Quantification of microplastic presence in Texas waterways and inflows to the Gulf of Mexico

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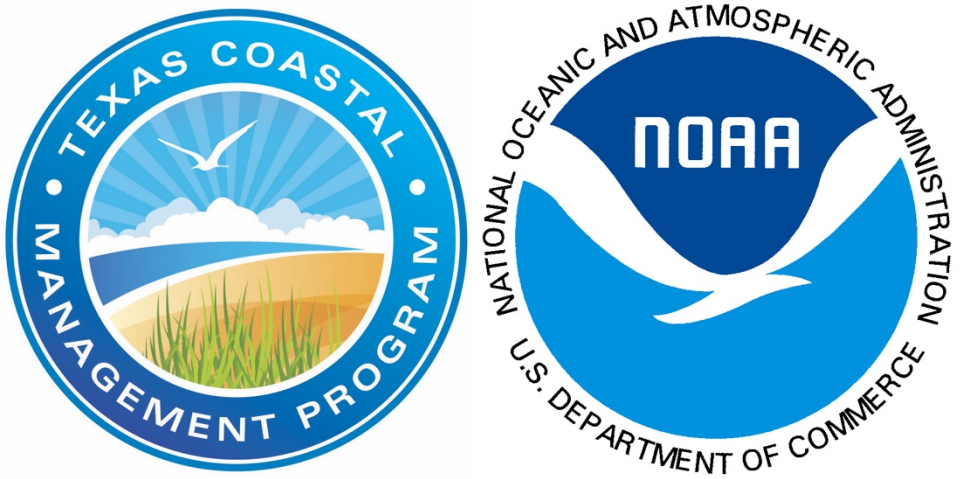

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EXECUTIVE SUMMARY

Plastics are a staple of everyday life; from packaging to toys, to clothing, plastic has become a constant in day-to-day life. It is easy to see why, due to its structural stability, ability to withstand breakdown, and wide range of applications. Plastic's prolific use has allowed for its entry into the environment at every level. Microplastics are plastic particles and fibers that exist, for this research, as being less than 5 millimeters in size. These come from macroplastics degrading to smaller sizes, as well as these products being manufactured at this size. Research has been conducted quantifying microplastics in the Gulf of Mexico through the Mississippi River and in Coastal areas, but Texas represents a data gap for study. For this project, water samples were collected at 19 locations from Galveston to the U.S., Mexico border using multiple methods; a peristaltic pump, Van Dorn Sampler, and grab samples. For this study water samples were collected using the above methods at both the surface and 60% of the depth at the time of collection. All samples were filtered through a 0.45 µm, cellulose, membrane filter followed by visual sorting of suspected microplastics, and materials confirmation using Fourier-transform infrared microscopy (µFTIR). Microplastics and cellulosic (semi-synthetic) materials were found in all coastal systems examined. No significant differences were observed between systems or when comparing concentrations at the water surface with those at depth. The total number of these materials discharged into coastal Texas annually is>100 trillion, although their total mass is estimated at 95 kg (209 lbs). These numbers provide insight into the scale of the problem but must be examined with ongoing and future studies that assess the impacts of microplastics and cellulosic materials on ecosystems and aquatic organisms.

OUTREACH EFFORTS

This project funded 2 M.S. students between Texas A&M University-Corpus Christi (Matt Watford) and Texas A&M University-Kingsville (Lauren Rodriguez), involved another M.S. student at TAMUCC and supported paid undergraduate research experiences for 12 students at TAMUCC. Matt Watford led the project and either oversaw or performed most of the work presented below. Lauren Rodriguez helped with aspects of work performed at TAMUCC but focused largely on a separate, but related study of microplastics in Nueces Bay. Some of her efforts are included below, but a separate comprehensive report for that work is included as an appendix. Undergraduate researchers played a minor role in fieldwork but were heavily responsible for sample processing. This included sample filtering and visual sorting of suspected microplastics under a microscope. During their efforts, undergraduates learned basic lab techniques and how to use the proper quality controls that ensure our microplastics research will pass peer-review.

Below the students, both undergraduate and graduate, supported by or who participated in this research are listed. Presentations given to the public or in an academic setting are also listed. With the data only recently being finalized, the results of this work will also be incorporated where appropriate into Environmental Chemistry (CHEM 4443) and Advanced Environmental Chemistry (CHEM 5417) in Fall 2021.

