote areas, from remote islands in the South Pacific to the Arctic Ocean (Nichols et al., 2021; Peeken et al., 2018), but abundance and flux in urban, suburban, and rural water bodies are understudied.

Robust spatial temporal datasets are essential to improve understanding of microplastic fate and transport. However, collecting extensive, empirical data is resource intensive and time consuming. Citizen science enables collection of large quantitative datasets that span space and time (Fraisl et al., 2020; Fritz et al., 2019). High school science classrooms are uniquely poised to take on this effort, allowing replicate samples to be collected from a wide variety of surface water sources across geographically diverse sites. Perennial sampling can happen every year and even through multiple seasons each year under the supervision of high school teachers, who serve as trained scientists. This work will yield valuable longitudinal data across an expansive geographic range.

Direct involvement of students in microplastic research will lead to several educational outcomes including increased environmental awareness, appreciation for scientific rigor, enhanced scientific literacy, and greater community and civic engagement. Studies show early exposure to basic environmental concepts engenders positive environmental attitudes (Ardoin et al., 2020; Bradley et al., 1999). A literature review examining survey results from different nations found education correlated with a willingness to adopt environmentally responsible behaviors (Garcia-Vazquez & Garcia-Ael, 2021). Furthermore, educational programming can be a part of a systems-based solution to the environmental problem of microplastics (Grünzner et al., 2023). The lab and supplemental materials (see Chapter 6 appendix) described in this paper offer a meaningful introduction to this growing and multifaceted problem and invites students to participate in finding solutions. This program is a collaboration of scientists and science

educators and is linked to Next Generation Science Standards (National Research Council, 2019), meeting requirements for HS-ESS3-4 Earth and Human Activity (Sarna et al., 2020).

In this project, students engage in exciting, exploratory ventures as they collect samples from water bodies, many of which have never been tested for microplastic contamination. Students apply creative problem-solving skills, cooperate with one another, examine potential bias, and learn about science as a dynamic process where unexpected findings spur further questions and investigations. This project helps students build an integrated and holistic perspective about the consequences of plastic pollution, giving them opportunities to consider policy, weigh economic consequences, and contemplate the impact of regulations. Finally, it encourages them to think about transboundary pollution and the uneven distribution of risks and benefits, which touches on social and corporate responsibility, and environmental justice. All told, this activity advances scientific knowledge while bolstering science literacy.

Methods

The first step is introducing the issue of plastic pollution to students. Education materials complete with definitions and preliminary questions are in the appendix (Chapter 6 Figure 1). Next, a local body of water is selected for testing. Both safety and accessibility must be considered. Appropriate safety protocols must be followed, particularly when working on bodies of water covered in ice (e.g., follow minimum ice thickness guidelines set forth by the DNR) or inclement weather. Based on an assessment of municipal developments, recent weather, season, local recreational and industrial activities, and type of water

body (river, pond, marsh), students should generate a qualitative hypothesis about the water body. If sampling two locations, geographical comparisons can be made. If sampling across seasons or before and after a precipitation event, temporal comparisons can be made.

Students must learn about the sampling protocol before traveling to the site. The standard operating procedure (SOP) can be found in the appendix (Chapter 6 Figure 2). Students and teachers complete the sampling data sheet on the sampling day. Depending on conditions and access, students can wade into water, sample from a bank or dock, or use an ice auger (Figure 6.1). Students should take numerous photos during sample collection to document their activities. For example, an inventory of potential contaminant sources can be taken (e.g. weathered buoys, beach litter, angling activities) including the source's proximity to the water body and a reference object (e.g., coin or ruler) for size. Finally, microplastics released from participants during sample collection are quantified with field blanks, which entail placement of a glass petri dish near the sampling site for the duration of sampling activities.

Once collected, samples are brought back to the classroom for processing. Again, students should be familiar with the processing protocol before they begin. A laboratory blank and a reagent blank (i.e. rinse water) are run to quantify background levels of microplastics during sample processing. Using photos and descriptions provided in the appendix (Chapter 6 Figure 3), students need clear guidance in identifying particles and fibers and categorizing each object examined as 1) confident positive, 2) potential positive, and 3)

confident negative. Multiple samples from a single water body can be reported independently and collectively, with an overall mean and standard deviation.

Hypotheses recorded before sampling should be revisited in light of findings, and new hypotheses and approaches discussed. Aside from data management, organization, and reporting, other topics warranting discussion are quality assurance quality control (QA/QC) measures (field and laboratory blank corrections), identification accuracy and bias, study limitations, and new research questions/future investigations inspired by findings.

Figure 6.1 Samples collected from various sites (public beach, stream, pond), during different seasons.

Lab Results

Multiple classrooms, ranging from middle school to post-secondary school, piloted the lab activity. All student groups encountered particles and fibers of interest, but there was uncertainty over how to confidently categorize suspected particles as either natural or synthetic. To measure accuracy, a trained scientist gathered samples (n = 2) alongside a classroom of citizen scientists (n = 7). Blank-corrected results for both students and scientists are in Figure 6.2. The scientist measured values

were generally within error of the student replicates. In general, microplastic abundance in surface waters is wide ranging and these values (~0.25 particles/L for "strongly microplastic" or ~1.0 particles/L considering both "strongly" and "maybe microplastic") are within values that have been documented for waters with greater land development (A. Thomas et al., 2024).

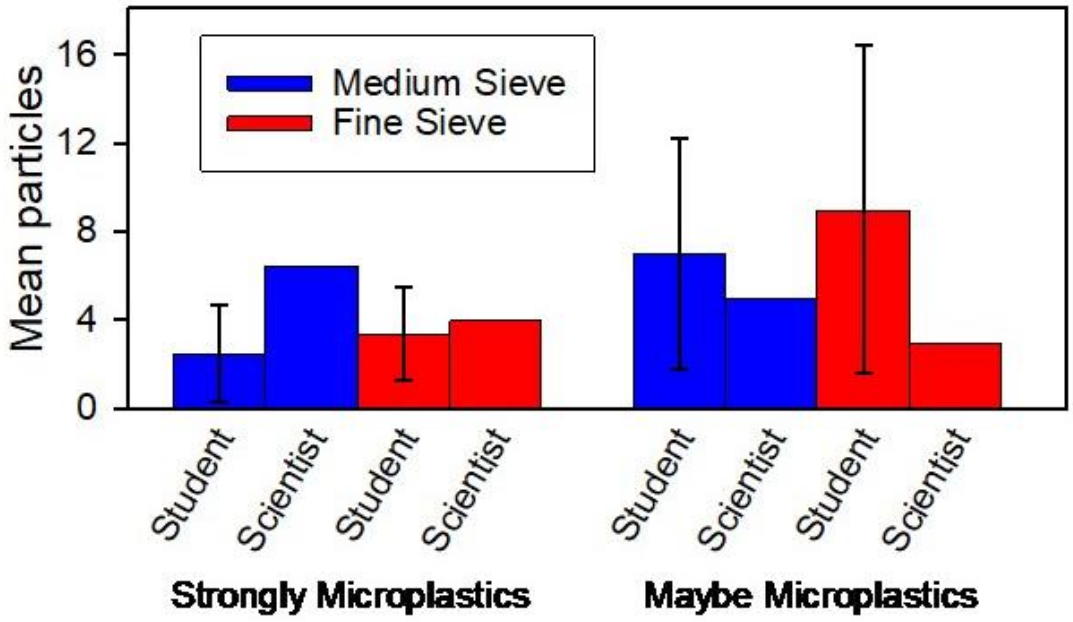

Figure 6.2 Results from samples collected by student (citizen) scientists and a trained scientist.

In addition to issues concerning the accuracy of particle identification, another challenge involves sieve clogging during sample collection. This dramatically slowed the rate of filtering. Furthermore, samples that clogged had an excess amount of sediment and/or biological material, which could obscure particles and fibers of interest when examined with a microscope. Finally, participants

experienced frustration when operating the dissecting microscope and while reducing sample volume through vacuum filtration.

Activity Assessment Results

To evaluate student knowledge and attitudes about microplastic pollution and access their understanding of scientific concepts, students answered twelve Likert scale questions before and after completing the activity (Figure 6.3). Participants piloting the lab activity were from a suburban private high school and public middle school, as well as an urban charter high school (n = 67). Analysis involved students that completed both the pre- and post-survey questions (n = 41). All survey responses were gathered in compliance with requirements set by the University of Minnesota's Institutional Review Board.

There were measurable changes in students' comprehension and expectation of science and the scientific method when comparing pre- and post-activity survey responses (Figure 6.3). For example, when asked to evaluate the statement, "When a scientist conducts an investigation, they know what they are going to find," *more* students disagree/strongly disagree after completing the activity (19.6 percentage points). Similarly, in reaction to the statement, "If you don't prove your hypothesis, the experiment was a failure," *more* students strongly disagree in the post-lab survey (19.5 percentage points). Finally, in the statement, "Results from a scientific study provide more questions than answers," *more* students strongly agreed after completing the activity (12.2 percentage points).

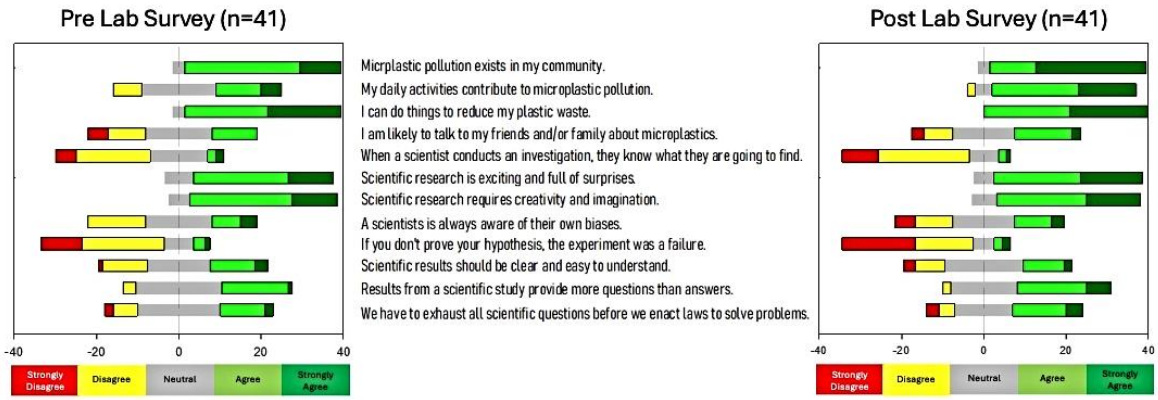

Figure 6.3 Quantitative analysis involved pre and post 12 Likert scale questions (n = 41).

There were large shifts around knowledge and understanding of microplastic pollution. Prior to the activity, most students agreed that microplastics were present in their environment. However, more students strongly agreed to both statements, "Microplastic pollution exists in my community" (41.5 percentage points) and "My daily activities contribute to MP pollution" (46.3 percentage points), after completing the activity. In addition, more students agreed/strongly agreed with the statement, "I can do things to reduce my plastic waste" (7.3 percentage point), while fewer students disagreed/strongly disagreed with the statement, "I'm likely to talk to my friends and/or family about microplastics" (9.8 percentage point) in post-survey answers.

1. What was your favorite part of this activity?
2. What was your least favorite part of this activity?
3. What was the most difficult part of this activity? Why?
4. What part of this activity would you have liked to spend more time on?
5. What surprised you the most about the results?
6. Did you know about microplastic pollution before you studied it in class? If so, how did you first hear about it?

Figure 6.4 Qualitative analysis involved 6 open-ended questions (n=47).

After completing the activity, students (n = 47) answered six short-answer questions (Figure 6.4). In response to question #1, a favorite part of the lab activity was being outside (34%) and collecting samples (28%). Students also enjoyed using the microscope (19%), doing something that felt real and hands on (8.5%), and working with their friends (6.4%). In response to question #2, the least favorite parts of the activity were exposure to bugs/mosquitoes (30%), waiting for turns to use equipment in the classroom (13%), confusing or inconclusive results (8.5%), presenting results to peers (8.5%), and filtering the samples (6.4%). Ten percent of participating students found no fault with the activity. In question #3, students identified using the microscope and distinguishing potential plastic particles from non-synthetic sediments and biota (45%) and filtering (10.6%) as the most difficult components of the activity. In question #4, when asked what part of the activity they would have liked to spend more time on, the most common responses were testing more locations and/or different bodies of water to compare the outcomes (32%) and spending more time outside collecting samples (23%). Students also expressed a desire to gain more confidence in identifying plastic (19%). In question #5, students were surprised by the quantity of microplastics discovered (72%). Of this total, one third expected to find more particles and were surprised at how few they discovered. The rest were expecting fewer and were surprised by the high number of particles discovered. Finally in question #6, 40.4% had prior knowledge of microplastics before participating in the activity, while 59.6% had not heard of microplastics before. Of those who had prior knowledge of microplastics, some students heard about it through the news or the

internet (42%), while others found out about it through classes/coursework (47%).

