Analysis of Plastics in Marine Mud and Gravel Sediments

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Abstract

Plastic pollution is a growing environmental problem and area of study with increasingly more millions of metric tons produced every year. These plastics are getting into our sediment and food web. Four marine sediment samples were taken from Quahog Bay, ME to analyze for microplastics (MPs) and microfibers (MFs): 2 from mud sediments and 2 from gravel sediments in the intertidal zone. The intertidal zone is a very influential marine ecosystem because of its proximity to land as well as it is a nursery ground for many marine organisms. The species diversity and MP concentration in this zone can tell us about the overall health of the environment. There were between 40 - 120 MFs and 70 - 220 MPs between all four samples. The gravel samples had more MPs while the mud samples had more MFs. Estuaries like the Quahog Bay that have a large tidal range (~9 ft) are more susceptible to higher MP concentrations. It is important to continue this type of sediment research to grow the sample size and learn more about how MP toxins in sediment can affect the rest of the ecosystem.

Introduction

Plastic pollution is one of the major environmental problems plaguing our world today. Plastic production has increased well past presumption, from ~1.5 Million metric tons in 1950 to ~368M metric tons in 2019 (*Statista,* 2022). When primary plastics break down into smaller pieces called secondary plastics they become more harmful to ecosystems, organisms, and humans. Microplastics (MPs) are secondary plastic fragments that range in size from ~1µm to 5 mm. Microfibers (MFs) are another type of secondary plastic that are very fine synthetic threads released when doing laundry and from fishing gear. MPs and MFs are floating in the surface waters of our oceans and causing the death of marine life. Plastic enters marine environments through runoff, wind, storms, and direct littering.

The intertidal zone, area above water level at low tide and underwater at high tide, is a very important and prosperous ecosystem. Unfortunately, this zone is very susceptible to MP pollution because of its proximity to the coastline. The plastic in the ocean that is not collected by ocean circulation into the five subtropical gyres is either consumed by marine organisms or deposited in marine sediment. Some plastic is discharged in feces from marine organisms which makes it easier to be transported and deposited to deeper depths (Zhao et al, 2017). Not all polymers are less dense than seawater, therefore some denser ones such as PVC are able to sink to the benthos rather than float in the surface waters (Pagter et al, 2020). Biological fouling and weathering from microbes and algae can impact the densities of microplastics which allows them to move from the euphotic zone to the seabed (Kaiser et al, 2017). Previous studies have determined that marine sediments are sinks for every size of plastic fragments (C. Martin et al,

2022). MPs are found in varying depths in marine sediment, although the bulk of the MPs are found within the top 5 cm of the sediment (J. Martin et al, 2017). Specifically, the seafloor in the intertidal zone is a large sink for MPs. The intertidal zone is a breeding ground for deep water species, habitat for species privatal in the wood web, shelter for baby organisms, and major signal for the health of the environment (Rist, 2022). Therefore, the proximity to land and the high levels of biological interactions that can cause MP sediment deposition make this zone particularly vulnerable. Intertidal zone sediment microplastic research is limited but important to learn the MP toxin transfer rate into organisms and throughout the food web. Additionally, this research provides evidence for if there are long term effects of MPs and how they degrade over time in a sink like the seafloor.

The objective of this study is to quantify microplastics and microfibers in the top 55 mm of intertidal marine sediment in the Quahog Bay in Harpswell, ME. Additionally, this research investigates if MPs and MFs are evenly dispersed throughout the four sites or if sediment type, grain size, and proximity to land affects the distribution. Muddy sediments are characterized by very small grain size and a higher presence of organic material. Gravel sediments are characterized by a large grain size, and a lower amount of organic material. It is hypothesized that the combined gravel and sand samples will have more MPs and MFs compared to the mud samples because they are closer to shore and have a larger range in grain size.