Participating Graduate Students

Matt Watford: M.S. in Environmental Science at Texas A&M University-Corpus Christi
Jessica Myers: M.S. in Coastal & Marine Systems Science at Texas A&M University-Corpus Christi
Lauren Rodriguez: M.S. in Environmental Engineering at Texas A&M University-Kingsville

Participating Undergraduate Students

Grace Grainer, Joel Dominguez, Cassandra Quiroz, Liz Horn, Nicko Barrera, Daniel Lansidel, Elizabeth Hamilton, Daniela Bergman, Tanner Thompson, Demori Lawrence, Jose Rojas, Maddie Holley

Academic Presentations

Watford, M., Myers, J., Geist, S., Conkle, J.L., Microplastic Quantification in Texas Waterways. 2nd Annual Texas Plastic Pollution Symposium. Galveston, TX, USA. 10/29/19.

Rodriguez, L., Ren, J., Conkle, J., Quantifying Microplastics loading to Nueces Bay, Corpus Christi, Texas. 2nd Annual Texas Plastic Pollution Symposium. Galveston, TX, USA. 10/29/19.

Conkle, J.L. Too many needles in the haystack: Studying pollution in Coastal Texas. Research presentation during an interview for the TAMUCC Center for Coastal Studies Director position. Corpus Christi, TX, USA. 08/02/2021.

General Public Presentations

STEM Summer Camp: 8/8/19 Matt presentation at Texas State Aquarium High School STEM summer camp program. Spent the day teaching the campers about basic science principles and discussing the methodology of research being conducted.

Teen STEM Café: 10/28/19 & 11/11/19 Matt presentation at Texas State Aquarium Teen Café Program. Discussed ongoing research with a group of teens and engaged in questions and discussions.

Texas State Aquarium Live Stream: 01/16/20 Presentation explaining microplastic basics and research going on in the state. (18 Attendees for the live program and additional viewers for the recorded program. Unfortunately, during the pandemic, the Texas State Aquarium let go of their educational staff and we can no longer view or report viewership numbers for the video)

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ACKNOWLEDGEMENTS

We would like to thank the Coastal Management Program of the Texas General Land Office for their generous support of this project as well as patience and understanding of delays caused by the global pandemic. We would also like to acknowledge and thank the additional graduate and undergraduate researchers who helped with this research: Lauren Rodriguez, Jessica Myers, Grace Grainer, Joel Dominguez, Cassandra Quiroz, Liz Horn, Nicko Barrera, Daniel Lansidel, Elizabeth Hamilton, Daniela Bergman, Tanner Thompson, Demori Lawrence, Jose Rojas, and Maddie Holley. In addition, we would also like to thank Charlie Guillen, founder of Boca Chica Beach Legends, for helping to sample the Rio Grande and Rachel Windham and Dr. Antonietta Quigg of Texas A&M Galveston for helping to sample Galveston Bay. Finally, thank you Will Wolfe for helping with the statistical analysis.

INTRODUCTION

Arguably the most widely used material, plastic is integrated into nearly all aspects of everyday life. This lightweight, corrosion-resistant, high strength, durable, material is derived from synthetic polymers and has forever altered the ecosystems of this planet (Nature Communications, 2018). Synthetic plastic polymers are comprised of long chains of repeating subunits created using both organic and inorganic raw materials.[1] Presently, the most widely used plastics are low- and high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET), which account for 90% of the total plastic production worldwide.[1]

Plastic production almost quadrupled during World War II (1939 - 1945) going from ~95,000 to ~370,000 tonnes being produced annually.[2] This growth accelerated with 368 million tonnes of plastic produced globally in 2019, increasing by almost 11 million tonnes annually since 2010.[3] Since its commercialization, ~5.7 billion tonnes of plastic have been produced worldwide with about 90% being single-use products.[4,5] Approximately 11% of globally generated plastic waste entered aquatic ecosystems in 2016, leading to between 19 and 23 million metric tons of plastic entering the aquatic systems every year.[6] Roughly 80% of marine plastic is from land and enters the environment from multiple sources, including atmospheric transport, beach litter, and watershed runoff. Direct entries to aquatic systems occur as well through sources such as aquaculture, shipping, and fishing activities.[7,8] One of the largest contributors to direct aquatic entry is the fishing industry, accounting for ~18% of the marine plastic debris, by quantity, found in the ocean due in large part to discarded fishing equipment such as nets.[7]

Microplastics exist throughout the global ecosystem and are commonly defined as plastics that are smaller than 5 mm in size.[9] Microplastics can be classified as either primary or secondary microplastics depending on the original production size. Primary source microplastics are plastics that are produced at less than 5mm in size. Sources of these include scrubbers in industrial cleaning products, as well as some pre-production plastic pellets and powder used as the feedstock to make consumer goods.[10] Secondary microplastics are plastics that were originally produced at the macro level that have since degraded to fibers or particulates.[11] Although plastic pellet presence at the water surface was originally documented in 1972 by E. J. Carpenter and K. L. Smith, microplastics are still a relatively new field of study, with many gaps in understanding the pollutant.[1]