Discussion

Through this activity, students experience the scientific method first-hand as they collect and process environmental samples, organize data, and interpret results. This work not only promotes cooperation, but it also encourages students to troubleshoot and adapt when faced with minor challenges inherent in science. Furthermore, unexpected findings offer participants the chance to apply critical thinking skills as they analyze the results and generate new research questions. This authentic experience helps students develop an empirical understanding of human error and bias while learning to appreciate the importance of QA/QC measures and the practical limits of scientific studies. The discussion below addresses the most common issues encountered while piloting this study.

One problem students may encounter during water sampling is water turbidity. Productive waters during the summer months and/or the disturbance of organic material and sediment during water sample collection contributes to this issue. Limiting the number of individuals in the water, sampling from the water's edge or from a dock, or by collecting samples before seasonal algal blooms helps mitigate this problem.

Many challenges encountered during sample processing stem from a lack of experience with the laboratory equipment and techniques. For example, students use a pump to draw water samples through cellulose filter paper. However, without practice and refined technique, particles of interest during vacuum filtration pulled around the filter

paper end up in the flask, thus evading counting and analysis. This results in an underestimation of particles. Processing practice samples will improve experimental outcomes, can save time, improve accuracy, reduce frustration, and improve laboratory skills. Practicing techniques reinforce the idea that consistent field and laboratory methods are essential for producing high-quality data.

The detection and identification of plastic particles pose a challenge, even for experienced researchers. Training materials with images and detailed descriptions of known plastic particles before sample analysis will enable students to classify particles and fibers into two categories: strongly microplastics, maybe microplastics. This dimension of the activity offers students a chance to contemplate standardization and bias (De Frond et al., 2022). For example, having students consider common materials that could be mistaken for plastic, such as blue jean fibers, can help them be more aware and discerning (Athey et al., 2020). A discussion focused on selection bias will help them think about how they might curb such bias. For example, two groups may count their own samples, swap with another group and count samples, and then compare results. Another idea is to use a hot needle test to judge whether particles are synthetic (i.e., melts) or natural (i.e., singes or nothing happens). Alternatively, applying dye to a sample that binds to synthetic polymers, or the surrounding matrix can help differentiate microplastic particles from mineral and biological material (Figure 6.5). Encouraging students to articulate uncertainty while processing samples can lead to important discussions about standardization and how high-quality results rely on clear and consistent selection criteria.

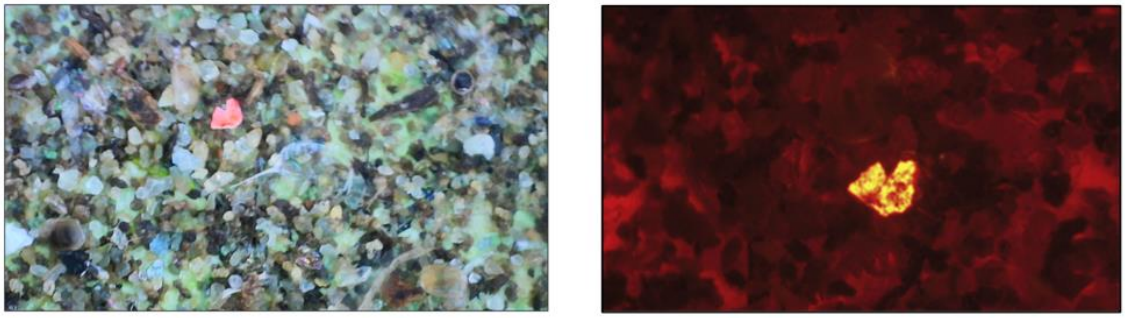

Figure 6.5 Photograph of a sample collected from Cedar Lake Farm Regional Park, Scott County, MN. Sample gathered with fine mesh sieve (106 µm). The sample was photographed using the wi-fi digital microscope included in the sampling kit (left). Fluorescent image of the sample after treatment with wet peroxide oxidation and Nile red staining (right). The brightly fluorescing fragment in the right-hand image is the same as the red fragment visible in the left-hand image.

An accurate count and characterization (i.e., morphology) of suspected plastic particles and fibers are linked to limits in study design and QA/QC measures. Giving students an opportunity to consider the types of plastics that may go uncounted will help them see potential gaps in the SOP. Certain colors, such as white or transparent, might go unnoticed. Students should also think about the magnifying power of the microscope they are using. Furthermore, certain size fractions, such as those smaller than the pores on the smallest sieve (106 µm), may also go uncaptured and uncounted. Finally, it is important to discuss how field, laboratory, and reagent blanks serve to quantify background contamination. For example, students should be asked how particles recovered in blanks should be incorporated into the data. They should also consider the consequences of carrying out a study without knowledge of background levels. They can also think about the possible sources of contaminants during sample collection and processing and how to safeguard against the unwitting introduction of

particles. Reflecting on these questions helps students understand the practical limitations and uncertainties inherent in science.

As evidenced by the qualitative survey responses, the challenges described above, such as distinguishing potential plastic particles from non-synthetic sediments and biota, led to frustration among participants. However, these experiences present authentic problems common in science, creating opportunities for students to engage their curiosity and find creative solutions. Such encounters foster discussions about the benefits and limits of science, highlighting the exploratory and iterative nature of research, where establishing new tools for microplastic detection is an active area of technological development. Additionally, hard-won discoveries can deepen awe and excitement.

An inconsistency in qualitative and quantitative survey answers may indicate a need to modify the survey format. For example, nearly 60% of students in the post activity qualitative questions said they had not heard of microplastics before participating in the lab, but the pre-lab quantitative question revealed nearly all participants agreed or strongly agreed with the statement, "microplastic pollution exists in my community." This may indicate the Likert scale question was a leading question and should be modified.

Regardless, measurable but modest shifts in knowledge and attitudes were evident in the quantitative pre/post surveys. It is interesting that there were slight positive shifts in the recognition of microplastics in local water bodies, in acknowledging a personal contribution to the problem, and the expression of self-efficacy around the issue, but there was no shift in their likelihood to talk about the

issue with friends and family. This could stem from a lack of confidence about their knowledge or a perception of controversy over the issue. Individuals may also feel the problem lacks urgency since half of the students were surprised at how *few* microplastics were discovered. Students might not see it as a priority topic as other issues are more compelling for discussion with friends and family. Finally, the schools involved in piloting the program drew from an urban/suburban population. Testing this program in schools with different demographic profiles may yield different results, especially since the breadth of media students consume and the type of educational programming they receive can vary dramatically.

Conclusions

Microplastics are pervasive global contaminants, and their ubiquity could serve to unite and integrate geographically disparate communities through this citizen science activity. A multitude of stand-alone exploratory experiments exist for secondary students, but this project offers a unique opportunity to connect individuals to a larger, regional network bound in purpose to track contaminants and discuss solutions based on findings. Over time, educators in a single area revisiting the lab seasonally or annually will generate a wealth of longitudinal data. Similarly, educators partnering with other regional schools for geographic comparisons could yield interesting results. Even as a stand-alone activity, the program gives students first-hand experience in generating scientific results while also allowing them to consider how data are produced and reported and how findings shape the behavior, attitudes, and policies in their local communities.

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Chapter 7: Conclusion

Plastic incrementally found its way into the modern world with promises of luxury and convenience. On the cover of a 1955 issue of LIFE Magazine, an iconic photo of a young family tossing disposable plates and cutlery into the air actively promotes 'Throwaway Living.' The caption informs readers that cleaning these items would take a housewife 40 hours, but now with the advent of disposable plastics, she was free from this drudgery. Attracted to visions of easy living and excited by the possibilities that polymer chemistry had to offer, many openly embraced this new material. However, some Americans having lived through scarcity and rationing during the Great Depression and WWII had to *learn* how to throw products away after a single use (Davis, 2024).

Whether received with enthusiasm or reluctance, plastic products began to slowly and fully incorporate into a contemporary landscape, replacing ivory piano keys, metal lighters, tortoise shell combs, and paper bags. They also introduced exciting inventions the world had never seen, such as the artificial heart, space suits, Etch A Sketch, and Legos. The transformation was so seamless and incremental there was no conspicuous occasion prompting society to evaluate or even consider the long-term consequence of mass-producing materials from non-renewable resources that would then be incapable of mineralization. The process is far from complete, as this industry is expected to continue expanding. For example, the global medical plastics market was 25.1 billion US dollars (USD) in 2020 (Amato-Lourenço et al., 2020) and it is projected to reach 50 billion USD by 2030 (Medical Plastics - Global Strategic Business Report, 2025).

Today, the benefits of plastic remain abundantly clear. However, these advantages must now be weighed against a growing body of scientific evidence that highlights the costs involved in attempting to meet so many human needs and desires with synthetic polymers. From a design standpoint, a drawback to plastic is its sustained structural integrity. Since plastic packaging with the shortest lifespan makes up nearly 40% of global production (Plastic Europe, 2023), this results in an accumulation of durable solid waste in the environment. The latest estimates put total annual environmental release of plastic at 10–40 Mt (Thompson et al., 2024).

Given these numbers, perhaps it is no surprise that litter was the primary force that initially shifted the public's perception of plastic. Much of this was driven by images of marine life fatally ensnared in ghost nets (Chelliah et al., 2024) and beach trash. In one report, a young Cuvier's beaked whale is mortally wounded by 88 pounds of plastic refuse impacting its stomach (Borunda, 2019). In another story, a marine biologist extracts a plastic straw from the bloodied nose of an olive ridley sea turtle near the shores of Costa Rica. The viral YouTube video of this wildlife intervention garnered an impressive 110 million views online (Figgener, 2015). Such graphic and distressing testimonials directly from the animal kingdom are incredibly potent in their ability to influence public opinion. While some 1,300 terrestrial and marine species are catalogued as having microplastics in their bodies (Thompson et al., 2024), there is a single animal among them that captures the most attention and generates the most distress. That animal is *Homo sapiens*.

There is now incontrovertible proof that microplastics are inside human bodies, but the total count, mass, variety, size, morphology, distribution, and elimination, based on routes of exposure and physiological pathways, are vaguely understood. On a population level, occupational exposure and impact on vulnerable groups, such as those with sensitivities or comorbidities, must also be considered. For example, microplastics have been isolated from the sputum of individuals experiencing respiratory disease (Huang et al., 2022), inflammatory bowel disease (Yan et al., 2022), and patients with cirrhotic liver (Horvatits et al., 2022b), but no clear causal relationship can be made between the disease and the exposure. Despite a collective desire and a keen sense of urgency to understand the hazards and health outcomes associated with microplastics exposure in humans, the scientific process is inherently meticulous and methodical, working on a protracted timeline. Furthermore, given the heterogeneous nature of this contaminant and variety of methods in which it can be studied, these efforts will likely result in a cumulative mass of variable and nuanced findings.

A significant challenge around this discipline lies in the fact that microplastics have seized the public's attention all while the most pressing health questions remain unanswered. A combination of scientific uncertainty and intense interest from non-scientists can create complications because it invites *all* members of the public, including lawmakers, environmentalists, industrialists, and concerned citizens to experience the scientific process in real time, with its inevitable twists and turns. Of course, if the public is intimately familiar with the scientific process, including peer review, a healthy

amount of internal debate, holding reasonable expectations about the limitations and certainty in findings, and some degree of scientific jargon, then they will not be vulnerable to misinformation or manipulation from bad-faith actors. However, past misunderstandings about the scientific process combined with the public's expectations that all scientific results are final and absolute, have resulted in confusion at best, and baseless conspiratorial suspicion at worst. This was seen in the science of climate change (McKie, 2019) and during the COVID-19 global pandemic (Andersen, 2024).