Methods

Instruments used

Four 250 mL beakers
Four safety goggles
Latex Gloves
INCU-Shaker Mini
Rocker 300 Vacuum Pump
1 liter vacuum trap
SAS Positive Pressure room (clean room)
Two pyrex glass bowls
Metal clamp
53 µM Sieve
Stainless Steel 3-Station Vacuum Filtration Manifold
Three vacuum filter reservoirs
Three vacuum filter funnels
Air Science Purair Ductless Fume Hood (negative pressure chemical hood)
Knife
1.0 M Sodium Hydroxide (NaOH)
Four Cytiva Whatman GF/F 47 mm filters
Zeiss Stemi 305 Microscope
Four glass petri dishes
Tin foil
Four 150 mm sterile Petri Dishes with covers
Stainless steel dissection tray
Ohaus Adventurer Scale
8.5 L Coleman Chiller Personal Cooler
Mud and Sand Auger 3-1/4” diameter.
0.9% Sodium Chloride Irrigation USP (NaCl)
MyWeigh KD-8000 Scale
250 mL squirt bottles
500 mL squirt bottles

Glass stirring rod

Collection of Sediment Samples

Before leaving to obtain the samples, the Mud and Sand Auger was marked for collection by measuring 50 mm from its base with a line in sharpie. Four beakers were labeled 1M, 2M, 1G, 2G for mud and gravel sediments. Then, they were rinsed with deionized (DI) water and weighted to measure the empty beaker weight using the Ohaus Adventurer Scale. A 25 ft Carolina Skiff boat was driven to the sample sites to collect sediment cores in the Quahog Bay. The four 250 mL beakers and the auger were rinsed with filtered DI water just prior to sample collection. The auger was placed into the ground vertically and pushed down until the marked line was at ground level. Then, the auger was pulled up with the sediment sample still in it. The pipe was pushed through the auger, allowing the sample to fall into the labeled beaker. The sample was covered with tinfoil to reduce contamination and placed into the cooler. This process was repeated for the following three samples at separate locations (Figure 1).

Figure 1: Google Map Image with labeled sample sites in Quahog Bay, Harpswell, ME.

Density Separation

It is important to note that gloves were worn for all lab work to limit sample contamination and protect the scientists from the various solutions. The samples were taken out of the cooler and weighed on a MyWeigh KD-8000 scale to measure the sample wet weights. The following equation was used to calculate sample wet weight: sample wet weight in beaker - empty beaker weight (Table 1). The samples were placed into a clean bowl inside the clean room where sodium chloride (NaCl) was slowly squirted into each beaker using a squirt bottle. As NaCl was added to each sample, a clean stirring rod was placed vertically into them. An analyst stirred each sample while pouring the NaCl to disperse the sediment. The samples were stirred twice in one afternoon: once when the NaCl was introduced and again before bedtime. Once each beaker was full and well stirred, the samples were left overnight to settle. NaCl was introduced to the sediment samples because of its high density in comparison to fresh water. This caused microplastics, microfibers, and lipids to separate from the mud, gravel, and sand, and float towards the top of the beaker. The samples were slowly stirred once more in the morning, but this time the settled sediment in the bottom of the beakers was not disturbed. More NaCl was added to the samples causing just the top layer of the beaker water to overflow into the bowls underneath. This allowed for the MPs, MFs, and organic matter that floated to the top to be separated from the sediment body. The beakers were then very carefully taken to the nearby sink where about 1 cm of the sample was poured down the drain. Then, the remaining sediment and NaCl contents was dumped outside. Inside the clean room, the samples were poured from the bowls into clean 250 mL beakers. Then, they were filtered through a 53 µm sieve into a clean bowl using NaCl. The 53 µm sieve was backwashed using 1.0 M of sodium hydroxide (NaOH) into a different clean bowl. The backwashed sample was then finally poured from the bowl into a new beaker. This was repeated for all samples. Once the final samples were in their beakers with NaOH, they were covered in tin foil and placed into the INCU-Shaker at 60℃ and 130 rpms for ~3 hours (Figure 2).

Sample type	Sample number	Sample Wet Weight in Beaker (g)	Empty Beaker Weight (g)	Sample Wet Weight (g)
Mud	1M	225	106	119
Mud	2M	221	108	113
Gravel & Sand	1G	206	108	98
Gravel & Sand	2G	226	106	120

Table 1: Marine sediment calculated sample weights (g).