Plastic is found throughout the aquatic environment, with one study finding that 92% of all interactions between organisms and marine debris involve plastic.[12] Microplastics exist from pole to pole and are capable of migrating (via currents and atmospheric activity) and accumulating throughout natural habitats, existing in pristine sediments and urban beaches alike.[1] A recent study found that plastic deposition rates at 11 protected areas in the U.S were between ~1,000 to 2,000 tonnes annually.[13] Even if environmental loading ceased, materials already in the environment would continue to fragment, producing smaller microplastics.

Microplastics exist throughout the water column and in sediment, creating the potential for consumption by organisms across multiple feeding guilds.[14] In order of occurrence, PE occurred the most frequently in both ocean and marine sediments, followed closely by PP and PS.[15] Nylon, polyester, acrylic, and other polymers were also found, albeit, less regularly than the other materials. Certain plastics in their raw form, including PS, PVC, and PET, have a density > 1

g cm^{-3} causing them to sink more quickly near point sources. Other plastics, such as PE and PP, have densities < 1 g cm^{-3} and float. Other factors affecting density, such as weathering and biofilm development, act to influence the environmental fate of these plastics.[15] Plastic formation postproduction, such as utilizing PS in its foamed form, can also change the density and eventual fate of the plastic.

Not only acting as a pollutant themselves, but plastics can also further transport other chemicals, acting as sources, sinks, or both for anthropogenic chemicals.[16] First, there are many chemical additives introduced to the plastic during manufacturing that can leach during normal uses or once it enters the environment. However, plastics also sorb substances, including chemicals that are persistent, bioaccumulate, and are toxic (PBTs) from the water column and sediment. and include harmful substances such as polychlorinated biphenyls and dioxins. The tendency of some plastics to float allows them to travel large distances, transporting sorbed chemicals away from sources. Wildlife that consume plastic is then at an increased risk for contaminant exposure and any associated effects.[16]

Research on gross quantification of microplastics is growing but limited in the Gulf of Mexico. One study conducted in Mobile Bay in Alabama found that microplastics were at every single sampling site, with more microplastics being found in higher salinity, marine-influenced locations, and PP and PE being the most abundant microplastics.[17] Another quantification study in Louisiana Continental Shelf waters found that microplastic concentrations were not statistically different from any of the most common taxa found using neuston net sampling.[18] Salt and freshwater studies have produced similar results, finding microplastics everywhere examined. Freshwater fishes and samples from Laguna Madre found that 8 and 10% respectively

had microplastics in their gut tracts.[19] A freshwater study focusing on the freshwater bluegill (*Lepomis macrochirus*) and longear (*Lepomis megalotis)* in the Brazos River Basin in Texas found that 45% of the fish's stomachs contained microplastics.[20] Another animal ingestion study in the Corpus Christi Bay focusing on blue crabs (*Callinectes sapidus*) found that 36% of collected crabs contained fully synthetic and semisynthetic fragments and fibers, averaging 0.87 items per crab.[21]

The objective of this research is to estimate microplastics in coastal stretches of Texas' rivers and bays and the discharge of this contaminant into the Gulf of Mexico through these systems. *Hypothesis:* Microplastics will be found in the coastal stretches of all the river samples as well as the bays and their discharge to the Gulf of Mexico. The highest microplastic concentrations will be correlated with nearby population densities and land use, specifically, Corpus Christi Bay, Galveston Bay, and Trinity River.

MATERIALS AND METHODS

Site Selection

Site selection was developed to incorporate the water flowing into the Gulf of Mexico through Texas. To generate a representative sample, site selection included nine major river systems, bays at river outfalls, and outfalls from bays to the Gulf of Mexico. The rivers sampled include the Trinity, San Jacinto, Brazos, Colorado, Lavaca, Guadalupe, Aransas, Nueces, and the Rio Grande. The 7 Texas bays sampled for microplastic presence and include Galveston, Matagorda, San Antonio, Aransas, Copano, Corpus Christi, and Baffin. Finally, three major outfalls from the bays to Gulf were sampled: Galveston Bay outfall, Matagorda Bay outfall, and the Corpus Christi Bay ship channel (Figure 1, Table 1). These selected sites formed eight watersheds. Galveston Bay