To illustrate this point, there is a particular pitfall to be mindful of in the discipline of microplastics research, and that is the emergence of 'unicorn' publications. These are works that receive an outsized amount of media attention when they are first released. However, as the overall body of knowledge matures, some of these works lose saliency within the scientific community, but to the public such findings endure. A good example of this is the claim that humans consume one credit cards' worth of plastic each week (Senathirajah et al., 2021). Another example is the claim that humans eat 50 plastic bags each year (Bai et al., 2022). No publications have been able to corroborate these findings. On the contrary, some papers actively refute these claims (Pletz, 2022). While such unicorns are relegated to mere horses within scientific circles, they can stubbornly persist in the public's mind. While it is safe to assume that deception is never the intent of any author, this serves as a good reminder that a careful balance must be struck between making scientific findings relatable without overstating, especially when communicating to a non-science audience.

Concerns about distorting science should not be dismissed. Concerns also should not eclipse the incredible opportunity provided by the public's willingness to engage in issues concerning human and environmental health. In a highly competitive attention economy, genuine curiosity and a desire to be informed of public health issues should be regarded as a precious resource that should neither be squandered nor treated recklessly. Instead, it is a chance to engage members of society in larger conversation about the utility of science and how it can be meaningfully applied to affect policy change. Results can foster self-efficacy for community members who are struggling with concern about exposure. They can also encourage innovation and practices that bring society to a more sustainable, circular economy.

A search with keyword 'microplastics' on Ovid Medline now reveals over 17,000 publications (Ovid MEDLINE, 2025). While research in this field is growing in bulk it is also expanding in breadth, through the integration of multiple disciplines. Given this wide landscape, this dissertation seeks to intersect the problem of microplastic pollution at multiple, discrete points. First, through methods. Science is dynamic by nature and methods are in constant need of maintenance and upgrades. Continuously improving methods obligates those who actively conduct research to use best practices. This work also inspires others to innovate by improving old techniques, developing new techniques, or applying known tools and in novel ways. Above all, it is crucial that methods uphold accuracy through the consistent application of quality assurance and quality control measures, as findings are only as powerful as methods are strong. This dissertation's second point of contact with microplastic research involves atmospheric fate and

transport. This work further clarifies the relationship between environmental conditions and the abundance, shape, and size of contaminant. With the help of this and other research, more accurate models can be constructed to predict particle fallout and entrainment on a global scale. The third point of contact involves direct human exposure. This work not only adds to a growing list of potential sources, it also examines for the first time a change in exposure that comes from using durable products. It may even provide the necessary feedback loop for manufacturers who want to create the best performing and safest products possible. The fourth and final point of contact involves public engagement through a citizen science program. This work will not only collect critical state-wide baseline contaminant levels that will inform understanding of its flow and fluctuations through time, it will also elevate science literacy and critical thinking while offering participants an opportunity to exercise agency. Finally, this project fosters an essential connection and investment by community members in the health of local watersheds.

The sheer scale of the global environmental issues of plastic pollution can be overwhelming to the public. It can cause people to pull back and become less engaged and informed as a means of protecting themselves from feeling powerless and trapped in systems that work against their values. One reason single-use plastics were able to proliferate for so long without criticism was the promise of recycling. Consumers concerned about the amount of waste they were generating through their purchase of single-use plastic had their conscience eased by the idea that they could simply recycle the cartons, bags, and bottles moving in and out of their homes. These items in North American

per capita total 139 kg/year, or 0.39 kg/day (Heller et al., 2020). This means a family of four is tossing out roughly 1.5 pounds of plastic each day.

Although many believe these materials are being recycled, some recent stories are shaking the public's confidence. One media organization fitted 47 plastic bags with trackers and then dropped them off at store repositories for recycling. The investigative reporters found most of the trackers ended up in incinerators and landfills. Two of them ended up in Malaysia and one ended up in Indonesia (Knowles & Pistone, 2023). Others have identified practical problems with recycling, especially around effective sorting, cost, and feasibility. In the U.S. the proportion of plastic items that can be converted into new materials is about 5% (Sullivan, 2022). Many are surprised to learn how low this amount is. In fact, a 2023 survey found 93% of Americans overestimate the amount of plastic that is recycled (Turek, 2024). This speaks to the effectiveness of marketing the idea of recycling. It also prevents actions to reduce production, improve product design, promote non-plastic substitutes, and improved waste management.

Some of these problems can be addressed through policy and regulatory measures. Furthermore, recent surveys show the public would likely support such policies. For example, a poll conducted by Oceana in 2022, reported 81% of Americans support national, regional, and local policies aimed to reduce single-use plastic. This concern about plastic pollution includes 93% of Democrats and 78% of Republicans, which is a staggering amount of bipartisan support (Valliant & Cranor, 2022). However, environmental and health policy is most effective when it is informed by reliable scientific findings brought by scientists

who possess a good perspective on the total body of work, and have the technical expertise required to understand the methods and limits in study design. By 2019, evidence of this pollutant's pervasiveness in seas and oceans was sufficient to motivate the United Nations Environment Assembly to adopt four resolutions involving marine litter and microplastics (United Nations Environment Programme, 2019).

While these actions demonstrate concern for one of the four Global Commons defined by the UN, it is still confined to large bodies of water. Also, given the number of chemical additives, sorbed environmental pollutants, and uncertainty about exposures to any one or combination of chemicals, there are many unknowns in the research that may persist for years if not decades. For this reason, it is an opportunity to apply the precautionary principle. This principle as defined in the 1992 Rio Declaration (BOARD, 1992), that when a threat is deemed menacing enough, full scientific consensus is not needed before preventative action is taken. This is also expertly described in a quote by epidemiologist, Sir Austin Bradford Hill (1965):

> *"All scientific work is incomplete – whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge that we already have, or to postpone the action that it appears to demand at a given time."*

Environmental justice is the final issue that must be addressed. It is at the very end of this dissertation, not because it is the least important, but because it is the most important. From manufacture to disposal, there are marginalized communities disproportionately burdened by the production and disposal of plastic. While the true cost

of plastic is beginning to emerge in the general public's consciousness, residents of 'fenceline' communities have long understood the price. This starts with the extraction of raw materials. A glut of natural gas borne by the Marcellus Shale region of North America has made the U.S. the top exporter of ethane since 2018 (Sicotte, 2020) and it is well known that those living in close proximity to hydraulic fracturing wells have a higher risk for negative health outcomes (Clough, 2018). Also, as societies transition to renewable energy sources, the decline in fossil fuel energy demand is projected to bolster future plastic production, which will only increase demand for raw ingredients that serve as building blocks for plastic (IEA, 2018). There are also risks associated with transportation. Most recently, in February of 2023, a train derailment in East Palestine, Ohio spilled 1.1 million pounds of vinyl chloride, a key ingredient in polyvinyl chloride. It is also a toxic gas associated with cancer of the liver, lung, and brain (Sun et al., 2023). The manufacturing of plastic products also exacts a toll on human health, mostly from occupational exposures (Azoulay et al., 2019; Landrigan et al., 2023), such as individuals working in textile factories or those handling toxic solvents. Finally, while it is understudied, there are growing human and environmental health concerns that stem from burning and burying plastic waste at the end of its life cycle. Most of these concerns stem from open-burning practices (Pathak et al., 2024), which occur less frequently in the United States. Still, emissions that come from the incineration of plastic garbage, with their associated chemical additives and comprise a growing proportion of solid waste, is

understudied. Finally, these areas are often home to low-income and/or disenfranchised community members (Patnaik et al., 2020).

Plastic does not remain inside national borders. The global plastics trade is robust. If it were a single nation, it would be the fourth largest exporter in the world, exporting $1.2 trillion in goods. This 'nation' would only be exceeded by China, the United States, and Germany (UNCTAD, 2022). What is important about these figures is that it includes the total life cycle of plastic, and plastic waste is sold as a commodity on the international market. Together, developed nations export plastic waste which accounts for almost 80% of the total global plastic trade in 2021 (UNCTAD, 2022). This is concerning as many of the nations receiving plastic waste lack the equipment and safety measures to handle it without creating further environmental harm. The practice of exporting waste from more affluent to less affluent nations is known as 'waste colonialism' and it is the act of externalizing harm and creating environmental injustice on an international scale. Residents of the world's sacrifice zones have been working to combat these practices. Members of these communities pay the cost of plastics production that has been externalized by industry and permitted by the rest of society. Addressing this issue will require action from the governments of importing nations, exporting nations, industries, and citizens together.

Microplastics research takes place mainly in the Global North. According to a scientometric analysis conducted by (M. Li et al., 2022b), the ten nations that produce the most microplastics publications are: China (20.23%), USA (15.22%), Germany (10.62%),

England (8.32%), Italy (7.14%), France (6.58%), Spain (5.99%), Netherlands (4.63%), Australia (4.56%), Canada (4.32%). Many researchers have pointed out the dearth of knowledge about microplastic contamination in the Global South (Luo et al., 2022; Munyaneza et al., 2022b; A. Xu et al., 2022). Seeing as how this contaminant knows no political boundaries it stands to reason that it ought to be studied in every nation. In fact, an argument can be made that there is a greater need for research in nations that are on the receiving end of waste colonialism.

In closing, Richard C. Tompson, who originally coined the term 'microplastics' in a 2004 publication, wrote a reflection on the past 20 years of this discipline. In this most recent publication he states 'microplastic pollution is the consequence of human decisions and actions, and understanding these social dynamics is key to designing effective solutions' (Thompson et al., 2024). The number of places where microplastics can be found on planet Earth is finite. For this reason, discovery papers are winding down. However, this could mark another pivotal moment, or significant shift in the discipline, where more research is dedicated to finding real and feasible solutions to this problem.

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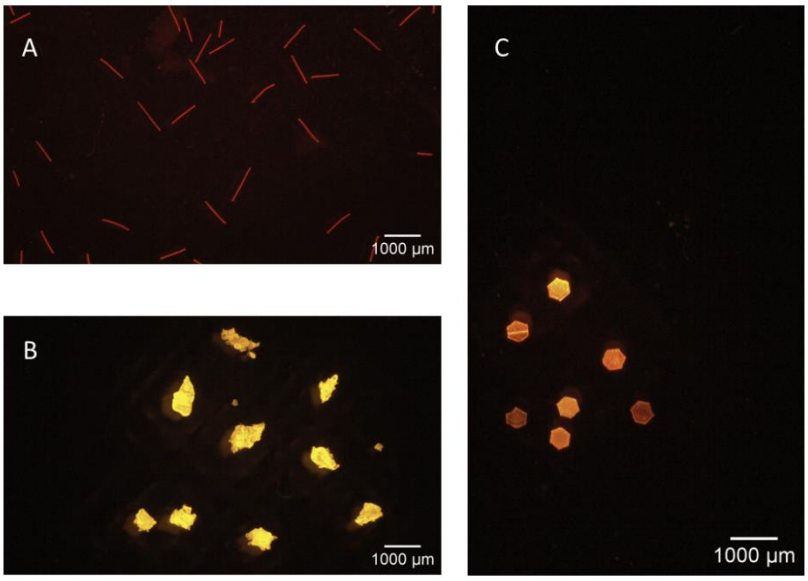
Appendices

Chapter 3



Chapter 3 Figure 1. A) Nylon fibers manufactured for an approximate uniform length of 1,000 µm and width of 19.3 µm, B) Irregular polyethylene fragments isolated from cosmetic products, variable in size and dimension, C) Symmetrical polyester fragment manufactured with a 500 µm diameter surface and a 76 µm depth.