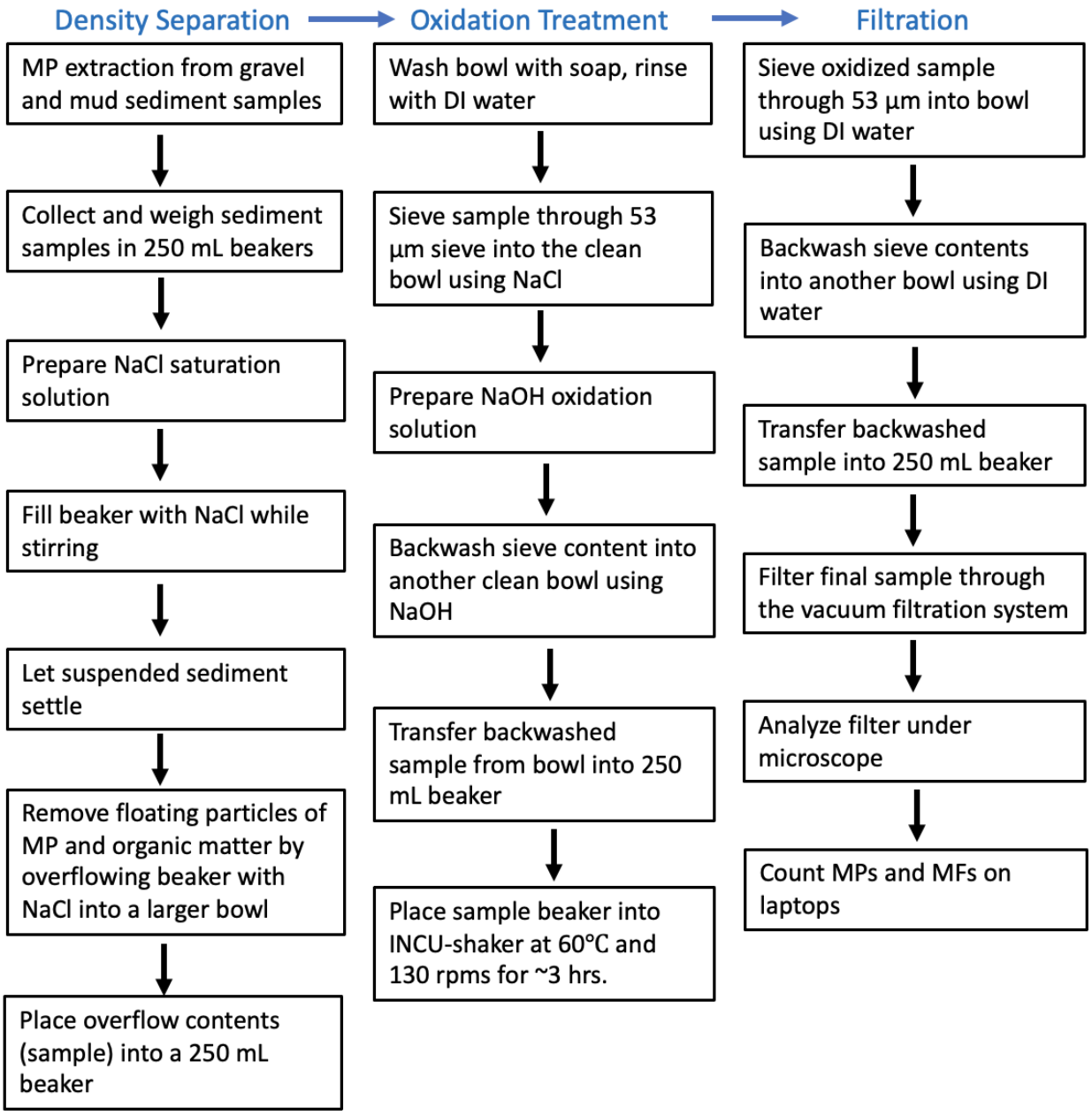

Figure 2: Flow chart of overall extraction, density separation, oxidation treatment, and filtration process of MPs and MFs in marine sediments. This process was adapted from Rivoira et al 2020.

Filtration of Sediment Samples

After 3 hours, the samples were taken out of the INCU-Shaker and placed into the clean room for filtration preparation. All glassware and tools were rinsed with filtered deionized (DI) water prior to use. Every sample was poured through the 53 µm sieve into a bowl underneath to filter out the NaOH. The sieve was then backwashed with DI water into a new bowl. Once each backwashed sample was poured from its bowl into four new beakers, they were poured into a vacuum filter reservoir. The sample traveled through the reservoir and the 47 mm filter into the

vacuum filter funnel that was fastened by a metal clamp. The sample then flowed through a tube to a 1-liter jug which captured all discarded solutions. The Rocker 300 vacuum pump powered this filtration process and was connected to the manifold by a tube. The manifold holds 3 separate filtration systems including the reservoir, filter, and funnel. All 3 filtration systems can flow at the same time and into the 1-liter discharge jug. Once all four samples were filtered, the filters were removed with tweezers and placed into labeled glass petri dishes.