watershed comprised samples from the Trinity River, San Jacinto River, Galveston Bay, and the Galveston Bay outfall. The Brazos River watershed is comprised of entirely the Brazos River and flows directly to the Gulf of Mexico. The Brazos River was the only sample in this watershed. The Matagorda/Lavaca Bay watershed is fed by two rivers, the Colorado and Lavaca River. These rivers flow to Matagorda Bay and then to the Gulf of Mexico through the Matagorda Bay outfall. Both rivers, the bay, and outfall were sampled for this watershed. The San Antonio Bay watershed is fed by the Guadalupe River to San Antonio Bay, both systems were sampled. The Copano/Aransas Bay watershed is fed by the Aransas River that flows into Copano Bay which feeds into Aransas Bay. The Aransas River and both bays were sampled for this watershed. The Corpus Christi Bay watershed is fed by the Nueces River that flows into the Corpus Christi Bay, and to the Gulf of Mexico through the Corpus Christi Bay outfall. All three systems; Nueces River, Corpus Christi Bay, and the Corpus Christi Bay outfall were sampled in this watershed. The Baffin Bay watershed receives few freshwater inflows making Baffin Bay largely an isolated watershed, it was the only sample site in this watershed. As with the Brazos River, the Rio Grande watershed is comprised of only the Rio Grande where it then flows directly into the Gulf of Mexico, it was the only sample site for this watershed.

Coastwide Microplastic Sampling

Samples were collected at 19 sites in Coastal Texas from July 23, 2019, through November 24, 2020 (Figure 1). Triplicates, 1 for every 17 samples, were taken at multiple sampling locations (Trinity River surface, San Jacinto River depth, and Corpus Christi Bay Outfall Surface) to assess sample variability. Triplicates were taken at Matagorda Bay and Baffin Bay but were unable to be processed due to time constraints. They will, however, be processed and analyzed for subsequent

publication. Additional sampling events took place at the Trinity River, San Jacinto River, Corpus Christi Bay, and Corpus Christi Bay Outfall. Additional sampling trips in Galveston Bay were planned but subsequently canceled due to Covid-19 and boat engine problems. River sampling points (coordinates in Table 1) were located at a safe, public access point nearest to the river's discharge into each bay. River samples were collected in the center of the river, where flows tend to be homogenous to ensure representative water samples. Bay sampling points were selected again using aerial imagery as points at or near the center of the bay to represent total levels and not be skewed by nearshore activities. Outfall sampling points were selected using aerial imagery as points that represented the primary outfall from the selected bays to the ocean.