Water Source	% Fiber	% Particle	Mean Fiber Size	Mean Particle Size
TAP	54.7%	45.3%	435	165
DI	56.8%	43.2%	385	162
RO	65%	35%	380	183
NP	50%	50%	341	112
HPLC	62.5%	37.5%	463	185

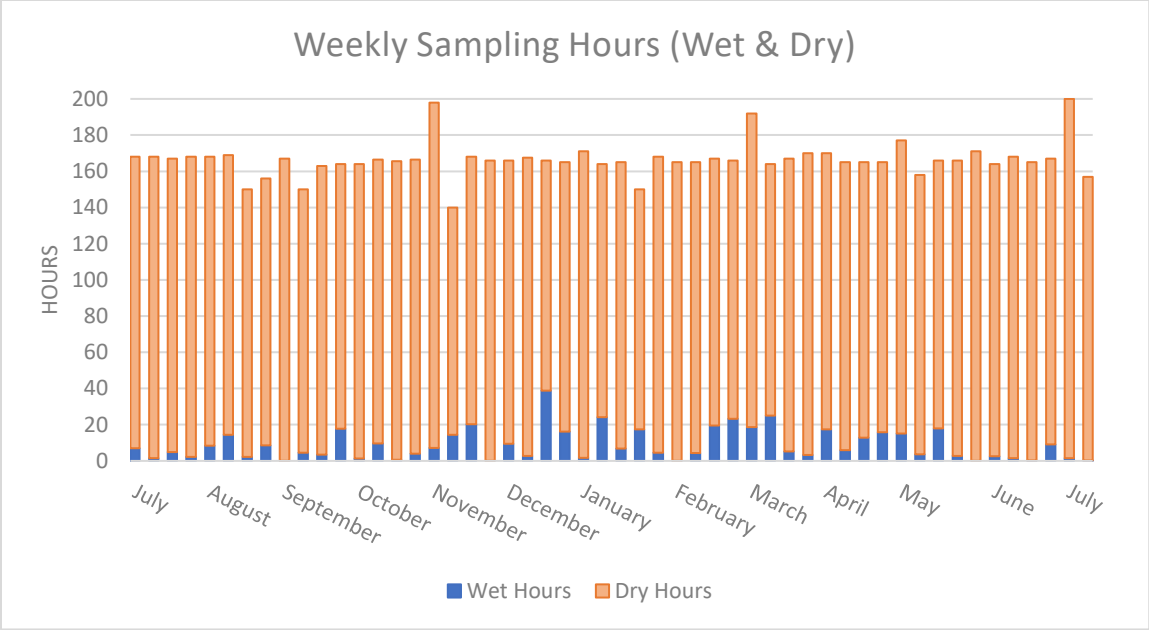
Chapter 3 Table 1. Morphology and size (µm) of contaminants in lab waters.

Dates	Wet Hrs.	Dry Hrs.	Particle Count	Fiber Count	Particle Flux	Fiber Flux	Total Flux
7/7 - 7/14		161.0	8	28	0.727	2.55	3.27
	7.1		21	15	43.6	31.1	74.7
7/14 - 7/21		166.6	4	90	0.351	7.91	8.26
	1.5		8	18	81	182	262
7/21 - 7/28		162.1	22	11	1.99	0.993	2.98
	4.9		43	84	128	251	379
7/28 - 8/4		165.9	21	26	1.85	2.29	4.15
	2.2		18	36	122	245	367
8/4 - 8/11		159.7	55	19	5.04	1.74	6.78
	8.4		19	30	33.3	52.6	85.9
8/11 - 8/18		154.5	34	23	3.22	2.18	5.40
	14.5		34	34	34.3	34.3	68.6
8/18 - 8/24		147.7	4	20	0.396	1.98	2.38
	2.3		6	7	38.2	44.5	82.7
8/24 - 8/31		147.4	22	29	2.18	2.88	5.06
	8.6		-1	49	-1.70	83.4	81.7
8/31 - 9/7		166.9	6	54	0.526	4.73	5.26
	0.1		0	5	0	732	732
9/7 - 9/13		145.5	8	48	0.804	4.83	5.63
	4.5		3	25	9.75	81.3	91.0
9/13 - 9/20		159.6	7	20	0.642	1.83	2.48
	3.4		29	57	125	245	370
9/20 - 9/27		146.3	18	28	1.80	2.80	4.60
	17.7		24	38	19.8	31.4	51.2
10/3 - 10/10		162.7	61	70	5.49	6.29	11.8
	1.3		9	13	101	146	248
10/10 - 10/17		156.9	11	50	1.03	4.66	5.69
	9.6		23	24	35.1	36.6	71.6
10/17 - 10/24		164.9	15	31	1.33	2.75	4.08
	0.6		1	23	24.4	561	585
10/24 - 10/31		162.5	7	58	0.630	5.22	5.85
	4		9	9	32.9	32.9	65.8
10/31 - 11/8		190.9	22	14	1.69	1.07	2.76
	7.1		8	8	16.5	16.5	33.0

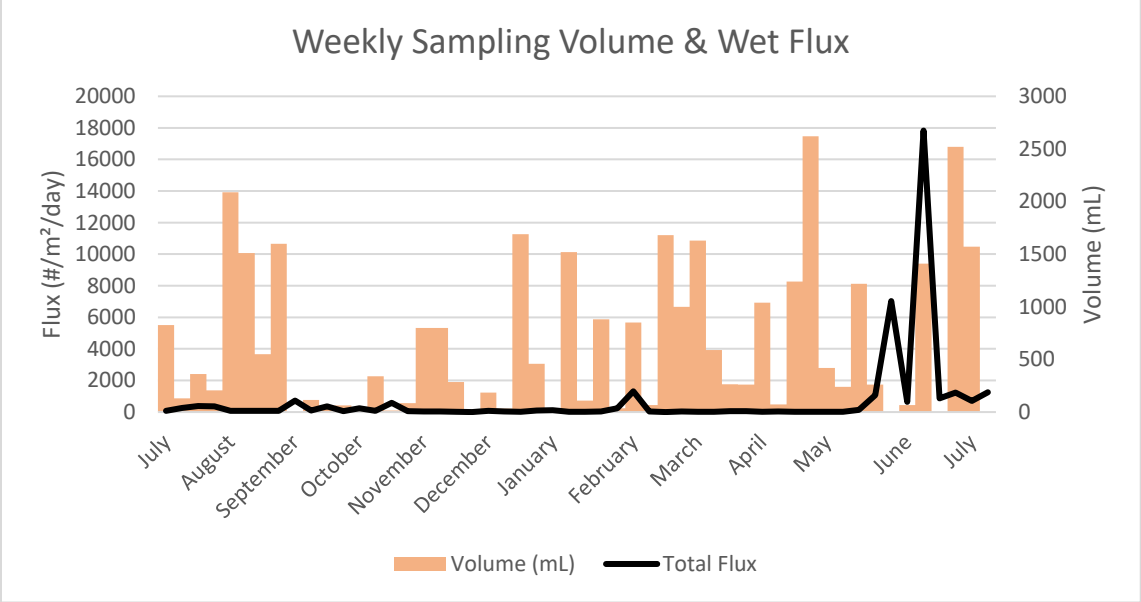
11/8 - 11/14		125.6	42	14	4.89	1.63	6.52
	14.4		24	12	24.4	12.2	36.6
11/14 - 11/21		147.7	52	45	5.15	4.46	9.61
	20.3		6	7	4.32	5.05	9.37
11/21 - 11/28		165.9	18	7	1.59	0.62	2.20
	0.1		-1	0	-146	0	-146
11/28 - 12/5		156.5	82	12	7.67	1.12	8.79
	9.5		38	9	58.5	13.9	72.4
12/5 - 12/12		164.8	48	9	4.26	0.799	5.06
	2.7		8	0	43.3	0.0	43.3
12/12 - 12/19		127.2	45	12	5.18	1.38	6.56
	38.8		38	9	14.3	3.39	17.7
12/19 - 12/26		148.8	29	5	2.85	0.492	3.34
	16.2		102	12	92.1	10.8	103
12/26 - 1/2		169.3	15	12	1.30	1.04	2.33
	1.7		10	3	86.1	25.8	112
1/2 - 1/9		139.8	22	9	2.30	0.942	3.24
	24.2		15	7	9.07	4.23	13.3
1/9 - 1/16		158.2	75	2	6.94	0.185	7.12
	6.8		0	5	0.000	10.8	10.8
1/16 - 1/22		132.5	58	3	6.40	0.331	6.74
	17.5		38	1	31.8	0.836	32.6
1/22 - 1/29		163.5	34	22	3.04	1.97	5.01
	4.5		65	8	211	26.0	237
1/29 - 2/5		164.9	11	4	0.976	0.355	1.33
	0.1		9	0	1317	0	1317
2/5 - 2/12		160.6	27	18	2.46	1.64	4.10
	4.4		2	6	6.65	20.0	26.6
2/12 - 2/19		147.4	3	8	0.298	0.794	1.09
	19.6		1	0	0.746	0	0.746
2/19 - 2/26		142.7	10	23	1.03	2.36	3.38
	23.3		24	33	15.1	20.7	35.8
2/26 - 3/6		173.3	5	5	0.422	0.422	0.844
	18.7		4	8	3.13	6.26	9.39
3/6 - 3/13		138.9	25	1	2.63	0.105	2.74
	25.1		22	4	12.8	2.33	15.2
3/13 - 3/20		161.7	95	9	8.60	0.814	9.41
	5.3		15	3	41.4	8.28	49.7
3/20 - 3/27		166.7	22	15	1.93	1.32	3.25
	3.3		10	1	44.3	4.43	48.8
3/27 - 4/3		152.6	76	16	7.29	1.53	8.82

	17.4		6	7	5.05	5.89	10.9
4/3 - 4/10		159.0	1	1	0.092	0.092	0.184
	6		6	5	14.6	12.2	26.8
4/10 - 4/17		152.2	69	13	6.63	1.25	7.88
	12.8		8	12	9.14	13.7	22.9
4/17 - 4/24		149.1	53	44	5.20	4.32	9.52
	15.9		9	8	8.28	7.36	15.6
4/24 - 5/1		161.8	14	8	1.27	0.723	1.99
	15.2		8	6	7.70	5.78	13.5
5/1 - 5/8		154.4	7	7	0.663	0.663	1.33
	3.6		3	2	12.2	8.13	20.3
5/8 - 5/15		148.0	793	79	78.4	7.81	86.2
	18		124	37	101	30.1	131
5/15 - 5/22		163.3	733	126	65.7	11.3	77.0
	2.7		86	112	466	607	1073
5/22 - 5/29		170.9	127	50	10.9	4.28	15.2
	0.1		36	12	5267	1756	7023
5/29 - 6/5		161.4	2548	101	231	9.16	240
	2.6		87	28	490	158	647
6/5 - 6/12		166.4	5282	247	464	21.7	486
	1.6		1772	178	16203	1628	17831
6/12 - 6/19		164.7	1038	151	92.2	13.4	106
	0.3		12	6	585	293	878
6/19 - 6/26		157.8	425	134	39.4	12.4	51.8
	9.2		711	59	1131	93.8	1225
6/26 - 7/4		198.4	75	180	5.53	13.3	18.8
	1.6		31	47	283	430	713
7/4 - 7/10		156.8	227	209	21.2	19.5	40.7
	0.2		4	13	293	951	1244

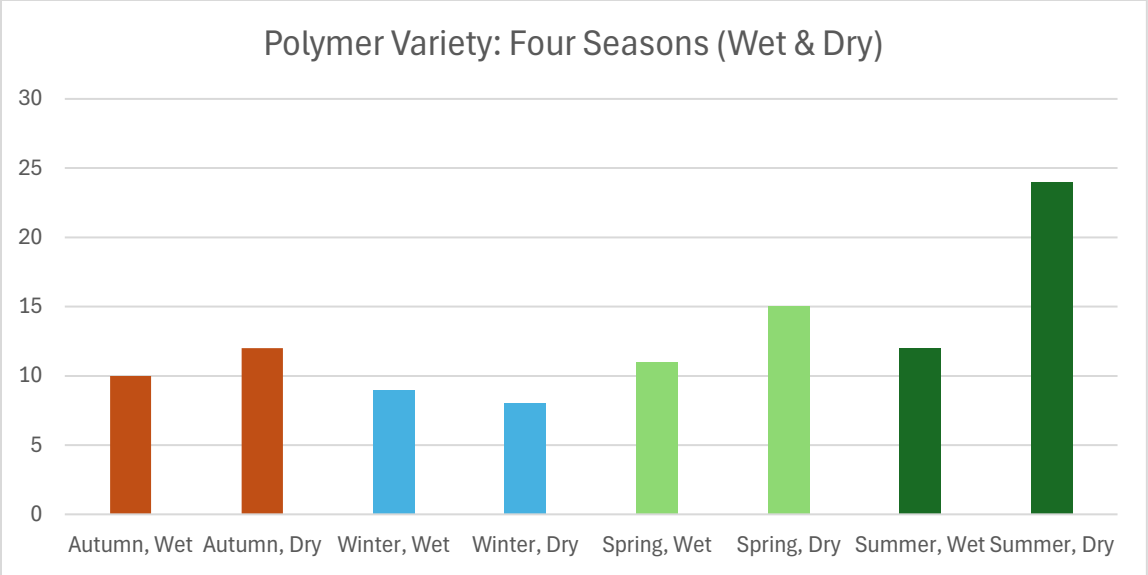
Chapter 4 Table 1. Raw data tables include wet and dry hours, particle counts, fiber counts, particle flux, fiber flux, and total flux from July 7, 2022 to July 10, 2023.