Microscopic Counting of Microplastics and Fibers

To count MPs and MFs, each sample was individually placed under the Zeiss Stemi 305 Microscope. On 1.0 zoom under the microscope, the microplastics were identified by looking for black spots. About 15 images were captured of each sample filter and then analyzed on laptops to individually count MPs and MFs. Microplastics were considered to be any densely colored fragment less than 5 mm but greater than 0.1 mm in length: the majority are black dots. Fragments less than 0.1 mm were not counted due to possible inaccuracy from the Zeiss Stemi 305 Microscope and human error. Microfibers were considered to be any thread-like fragment. Microplastics were not considered to be the shiny and reflective marks or the yellowish- green fragments. The reflective marks are inferred to be light from the microscope shining through the filter. The yellowish-green fragments are theorized to still be organic matter that did not dissolve in the sodium hydroxide.

Results

Microplastic and microfiber concentrations varied significantly based on sediment type. Sample 1M, the first mud sample, has the most microfibers while sample 1G, the first gravel sample, has the most microplastics. The gravel samples have a total of 82 microfibers and 390 microplastics. The mud samples have a total of 193 microfibers and 185 microplastics (Table 2). This aligns with the calculated MF and MP per gram values. The MFs/g calculations range from 0.334 to 0.994 MFs/g between all four samples, while the MPs/g calculations have a larger range of 0.666 to 2.232 MPs/g. Sample 1M has the most MFs/g at 0.994 MFs/g and 2M is the next largest at 0.666 MFs/g. Sample G1 has by far the most MPs/g at a value of 2.232 MPs/g with the next largest sample being G2 at 1.429 MPs/g (Table 3). Therefore, the gravel samples have more MPs and inversely, the mud samples have more MFs.

The average MF length varied from 5 to 10 mm between all four samples. The average MP length was relatively constant at 1 mm, with only sample 2G being 0.5 mm larger (Table 2). Sample 1M has 118 MFs that range from 2 - 22 mm, with an average of ~5 mm. Sample 1M has 111 MPs that range from 1 - 3 mm with ~1 mm average. Sample 2M has less MFs and MPs than Sample 1M. 2M has 75 MFs, ranging from 5 - 35 mm with an average of ~10 mm and 74 MPs ranging from 0.5 - 2 mm with an average of 1 mm. Sample 1G has the most MPs out of all the samples with 219 MPs ranging in length from 0.5 - 2 mm, averaging ~1 mm. Sample 1G has 42 MFs between 2 - 25 mm with an average of ~7 mm. Sample 2G has the least MFs out of all four samples with a value of 40 MFs. They range from 2 - 28 mm, averaging ~10 mm. There are 171 MPs in Sample 2G ranging from 1 - 3 mm with an average of 1.5 mm.

Sample Type	Sample Number	Number of Microfibers	Number of Microplastics	Average Microfiber	Average Microplastics

				Length (mm)	Length (mm)
Mud	1M	118	111	5	1
Mud	2M	75	74	10	1
Gravel & Sand	1G	42	219	7	1
Gravel & Sand	2G	40	171	10	1.5

Table 2: Number of microfibers and microplastics in each sample with an estimated average length for each.

Sample Type	Sample Number	Microfibers per gram	Microplastics per gram
Mud	1M	0.994	0.935
Mud	2M	0.666	0.657
Gravel & Sand	1G	0.428	2.232
Gravel & Sand	2G	0.334	1.429

Table 3: Calculated number of MF/g and MP/g in each sample.

All samples regardless of sediment type showcase colorful microfibers. The following colors are seen: red, light blue, dark blue, purple, and black. In all samples, the primary MF color is black. The mud samples showcase 0.340 MFs/ g more MFs than the gravel samples (Table 3). A large blue MF with a length of ~16 mm is seen in Sample 1M (Figure 3A). Sample 2M showcases a slightly larger than average sized red MF, specifically 14 mm long (Figure 3B). Sample 1G has one of the largest MFs measured in all the samples with a length of ~25 mm (Figure 3C). Sample 2G has clusters of MFs (Figure 3D). The light blue MF within this cluster was also one of the longest measured at ~25 mm (Figure 3E).