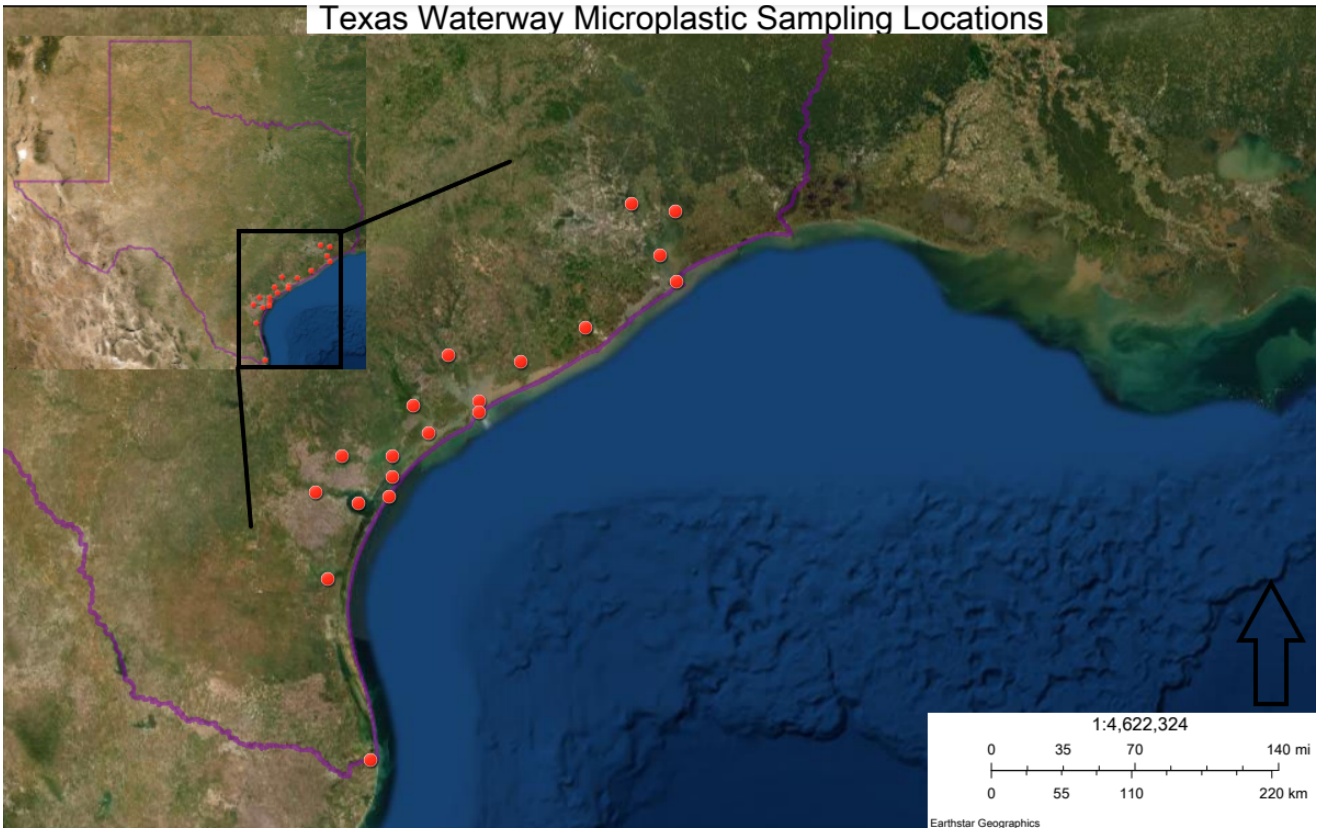

Figure 1. Microplastic sampling sites throughout the state of Texas.

Samples (~4 L) at the surface and 60% of the water column depth, were collected from a boat or kayak depending on location. Surface samples were collected via grab sample. Grab sampling was used for all surface water collections aside from the Galveston Bay samples where

Table 1. Sampling Coordinates, sample depth, and watersheds.

Sample Location	Coordinates	Sample Depth (m)	Watershed
Trinity River	29°50'12.28"N, 94°45'49.05"W	5 m	Galveston Bay
San Jacinto River	29°53'27.76"N, 95°6'42.03"W	4 m	Galveston Bay
Brazos River	29°1'51.22"N, 95°28'39.34'W	12 m	Brazos River
Colorado River	28°47'15.56"N, 95°59'43.50"W	4 m	Matagorda/Lavaca Bay
Lavaca River	28°49'55.78"N, 96°34'38.90"W	2.5 m	Matagorda/Lavaca Bay
Guadalupe River	28°28'35.19"N, 96°51'42.32"W	3 m	San Antonio Bay
Aransas River	28°7'37.20"N, 97°25'39.95"W	2.5 m	Copano/Aransas Bay
Nueces River	27°52'2.79"N, 97°38'22.42"W	2.5 m	Corpus Christi Bay
Rio Grande River	25°57'29.03"N, 97°12'16.58"W	2 m	Rio Grande
Galveston Bay	29°31.9234’N, 94°52.8075'W	2.5 m	Galveston Bay
Matagorda Bay	28°30.683'N, 96°19.781'W	3 m	Matagorda/Lavaca Bay
San Antonio Bay	28°17.143'N, 96°44.276'W	1.5 m	San Antonio Bay
Aransas Bay	27°58'28.56"N, 97°1'26.04"W	2 m	Copano/Aransas Bay
Copano Bay	28°7'27.16"N, 97°1'41.95"W	2 m	Copano/Aransas Bay
Corpus Christi Bay	27°47'14.52"N, 97°17'46.14"W	3 m	Corpus Christi Bay
Baffin Bay	27°15.214'N, 97°32.650'W	2 m	Baffin Bay
Galveston Bay Outfall	29°22.7779'N, 97° 3'3.71"W	5 m	Galveston Bay
Matagorda Bay Outfall	28°26.089'N, 96°20.179'W	8 m	Matagorda/Lavaca Bay
Corpus Christi Bay Outfall	27°50'25.78"N, 97°3'3.71"W	10 m	Corpus Christi Bay

the height from the boat to the water surface was prohibitive and required the use of the Van Dorn sampler (Figure 2). To collect the grab samples, the cubitainer was dipped into the water just below the surface, allowing the water to fill the sample container. All sub-surface samples were collected with a Van Dorn sampler at 60% of each site's depth. Total depth was determined using either the boat's depth finder or by lowering the Van Dorn sampler to the bottom. The sampler can collect ~1.2 L at a time, therefore multiple samples were collected and composited to reach the desired 4 L volume. To minimize contamination carryover between samples, the 1.2 L acrylic tube was rinsed with 500 mL of DI water after each sample. Carryover is a major concern with microplastic sampling methods. Originally, we planned to use a peristaltic pump with PE tubing to collect samples. After the collection of several samples, it became obvious that there was a significant amount of suspected microplastic materials remaining in the tubing that would create problems when assessing and validating our data, in addition to an increase in labor