Chapter 4 Figure 1. Weekly sampling hours (wet & dry).

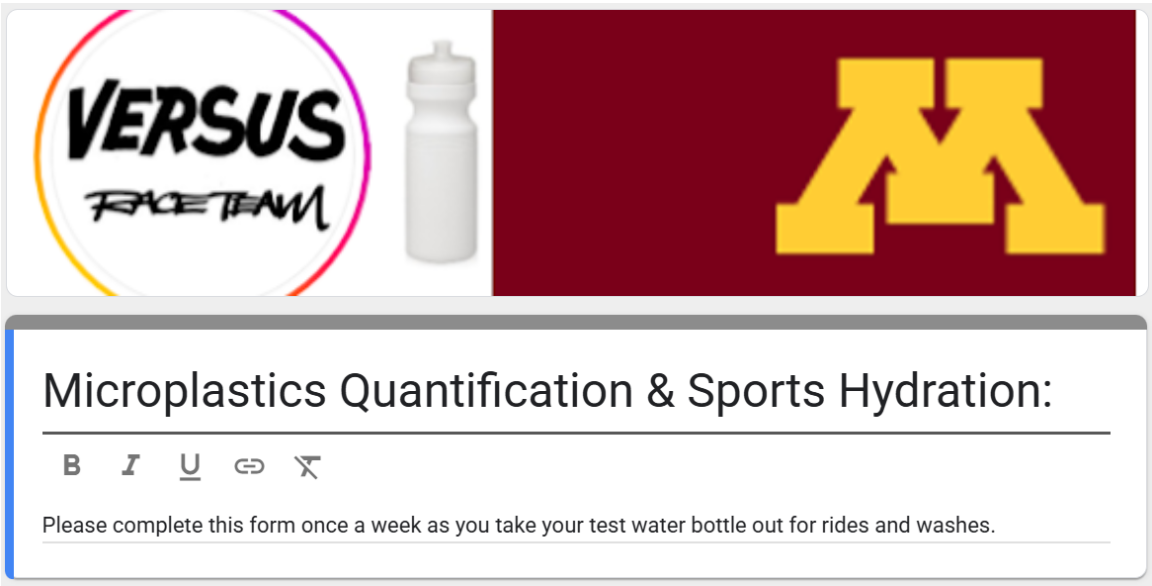


Chapter 4 Figure 2. Weekly sampling volume and wet flux.



Chapter 4 Figure 3. FTIR summary results, polymer variety across four seasons (wet and dry fallout).

Chapter 5



Please enter the name of your test bottle. *

Short answer text

How many hours this week did you ride with the test bottle? Round to the nearest integer. *

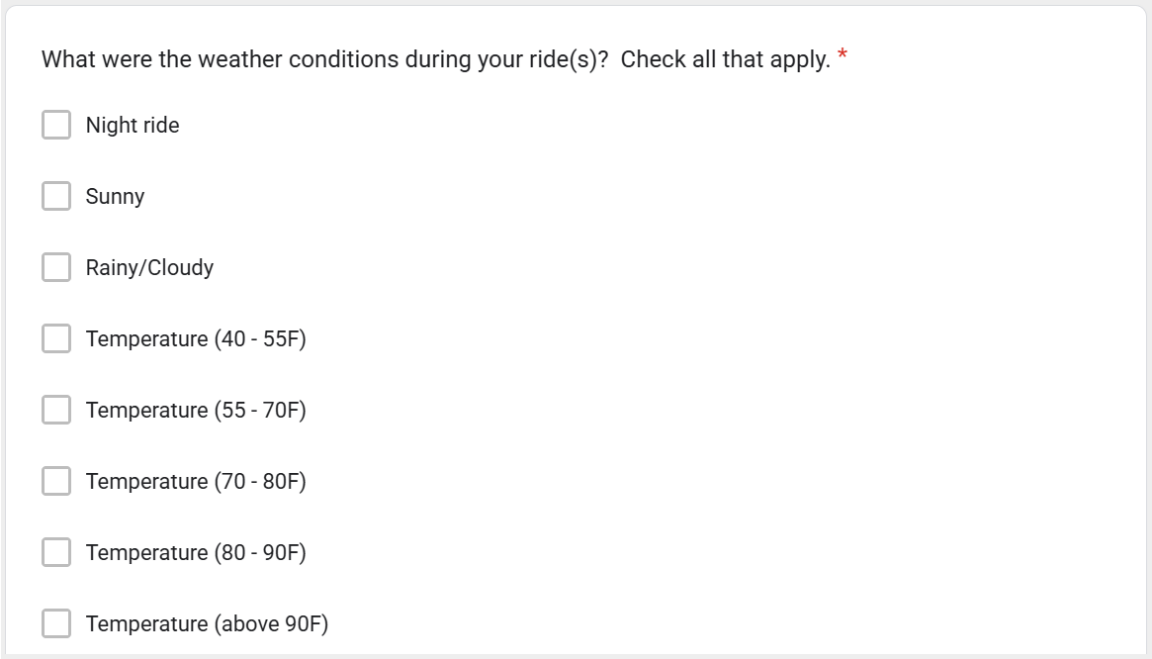
Short answer text

What did you ride on? *

- [ ] Pavement
- [ ] Gravel
- [ ] Trail
- [ ] Trainer
- [ ] Other...

After the ride(s)... *

- [ ] The bottle was hand washed.
- [ ] The bottle was washed in a dishwasher.
- [ ] The bottle was ONLY rinsed with tap water.
- [ ] The bottle was not washed nor rinsed.
- [ ] Other...



Chapter 5 Figure 1. Captured images of the bottle use and care survey completed weekly by members of Versus cycling team.

Test Bottle (Particles)	IR Identification (Open Specy Library)	% Match
Plastic A-1	honeycomb middle wall (organic matter)	65%
	3-pentatone	63%
	ethylene vinyl acetate (polyethylene)	64%
	alkyd resin (polyhaloolefins vinylhalides)	68%
Plastic A-2	fur mohair angora goat (organic matter)	70%
	recent pollen2 olea (organic matter)	65%
	polyurethanes isocyanates (paint)	65%
Plastic A-3	poly(ethylene)	66%
	empty well (mineral)	60%
	alkyd resin (polyhaloolefins vinylhalides)	66%
Plastic B-1	keratin peacock feather (organic matter)	81%
	cellulose (organic matter)	84%
	cotton (organic matter)	81%
Plastic B-2	cellulose (organic matter)	84%
	poly(vinyl chloride)	73%
	cotton (organic matter)	81%
	polyamide	84%
Plastic B-3	polycarbonate	79%
	empty well (mineral)	59%
	poly(propylene)	81%
Plastic C-1	wool cashmere Kazakhstan (organic matter)	79%
	crangon chitin exuvie (organic matter)	64%
	poly(acrylamide)	75%
	poly(ethylene)	83%

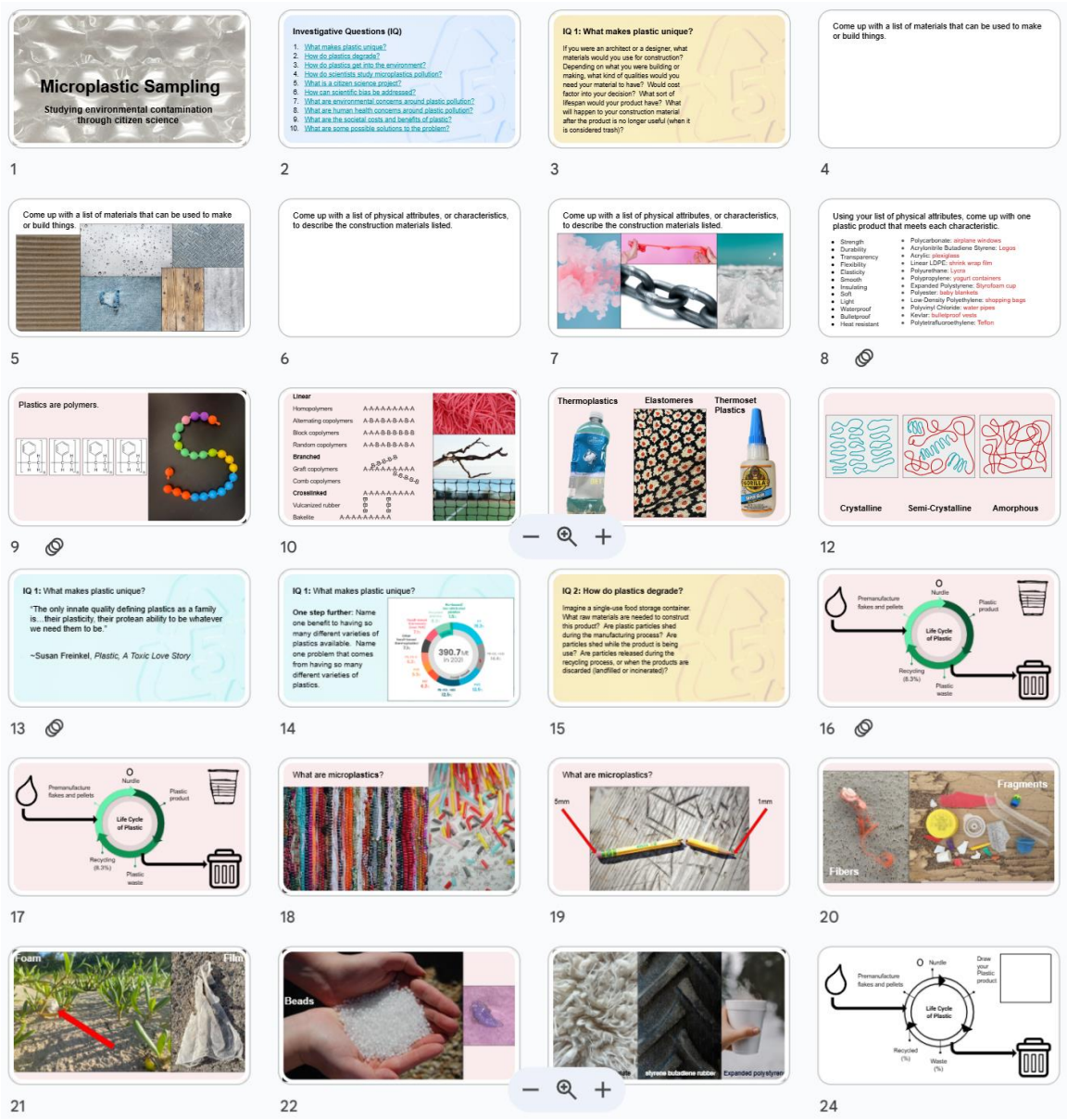
	poly(ethylene)	87%
Plastic C-2	poly(ethylene)	76%
	poly(ethylene)	85%
	poly(ethylene)	86%
	poly (ethylene) eva	71%
Plastic C-3	low density polyethylene	75%
	ethyl acetate (mineral)	46%
	poly(ether) keytones	59%
	low density polyethylene	66%
Metal A-1	zircon (mineral)	71%
	fluorite (mineral)	66%
	fluorite (mineral)	74%
	cotton (organic matter)	68%
Metal A-2	empty well (mineral)	59%
	1-bromo-3-chloropropane (mineral)	63%
	empty well (mineral)	73%
	scoured wool not made rough (organic matter)	82%
Metal A-3	2-bromopropane (mineral)	65%
	fluorite (mineral)	74%
	empty well (mineral)	65%
	keratin peacock feather (organic matter)	75%
Metal B-1	Ethylene vinyl acetate (polyethylene)	77%
	black tennis racket grip (polyurethane)	76%
	empty well (mineral)	56%
Metal B-1	coal (mineral)	63%
	polycarbonate	53%
	butadiene isoprene (natural rubber)	52%
	acronal (mineral)	66%
	kolophonium-rosin (mineral)	68%
Metal B-1	coal (mineral)	72%
	coal (mineral)	68%