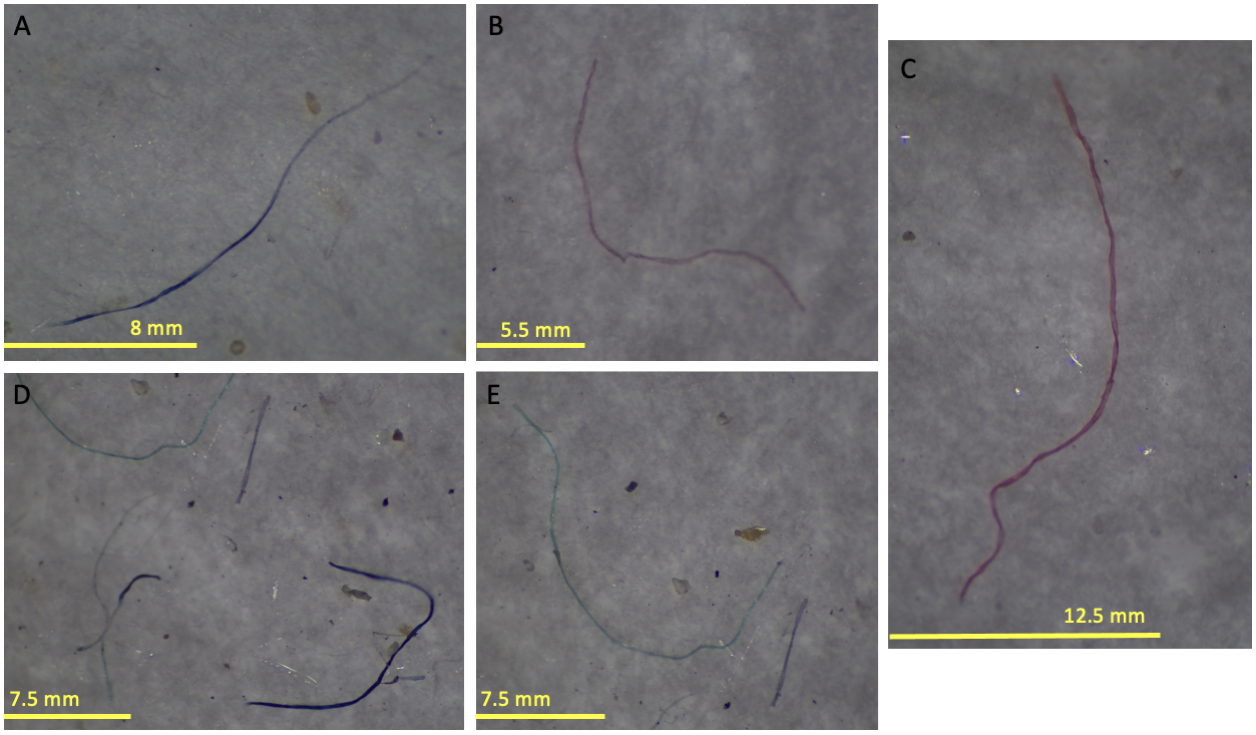

Figure 3: Microfibers from samples 1M (A), 2M (B), 1G (C), and 2G (D & E) under the Zeiss Stemi 305 Microscope on 4.0 zoom. The blue MF in (E) is also seen in the top left corner of (D).

The microplastics in all samples were primarily black in color and averaged ~1 mm in length. Looking through the 305 Microscope on 4.0 zoom, the MPs had slight variations in color. A couple red MPs were identified in Sample M2. The MPs differ in shape and seem to be irregular (Figure 4). Sample 2M showcases an average sized MP with a length of 1 mm (Figure 4B), while the other images in this figure display larger than average MPs between 1.5 - 2.5 mm. Sample 1M and 2G have the largest range in MP length which is 1-3 mm. Sample 2G exhibits one of the largest measured MPs with a length of 2.5 mm (Figure 4D).