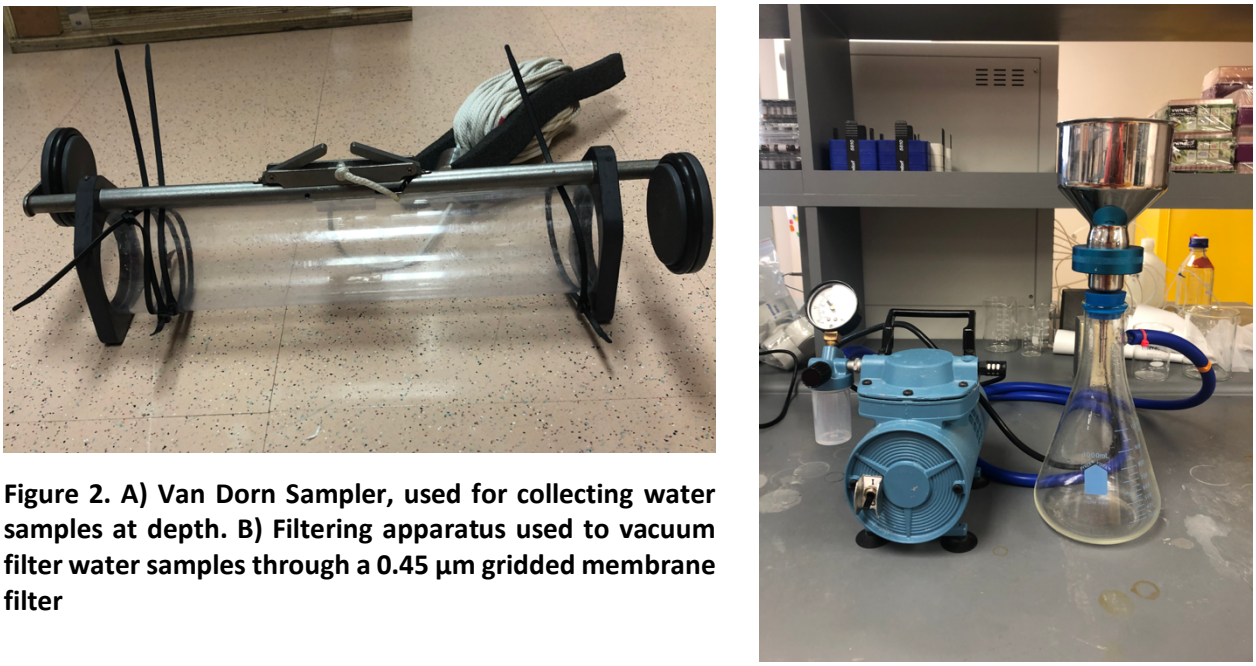

Figure 2. A) Van Dorn Sampler, used for collecting water samples at depth. B) Filtering apparatus used to vacuum filter water samples through a 0.45 µm gridded membrane filter

required. Therefore, the Van Dorn sampler was purchased, and all previously sampled sites were re-sampled using the new method.

Filtering

Samples were filtered using a stainless-steel vacuum filter (Figure 2) with a 0.45 µm pore size gridded cellulose membrane. Due to the suspended solids in the water collected, multiple membrane filters were used per sample. While suspended solids varied, each membrane filter was used to filter ~200 mL of water. After that volume was filtered, the stainless-steel funnel was carefully washed down onto the filter with DI water to ensure no materials remained on the side of the funnel. Membrane filters were then placed onto a 60 mm diameter, polystyrene petri dish and immediately closed to reduce contamination. When the entire sample was filtered, its water volume was recorded. Each sample container was then rinsed twice with 400 mL of UltraPure water to capture any remaining materials. The used filters were then dried at 60 ˚C for a minimum of 4 hours.