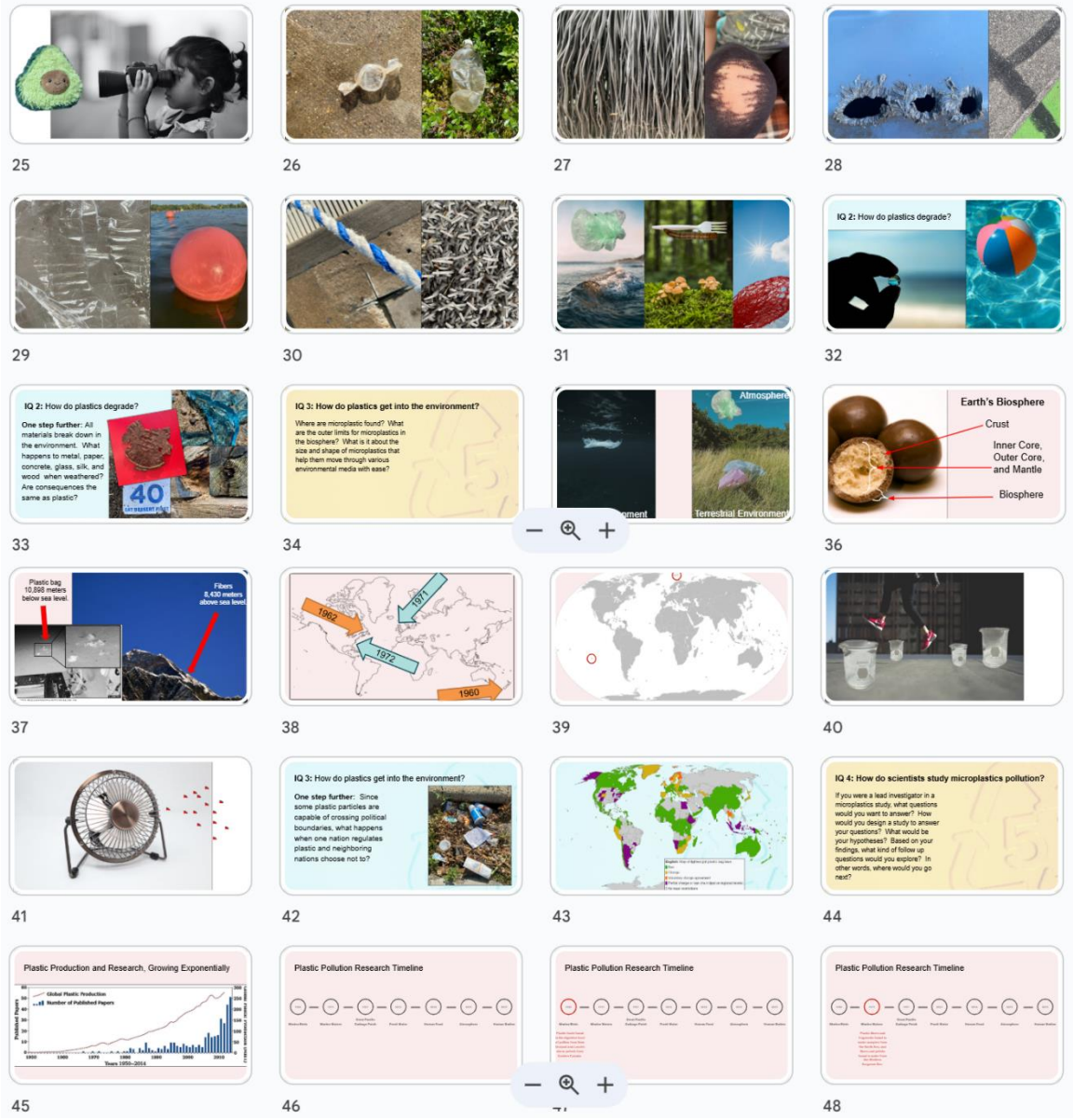
Test Bottle (Components)	**IR Identification (Open Specy Library)**	**% Match**
Plastic A Lid	poly(ethylene) low density PE	77%
Plastic A Lid	poly(ethylene)	74%
Plastic A Nozzle	poly(ethylene), paraffin	86%
Plastic A Body	poly(ethylene) low density PE	71%
Plastic B Lid	polycarbonate	61%
Plastid B Straw	polysiloxanes (silicone rubber)	80%
Plastic B Body	poly(propylene)	94%
Plastic B Body	poly(propylene)	91%
Plastic C Nozzle	l-glutamic_acid_sigma (mineral)	56%
Plastic C Body	poly(ethylene)	86%
Plastic C Body	poly(ethylene)	81%
Plastic C Body	poly(ethylene) low density	88%
Metal A Lid	polypropylene	92%
Metal A Nozzle	l-glutamic_acid_sigma (mineral)	66%

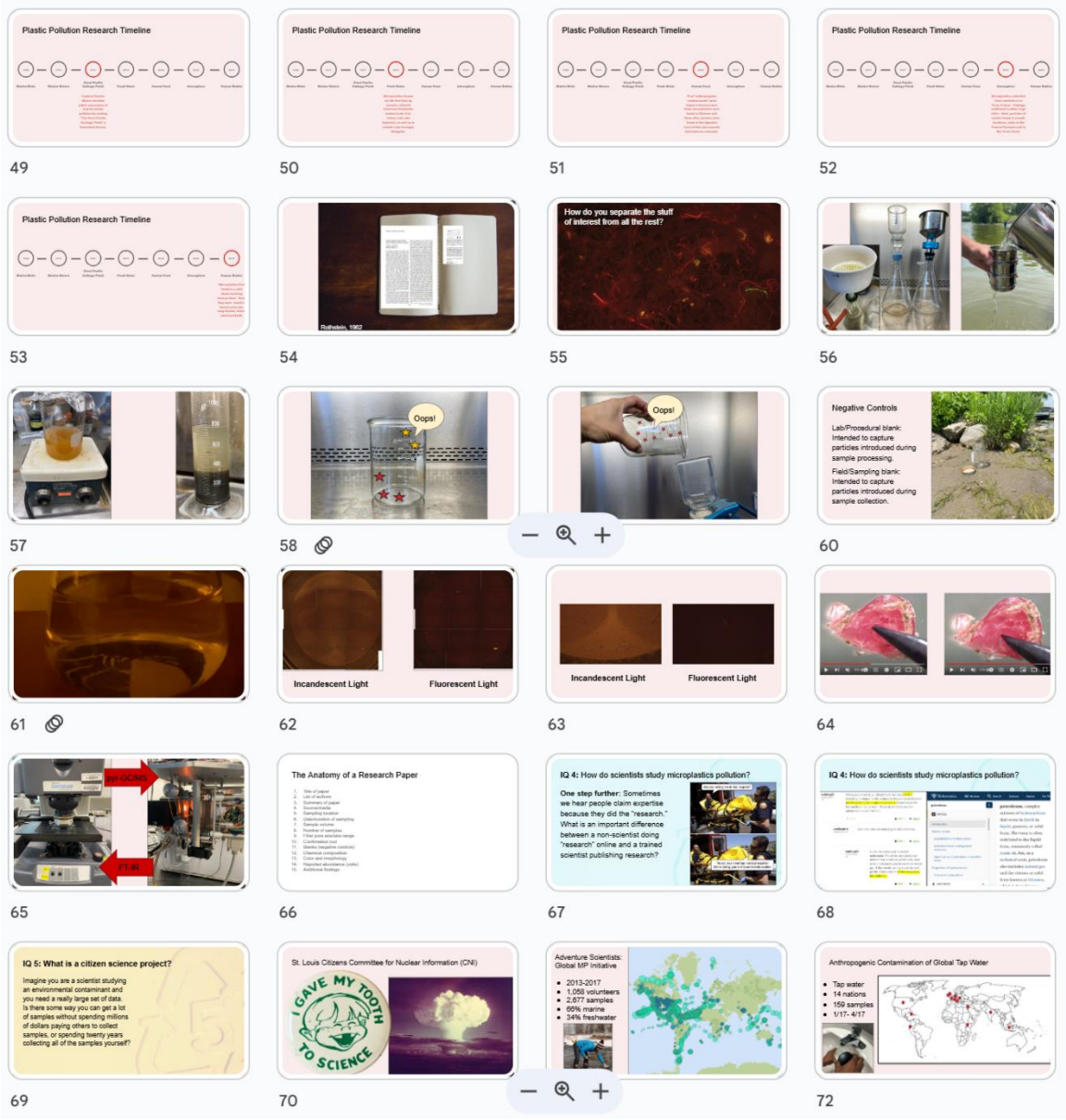
Metal A Straw	polysiloxanes (silicone rubber)	87%
Metal B Lid	polypropylene	97%
Metal B Straw	polysiloxanes	73%
Metal B Straw	polysiloxanes	65%

Chapter 5 Table 1. Chemical characterization results with FTIR for test bottles, both contaminant particles and bottle components.

Chapter 6




25
26
27
28
29
30
31
IQ 2: How do plastics degrade?
32
IQ 2: How do plastics degrade?
One step further: All materials break down in the environment. What happens to metal, paper, concrete, glass, silk, and wood when weathered? Are consequences the same as plastic?
40
33
IQ 3: How do plastics get into the environment?
Where are microplastic found? What are the outer limits for microplastics in the biosphere? What is it about the size and shape of microplastics that help them move through various environmental media with ease?
34
Atmosphere
Terrestrial Environment
Earth's Biosphere
Crust
Inner Core, Outer Core, and Mantle
Biosphere
36
Plastic bag 10,898 meters below sea level
Fibers 8,430 meters above sea level
37
1962
1971
1972
1960
38
39
40
41
IQ 3: How do plastics get into the environment?
One step further: Since some plastic particles are capable of crossing political boundaries, what happens when one nation regulates plastic and neighboring nations choose not to?
42
43
IQ 4: How do scientists study microplastics pollution?
If you were a lead investigator in a microplastics study, what questions would you want to answer? How would you design a study to answer your questions? What would be your hypotheses? Based on your findings, what kind of follow up questions would you explore? In other words, where would you go next?
44
Plastic Production and Research, Growing Exponentially
Global Plastic Production
Number of Published Papers
Published Papers
Plastic Production (million metric tons)
Years 1950–2014
45
Plastic Pollution Research Timeline
46
Plastic Pollution Research Timeline
Plastic Pollution Research Timeline
48


Plastic Pollution Research Timeline
49
Plastic Pollution Research Timeline
50
Plastic Pollution Research Timeline
51
Plastic Pollution Research Timeline
52
Plastic Pollution Research Timeline
53
Rutherin, 1982
54
How do you separate the stuff of interest from all the rest?
55
56
57
Oops!
58
Oops!
Negative Controls
Lab/Procedural blank: Intended to capture particles introduced during sample processing.
Field/Sampling blank: Intended to capture particles introduced during sample collection.
60
61
Incandescent Light
Fluorescent Light
62
Incandescent Light
Fluorescent Light
63
64
μyr-GC/MS
FT-IR
65
The Anatomy of a Research Paper
66
IQ 4: How do scientists study microplastics pollution?
One step further: Sometimes we hear people claim expertise because they did the "research." What is an important difference between a non-scientist doing "research" online and a trained scientist publishing research?
67
IQ 4: How do scientists study microplastics pollution?
68
IQ 5: What is a citizen science project?
Imagine you are a scientist studying an environmental contaminant and you need a really large set of data. Is there some way you can get a lot of samples without spending millions of dollars paying others to collect samples, or spending twenty years collecting all of the samples yourself?
69
St. Louis Citizens Committee for Nuclear Information (CNI)
I GAVE MY TOOTH TO SCIENCE
70
Adventure Scientists: Global MP Initiative
2013-2017
1,058 volunteers
2,677 samples
66% marine
34% freshwater
Anthropogenic Contamination of Global Tap Water
Tap water
14 nations
159 samples
1/17- 4/17
72

73

74

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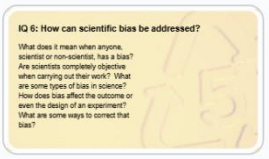