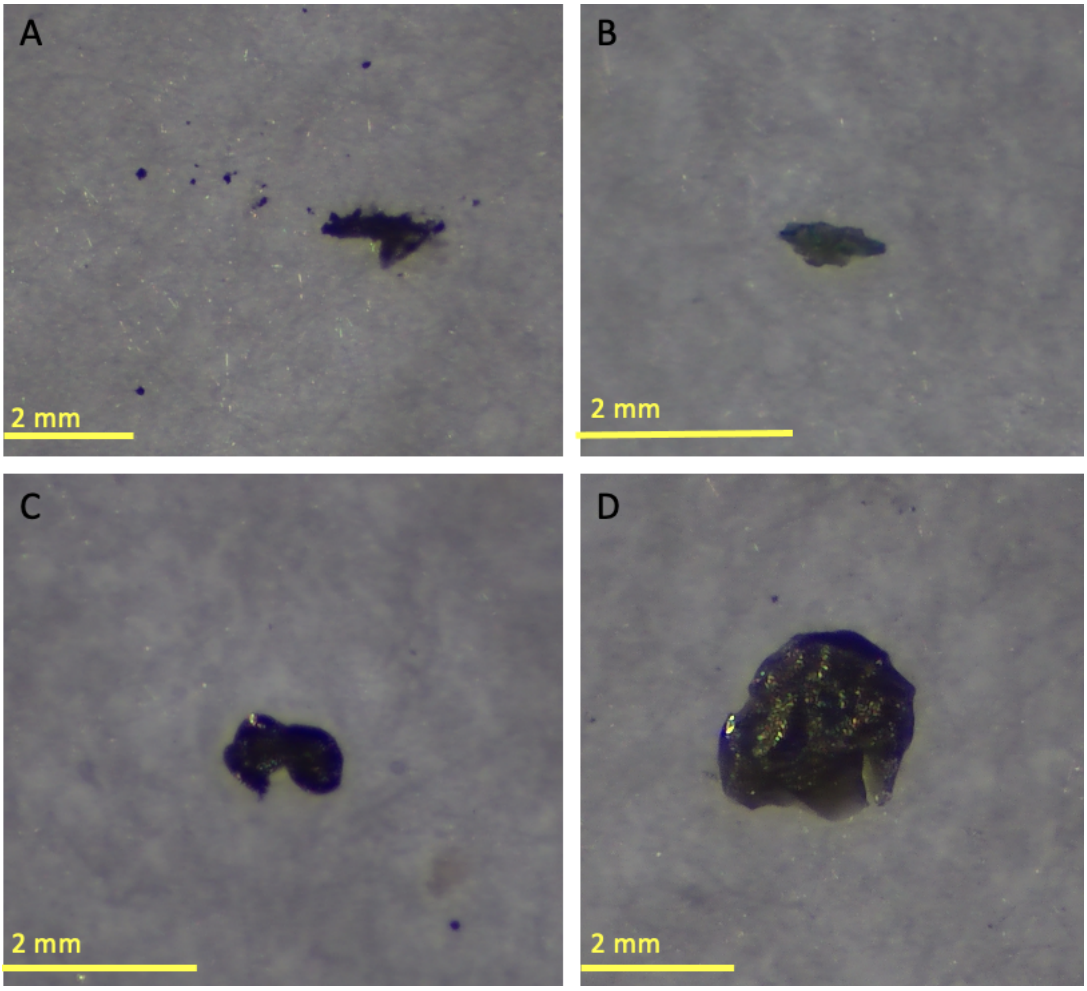

Figure 4: Microplastics from samples 1M measuring 2 mm (A), 2M measuring 1 mm (B), 1G measuring 1.5 mm (C), and 2G measuring 2.5 mm (D) under the Zeiss Stemi 305 Microscope on 4.0 zoom.

Discussion

This study presented a patchy distribution of four sample sites throughout the Quahog Bay, exhibiting relatively low concentrations of microplastics and microfibers in gravel and mud sediments in intertidal zones. All MPs and MFs found were classified as secondary sources: no pellets, spheres, or full plastics were recognized. This analysis measured between ~0.33 - 0.99 MFs/g and ~0.66 - 2.23 MPs/g which is lower than ranges observed in other studies. Although, other studies usually have a larger sample area, more analyzed samples, and larger samples. Most of the fibers observed in this research were black, but red and blue fibers were also heavily seen. This is most likely a result of the lobster and fishing industries in the Gulf of Maine, specifically the Quahog Bay. These MFs are potentially secondary fragments from their gear: ropes, nets, etc.