Visual Sorting

Each membrane filter was visually inspected and materials that resembled plastic were identified, separated, stored, and characterized. At this phase in sample processing, these materials are referred to as “suspected microplastics” because they have not yet been chemically verified. Suspected microplastics were visually identified using a Meji Techno EMZ-8TR stereo microscope at ~20-35x magnification and placed in a 2.5 cm, 4 mL acrylic screw top vial partially filled with a 70% ethanol solution. Visual identification of microplastics was aided using a key produced by Paul Helm, Kennan Munno, MERI Guide 2017, and the Rochman Lab (Appendix 1). Each suspected microplastic was recorded and logged by its color and shape. Colors included clear, white, red, blue, black, green, brown, yellow, orange, purple, pink, and multicolored. The

two shapes categorized for this study were fibers and particulates. Fibers are cylindrical and spindly, while particulates can be a variety of shapes, but are typically the result of fragmentation from larger plastic debris. Spheres and films are also common shapes discussed in the peer-reviewed literature, but none were found in our samples.

Fourier-transform infrared microscopy (μFTIR) Analysis

Materials analysis methods using Fourier-transform infrared spectroscopy were modeled after.[22] Suspected microplastics were analyzed using a Thermo Fisher Nicolet iS10 FTIR and Thermo Fisher iN5 microscope (μFTIR) equipped with a germanium tip. This instrument was run using attenuated total reflectance (ATR) with each sample spectrum being a composite of 100 sequential runs to improve accuracy. Background (200 runs) was sampled before the analysis of every sample. The samples were run in the IR region and the detector was MCT/A. Additionally, to aid in material identification for items with an ambiguous spectrum, particularly cellulosic items, a picture of each material was taken before analysis. Each item's spectrum was compared with spectra in the on-instrument libraries. This comparison generates a list of materials ranked based on their percent match to the sample material spectra. The matching information helps to initially narrow down possibilities and assess the need for additional analysis of the samples. Library matching cannot be relied on alone because it is limited by the materials that have been input into the library, which may not include the material being analyzed. Additionally, environmental weathering and biofilms may also affect the spectrum of a suspected microplastic resulting in a low percent match or even a mismatch. Therefore, library matching information was considered along with a visual examination of key peaks and valleys in the spectrum that are associated with commonly found microplastic and cellulosic materials. If ambiguity remained

after visual inspection and library matching, the sample was re-analyzed. If a second run did not provide clarity, a third run was performed after re-aligning the sample with the ATR tip. For some items, the picture taken before analysis can also be used to aid in material determination, which is particularly beneficial for cellulosic materials. This multi-step approach to materials identification uses several lines of evidence to support material identification of each item analyzed.

Due to the large number of suspected microplastics identified in the samples, all materials could not be analyzed. Therefore, suspected microplastics within each sample were subsampled at a rate of 10%+ based on their color and shape determined during sorting. For example, if a sample had 6 red fibers, 12 blue fibers, 17 green fibers, and 1 green particle, the analysis would include 1 red fiber, 2 blue fibers, 2 green fibers, and 1 green particle. This results in the verification of >10% of suspected microplastics within each sample. Materials verified using the µFTIR were grouped into three broad categories: Microplastic, Cellulosic and Other. More details about these three categories are presented in the results and discussion.

Sample Processing QA/QC

Microplastics, particularly fibers, are ubiquitous in the air due to shedding from textiles. Fibers were expected to be the main form of microplastics found in this study, therefore all feasible steps were taken to reduce and account for contamination of our samples. First, 100% cotton lab coats were purchased, dyed red, and worn during all sample filtering, sorting, and analysis. Additionally, much of this research was conducted during the COVID-19 pandemic, which required the use of face masks. Our lab made red, 100% cotton face masks. However, the design required the use of red paracord, which was nylon. Red was chosen because previous

microplastics work in our lab found few red fibers in samples. Lab coats and masks were expected to shed, but because they were red, it would be easier to assess the sources of these fibers than if lab coats and masks were white or other colors that have been commonly found in samples and potentially blanks. Other airborne contamination was also controlled or mitigated when possible. This included plastic sheet curtains for doors in our microscope and µFTIR analysis rooms. These sheet curtains move less air than an opening or closing door, which could potentially disturb an open sample as well as dust and particulates on surfaces in those rooms. Dust and particulates on surfaces were also reduced through regular cleaning using a "Swiffer" at the end of workdays. Fibers from these lab coats, face masks, face mask paracord, and Swiffer were analyzed using the µFTIR as described below and added to an in-house library to help identify these potential contaminants in our samples and blanks.