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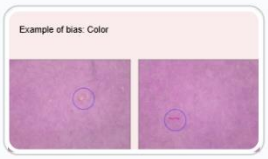

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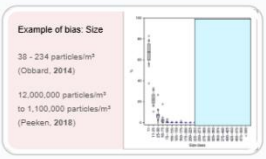

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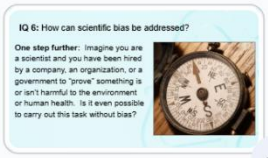

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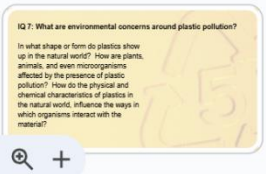

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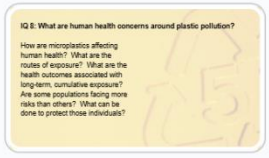

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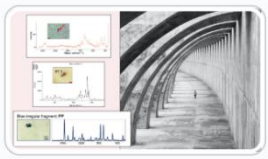
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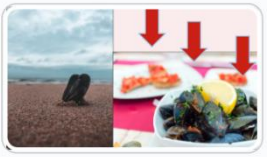
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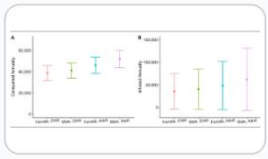
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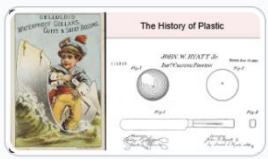

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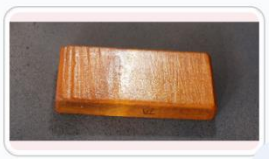
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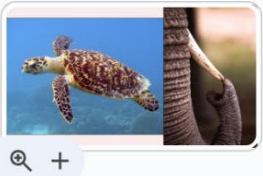

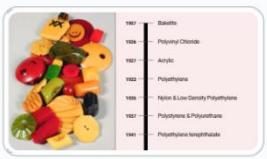
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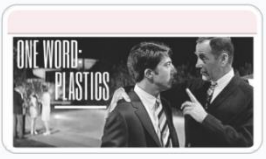

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Chapter 6, Figure 1. Ten-module curriculum for educators using citizen science program with their students.

Citizen Science Microplastics Sampling Project SOP

Goal

- Determine the quantity of microplastics in lake water to estimate the number of particles per liter.

Concepts and Experience You Will Gain

- Field sampling
- Vacuum filtration
- Particle counting
- Experimental controls/blanks

Table of Contents

Supplies

Materials provided per student group.

1. Metal bucket
2. 3 Sieves (3" diameter)
   - 4 mm (#5)
   - 355 µm (#45)
   - 106 µm (#140)
3. Stainless Steel Tweezers
4. DI Water Squeeze Bottle
5. 100% Cellulose Filter Paper
6. Petri Dish
8. Wi-Fi Microscope

Sampling Data Sheet

Name of Citizen Scientists' High School or Organization	
Date samples collected	
Name of water body	
City and state where water body is located	
Description of the sample collection location (e.g., "about 10 feet from shoreline on north side of lake")	
Latitude and Longitude of sampling location	
Type of water body (e.g., lake, stream, holding pond, marshy area etc.)	
Time of sample collection	
Outdoor temperature during sample collection	
Description of the weather conditions during sampling (e.g., windy, clear sunny day)	
Air quality index during sample collection https://www.pca.state.mn.us/air-water-land-climate/current-air-quality-conditions	
Please insert photo here of the sampling location	
Volume of water sampled (e.g. "all students sampled 10 buckets full" or "~ 20 liters")	

Field Sampling

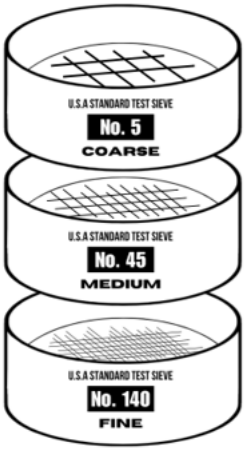
You will work in pairs. You must complete the sampling process with bare hands to avoid contaminating the water with microplastics from gloves.

Supplies

- 3 sieves
- Metal bucket
- Aluminum foil
- Ice auger (if sampling from a frozen water body)
- Waders or water shoes (if sampling in warmer months)
- Permanent markers
- Writing utensil
- Cleaned petri dish wrapped in aluminum foil
- Sample labels

Time: ~15 minutes to pour 10 buckets of water (~20 liters with our 2 qt buckets shown above) through sieve stack

Field Sampling



1. Fill out the sampling data sheet.
2. Open clean petri dish and set both halves open to air on the ground near the sampling location – this is the field sampling blank.
3. Choose your sampling location. *It is important to sample the water without disturbing the sediment. Cut a hole in the ice or wade out far enough into the water body that you can easily sample without disturbing the sediment.*
4. Rinse the metal bucket three times with the lake water to remove any microplastics that might already be present from prior handling and transport. *Take care not to disturb the lake sediment during rinsing and ensure you are standing downwind of where you intend to sample.*
5. The sieves should be clean prior to the sampling and wrapped in aluminum foil. Unwrap sieves immediately prior to sampling.
6. Stack the sieves so the finest mesh sieve is on the bottom, medium mesh is in the middle, and coarse mesh is on top (see image on right)
7. Have one person dip the metal bucket into the water and fill it to the brim while the other person holds the sieve stack.
8. Slowly pour a small volume of the sample through sieves to allow the finer mesh to be wet.

9. After wetting the sieves, carefully pour the remainder of the full bucket of water through the sieves.
    - Take care to pour slowly with a relatively narrow stream in the center of the sieves (see image) to avoid spilling.
    - Watch for water coming out between the middle and bottom sieve as the flow rate for the bottom sieve may be slower than the middle and top.
    - Check the bottom sieve for clogging after passing a few buckets through.
    - Consider giving it time for it to work its way through the filter. If the movement of water has significantly stalled, write down the number of buckets passed through the sieve stack and proceed to the next step.
10. Pour 9 more buckets of water through the sieves (aiming for 10 buckets total or ~20 L with the 2 qt buckets shown).
    - *Take care to refill the bucket a few feet away from where filtration is happening instead of directly below the sieves to prevent sampling the same water repeatedly. Make sure the total volume of water that was poured through the sieves is recorded on the sampling data sheet.*
11. Wrap the stack of sieves with aluminum foil and label the aluminum foil with the name of the water body, your initials, and the date *(Example: Cedar Lake, EMM Feb 15th, 2023)*
12. Wrap petri dish (field sampling blank) with aluminum foil and label it as "field blank" and the name of the water body, your initials, and the date.
13. Bring the aluminum foil wrapped sieve stack, blank, and the rest of the sampling kit back to the classroom.

Filtration

Students can work in groups of two or more to filter the samples depending on equipment constraints.

Safety

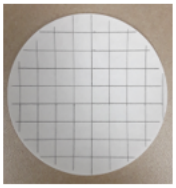
Goggles and closed toe shoes must be worn in the lab at all times. Handle glassware carefully to prevent broken glass. There are no chemical hazards present. Handle and filter samples with bare hands to avoid possible microplastic contamination from gloves. Wash or sanitize hands after working with natural water samples in case there are harmful bacteria present.

Supplies

- Deionized, Milli-Q, or filtered water
- Filters (either pre-gridded as shown above or regular lab filter paper)
- Permanent markers)
- Buchner funnel or glass filtration assembly with frit and metal clamp
- Filter flasks
- Vacuum filtration tubing
- House vacuum, hand pump, manual siphon, or water spigot designed for attaching tubing for vacuum filtration
- 100% cellulose filter paper
- Ruler with 1 cm markings
- Pencils
- Goggles

Time: ~15 minutes to pour 10 buckets of water (~20 liters) through sieve stack

Preparation



1. Either use provided gridded filter paper or draw 1 cm grids onto six filter papers using a ruler and mechanical pencil. *Individual filter papers are needed for the field sampling blank, laboratory blank, reagent blank, medium sieve, and small sieve.*
2. Place a new piece of aluminum foil (a piece ~2.5 times the size of the filter paper) on the lab bench next to where each group of students will be working and place a single gridded piece of filter paper on top of the tinfoil. Keep this filter paper sitting out for the entire duration of the experiment. This is your laboratory blank and will allow you to account for any microplastics contributed from the room air and synthetic clothing where filtering is done.
3. Fill the plastic squeeze bottle from the kit with deionized or Milli-Q water.
4. Hold the metal bucket over a sink and use the squeeze bottle to rinse it three times. This will help remove any microplastics that might already be on it from prior handling and transport.
5. Hold the Buchner funnel or glass filtration assembly components over a sink and use the squeeze bottle to rinse three times with deionized water. This will help remove any microplastics that might already be on it from prior handling and transport.

Filtration

6. Remove the top sieve (largest size mesh #5) from the stack. Write down what you see, this largest sieve size is used to remove large particles (rocks, leaves, bugs, or bigger plastics pieces we call “macro-plastics” or “meso-plastics”).
7. Remove the next, medium sized, sieve (#45) from the stack.
8. Turn the sieve upside down and rinse it with the DI water squeeze bottle into the metal bucket *(be careful to get all the water into the metal bucket)*. To rinse effectively, raster the stream of DI water back and forth over the surface of the sieve. Do this step at least three times. *You must take a lot of care to look at the sieves and make sure you are recovering all of the particles you can see. Sometimes particles/fibers get stuck as shown in the circle. Use a tweezers to remove the fibers/particles and rinse them into the metal bucket.*
9. Assemble the filtration equipment (ceramic Buchner funnel or glass filtration assembly with frit and metal clamp – either is fine)
10. Turn on the vacuum and pre-wet the filter paper by squirting some deionized water onto it using the squeeze bottle.
11. Pour the water from the metal bucket onto the filter paper. BE CAREFUL to make sure the stream of water flows through the center of the filter paper and does not pool. Water flooding over the sides of the filter paper can lose microplastics and reduce the accuracy of the measurement.
12. Rinse the metal bucket with DI water using the squeeze bottle and pour this water onto the filter paper too. Do this three times.
13. Use the squeeze bottle to thoroughly rinse the sides of the filter funnel with deionized water to capture any microplastics that may have stuck to the sides.
14. Once filtration is done, carefully remove the filter paper and place it into a clean piece of aluminum foil that is more than double the size of the filter paper. Fold the aluminum foil in half to seal the filter paper inside and then fold each of the three raw edges of the foil multiple times to create a foil packet. Take care to keep the filter facing up and try not to smash the foil onto the collected particles.
15. Label the foil with the name of the water body, your initials, the date, and the name of the sample. *(Example label: Cedar Lake, EMM Feb 15th, 2023, field sampling blank)*
16. Repeat steps 6-14 for the remaining sieve.

Field Blank

One per class

17. Repeat steps 6-14 for the petri dish. The two halves of the petri dish can be rinsed into the metal bucket with the squeeze bottle just like the sieves.

Reagent Blank

One per group of students that shared the same squeeze bottle.

18. Place a new filter paper onto the filter funnel.
19. Refill the squirt bottle with DI water, unscrew the cap, and then pour the entire contents over the new filter paper, being careful to make sure the stream of water stays in the center of the filter paper.
20. Remove the filter paper from the holder (carefully so it does not tear) and place it into a clean piece of aluminum foil that is more than double the size of the filter paper. Fold the tinfoil in half to seal the filter paper inside and then fold each of the three raw edges of the foil multiple times to create a foil packet.
21. Label the foil with your initials, the date, and the words "squeeze bottle blank".

Laboratory Blank

One per group of students that shared the same squeeze bottle.

22. Locate the filter paper that was set out on the benchtop during the entire experiment. Fold the tinfoil in half to seal the filter paper inside and then fold each of the three raw edges of the foil multiple times to create a foil packet.
23. Label the foil with your initials, the date, and the words "laboratory blank."

Check Point

Each group of students should now have four samples in total: two from the sieves (#45 and #140), one laboratory blank, and one reagent blank.