The sample sites in the Quahog Bay are high sand fraction locations which suggests high energy and strong currents. The tidal range (low to high tide) in the Quahog Bay is ~9 ft which is relatively large. The Gulf of Maine has the largest tidal range on Earth at ~50 ft in the Bay of Fundy which is just north of the Maine coast near Nova Scotia, Canada. Therefore, other

studies that sample higher mud fraction sediments indicating low energy environments are not directly comparable. After analyzing these results, it is hypothesized that since the Quahog Bay opens to the Atlantic Ocean these waters consequently have a high flushing time. Meaning, it takes a long time for the water mass to be replaced in the coastal environment between one estuary and another. Flushing time only accounts for the average amount of time the water mass is in the environment (Matso, 2018). This high flushing time in the Gulf of Maine most likely impacts how MPs and MFs settle in the intertidal sediment (Pagter et al, 2020).

More local reasons for one sediment type to have more MPs and MFs compared to the other include proximity to land and therefore runoff. Sample 1M is surrounded by the most coastline (Figure 1) and has by far the most MFs (Table 2). Sample 1M has 118 MFs: ~3X more than both gravel samples with values in the 40s and 43 more MFs compared to the other mud sample (Table 2). Sample 1M also has the highest MFs/g value (Table 3). The more runoff the shoreline is exposed to the more plastics are able to settle into the sediments. It would be interesting to examine if there is a positive correlation between runoff and/ or island pollution and microplastics concentration in the intertidal sediment in those areas.

Grain size would also be interesting to further investigate and measure. With the gravel samples having a larger average grain size from their gravel component, there seems to be a positive correlation between large grain size and more MPs. The mud samples having a smaller grain size are positively correlated with more MFs. More research is needed to discover an explanation for this correlation. It was hypothesized that the larger range in grain size between gravel and sand in the gravel samples would entrap more microplastics and microfibers. This was only half true in our study, with the gravel samples having more MPs but less MFs compared to the mud samples.

NaCl was used in this study for density separation between the sediment and microplastics. Although, other solutions should be tested to see which results in the most accurate and precise extraction. Different polymers have different densities, therefore using only 1 solution may be an underrepresentation of the numbers of MPs and MFs abundance. Additionally, the variety of polymers with different densities may be a result of ocean processes, specifically biofouling, a process where the growth of organic matter on a plastic fragment over time causes it to increase in density, potentially leading to the plastic surpassing a density capable of keeping it afloat. Lighter polymers are transported to the sediments along the shoreline more commonly than denser polymers. This is due to the larger amount of organic buildup needed to sink them, often requiring more time then it would take for the plastic to reach the shoreline (Kaiser et al, 2017).

Conclusion

Microplastic research is a growing area of study due to the relatively new discovery that they are everywhere on our planet: from the deepest ocean trenches to the air we breathe. Specifically, when exposed to sunlight, plastic burns and releases toxins into the air resulting in ambient air pollution. MPs directly affect organism health, especially marine organisms. Marine sediment is the core of marine ecosystems especially for intertidal species. As MPs degrade in the soils after deposition, they release harmful chemicals into the surrounding sediment. Phthalates and Bisphenol A (BPA) is one chemical that is particularly damaging to sediment organisms: vertebrates and invertebrates (Nature Action). Future research should analyze

which specific chemicals are heavily affecting intertidal organisms. Additionally, large sample sizes surveying MP concentrations in varying types of marine sediment would be beneficial for a more complete investigation.

Different types of marine environments have varying amounts of MP concentrations with estuaries and fjords having the most: 4 -140X more MPs compared to deep sea environments (Harris, 2020). As seen in Figure 14B of Harris, 2020, tidal dominated estuaries, like the Quahog Bay, are in the middle of environments in terms of MP trapping efficiency. Fjords are the most efficient for trapping MPs while strand plain environments are the least efficient. Therefore, our shorelines and intertidal organisms are the most susceptible to detrimental MPs with ~40 million tons of MPs washing up, depositing, or resurfacing there (Ritchie & Roser, 2020). The intertidal zone is integral to protect because it is the basis for many ecosystems: it controls the balance between land and sea. This zone is the breeding ground for many deep ocean species as well as shallow water species. Intertidal species are a key portion of the marine food web and a critical measure for overall ecosystem health (Dotson, 2019). If the intertidal ecosystem, sediment, and organisms become toxin as a result of high MP concentrations, then the entire marine environment will be at risk. This begins in the sediment. It is very important that we clean up our shorelines through trash pickup, at any scale possible, to limit the degradation of macroplastics to microplastics and nanoplastics.

Credit Authorship Contribution Statement

Teagan Cunningham: Methodology, Investigation, Writing - original draft, review, and editing.
Mikayla Wallace: Methodology, Writing - review and editing.
Nash Holley: Methodology

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