Analysis

Notes: Students can work on analysis individually. Directions for hot needle test developed using Beckingham, B., Apintiloaiei, A., Moore, C. et al. Hot or not: systematic review and laboratory evaluation of the hot needle test for microplastic identification. Micropl. & Nanopl. 3, 8 (2023). https://doi.org/10.1186/s43591-023-00056-4

Helpful videos on the hot needle test can be found here: https://bit.ly/3KzgyTU

Safety

Goggles and closed toe shoes must be worn in the lab at all times. Use light microscopes with care. Use caution when handling hot metal for the hot needle test to avoid burns.

Supplies

- Blanks and filtered samples
- USB or Wi-Fi Microscope (may require computer or mobile phones)
- Soldering iron or needle and lighter or Bunsen burner
- Blank sheet of paper
- Ruler with 1 cm marks
- Writing utensil

Particle Counting

1. Open the desired foil packet and set it on the bench next to the microscope while you work with the filter paper grids facing up.
2. Draw a grid of 2 x 2 cm squares on a piece of paper so you can record your particle counting data. Label one side "top."
3. Carefully place one of the filter papers under the light microscope and label one side "top"
4. Focus the light microscope and examine each grid individually for small (1 µm – 5 mm) fibers and particles. Particles may be difficult to see against the white filter paper. Record the number of particles you see in the corresponding box on the piece of paper you prepared for this...Particles should be categorized into one of three categories: 1) confident positive, 2) potential positive, and 3) confident negative.

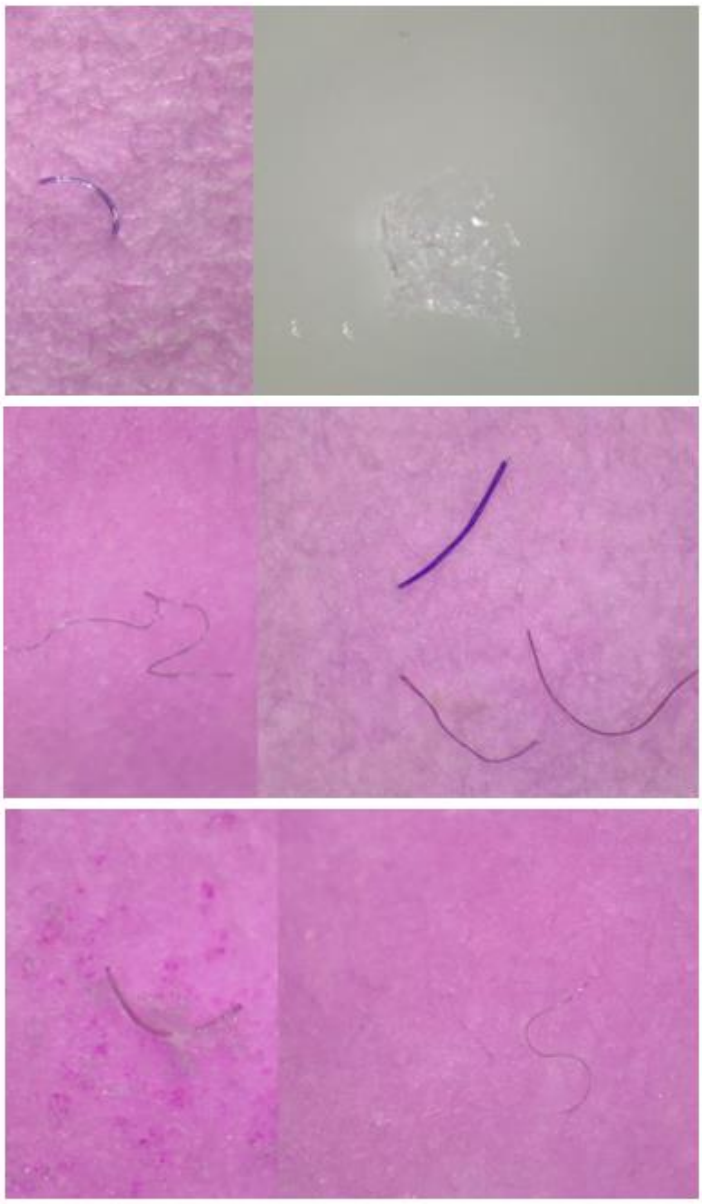
The images below give some guidance on particle identification strategies:

Chapter 6, Figure 2. Standard operating procedure for educators using citizen science program with their students.

Microplastic Visualization

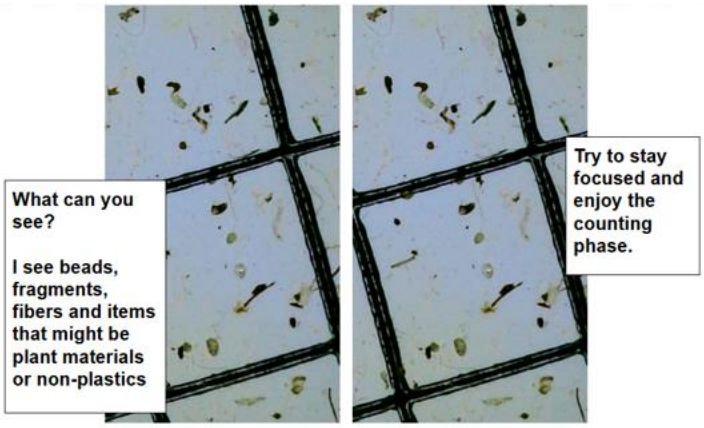
Microplastics come in various shapes, or morphologies. These include fragments, foams, fibers, and beads/spheres.

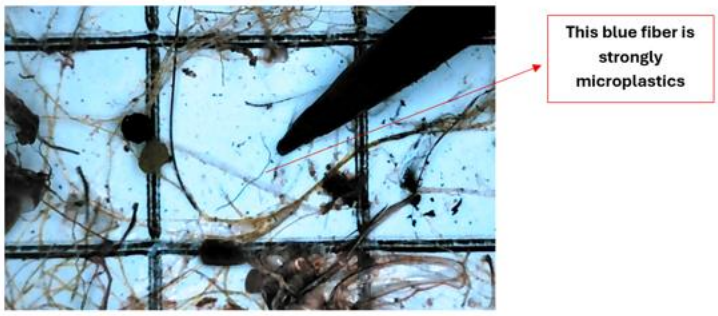
When looking at samples, keep an eye out for particles that are transparent. Move them around with the tweezers to get a better view. When examining fibers, those with a blunt end, a uniform diameter throughout (look like they were extruded), and/or a smooth or shiny surface are more likely to be made of plastic.

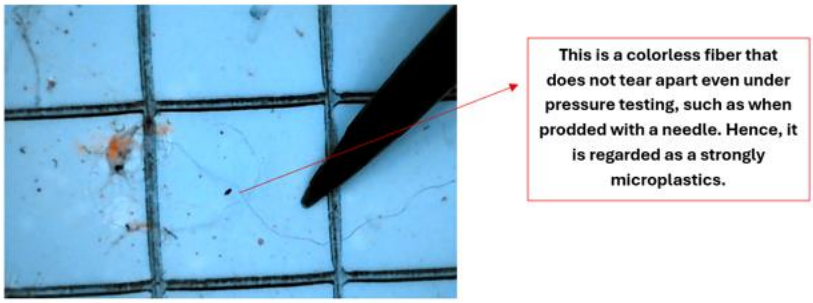


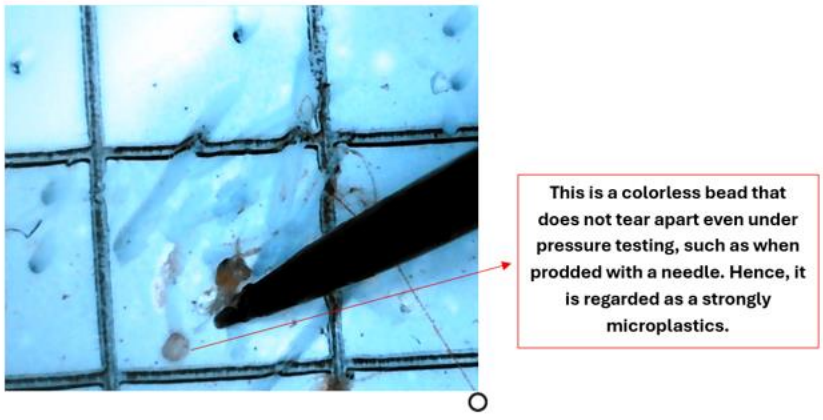
Visual and Counting Phase - determining if its a plastic or a natural particle will be the most challenging part - use color, texture and morphology as clues

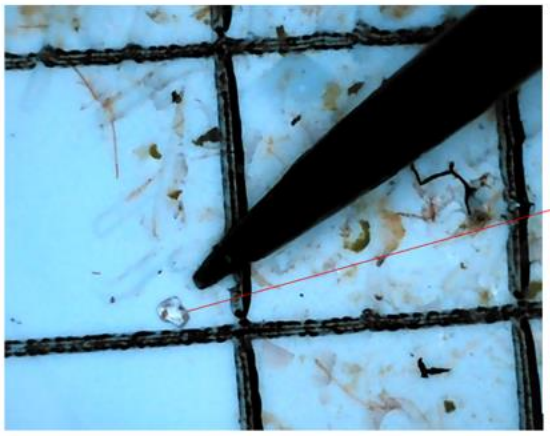
Visual and Counting Phase

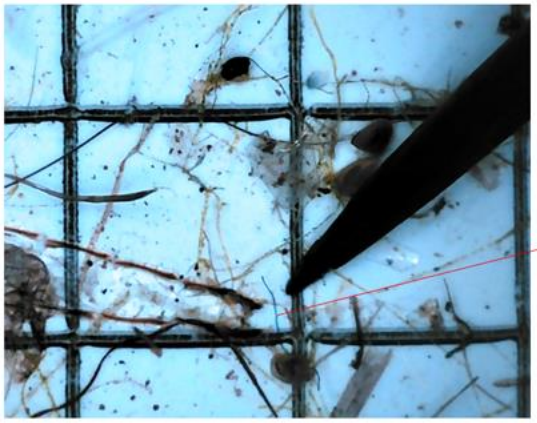

What can you see?
I see beads, fragments, fibers and items that might be plant materials or non-plastics
Try to stay focused and enjoy the counting phase.

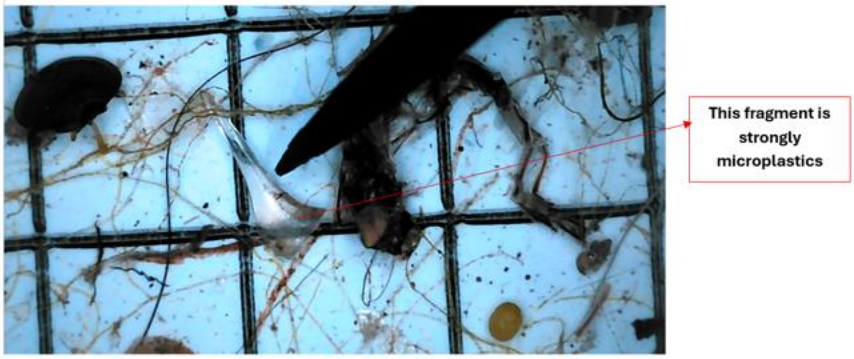

This blue fiber is strongly microplastics


This is a colorless fiber that does not tear apart even under pressure testing, such as when prodded with a needle. Hence, it is regarded as a strongly microplastics.


This is a colorless bead that does not tear apart even under pressure testing, such as when prodded with a needle. Hence, it is regarded as a strongly microplastics.


This colorless fragment is strongly microplastics


This blue fiber is strongly microplastics



Note on Sample Observation and Classification:

As you go through your samples, please keep in mind that each filter may appear different depending on the sample matrix. Filters may contain more biological or organic matter than what is shown above. The goal is not necessarily to find perfect matches, but to remain curious and maintain a cautious, investigative mindset.

Carefully examine each grid square and gently probe the particles. Try to distinguish between those that are strong candidates for microplastics (strongly microplastic) and those that are uncertain (maybe microplastic). This comparison is a valuable part of the learning experience.

While observing, feel free to record any noticeable biological materials (e.g., algae, plant fragments, insects) as part of your observations.

Keep a critical eye, stay engaged, and do not hesitate to ask your teacher questions as you make your assessments.

Chapter 6, Figure 3. Images used to guide citizen scientists as they categorize found particles as either "maybe microplastic" and "strongly microplastic."