Equipment and sample blanks were generated at a rate of one for every 13 samples. Equipment blanks were taken by cleaning the Van Dorn sampler with DI water as described above. Then, one end of the Van Dorn sampler was closed and the sampler was filled with UltraPure water. The second end was then closed, and the sampler was shaken vigorously for 20 seconds. Finally, one end was opened, and the water was poured into a cubitainer. Sampling blanks were recorded while sampling by filling a cubitainer with UltraPure water and leaving the container open while sampling to capture a representative amount of contamination that the sample might receive. Multiple blanks were generated during sample processing and analysis. The filtering blanks consisted of a 0.45 µm membrane filter placed in a petri dish that was left open to account for potential sample contamination during filtering. One filtering blank per water sample was used for an easy 1:1 comparison. Similarly, to the filtering blank, one sorting blank

was used per sample. This was done the same as the filtering blank, by leaving an open filter next to the processing for the entire duration of the sorting, creating two blanks per sample.

Data Analysis

For the data collected, there were 3 groupings that were characterized statistically: surface vs. depth, differences between rivers, bays, and outfalls, and lastly, watersheds. These 3 categories were analyzed for both microplastics and cellulosic items. Using the Skewness-Kurtosis to test normality, with the standards of both skewness and kurtosis values to be between –2 and 2, it was found that both surface and depth for microplastic values were normally distributed, while neither surface nor depth for cellulosic values were normal. As with surface and depth, the river, bay, and outfall distributions for microplastic values were normally distributed, as were the outfall values for cellulosic materials, however, the rivers and bays were not for cellulosic materials. Within the watersheds for microplastic values, Galveston Bay, Matagorda/Lavaca Bay, San Antonio Bay, and Corpus Christi Bay were all normal, while Copano/Aransas Bay was not. The Brazos River watershed, Baffin Bay watershed, and Rio Grande watershed were not tested due to not having enough samples. For watersheds dealing with cellulosic materials, only Matagorda/Lavaca Bay and Copano/Aransas Bay watersheds were normal, while Galveston Bay, San Antonio Bay, and Corpus Christi Bay watersheds were not. Again, the Brazos River, Baffin Bay, and the Rio Grande watersheds had too few values to test. To analyze differences between surface and depth, an independent two-sample t-Test was run. This test was run for the microplastic category only due to the data being normally distributed. The t-value was .21 with a p-value of .84, showing that there was not a significant difference between the two groups. To analyze the multiple categories of waterbody type and the different watersheds, an ANOVA

single factor test was utilized. For microplastics in rivers, bays, and outfalls, the F-value was .20, F-Crit 3.21, and p-value .82, indicating that there were no significant differences between the groups. Analyzing the watersheds for differences in microplastic values, again utilizing an ANOVA single factor test, no significant differences were found with an F-value of 1.33, F-Crit value of 2.26, and a p-value of .26. When running the differences between cellulosic materials in different watersheds, the ANOVA single factor test results showed there were significant differences between the watersheds with a p-value of 3.16E-6. Further analysis using the Tukey test, however, showed that the only differences were between samples that were not normally distributed and thus discounted.

Nueces Bay

A focused and more intensive study of microplastics in Nueces Bay was also conducted and is summarized in the results and discussion below, but the full extent of the project is described by a separate report in Appendix 4. For this study samples were collected at 8 sites, 1 in the Nueces River near Hazel Bazemore Park and 6 in Nueces Bay. Sites were sampled in duplicate on August 28, 2019, and August 1, 2020. Methods for sample collection, processing, and analysis are similar to those described above for the coastwide microplastic study. Additional details can be found in Section 2 of Appendix 4.

RESULTS

Coastwide Suspected Microplastic & Blank Summary

For the coastwide study, a total of 52 water samples were collected across the 19 sampling sites. From these samples 4,999 suspected microplastics were found, averaging 91.8 ± 64.7 per sample. Fibers (77%) dominated particulates (23%) in our samples. The most common suspected

microplastic colors were clear (44%), black (15%), red (14%), and blue (13%), accounting for 86% of materials sorted from samples. Within suspected microplastic fibers, these percentages are roughly similar with 44% clear, 18% red, 16% blue, and 13% black. For suspected microplastic particulates the most common colors were clear (45%) black (21%), yellow (10%), and white (8%).

With all microplastic research, contamination can be minimized but is only eliminated under highly controlled conditions that go beyond the capabilities of most researchers. As noted above in the methods, many steps were taken to reduce sample contamination. To quantify and account for the potential contamination of samples blanks were used. While suspected microplastics were analyzed at a rate of 10%+, every item found in blanks was analyzed using the μFTIR. If an item found in the blank matched the color, shape (particulate or fiber), and material of an item from its associated sample, that item was subtracted from the final concentration of the sample. This 3-factor match for blank correction reduces the potential to over-correct contamination in samples.

While mitigation efforts likely reduced the total number of items in blanks, 329 suspected microplastics were found across the 122 blanks produced in this study, with 88% being fibers. The use of red lab coats and masks during lab processing was helpful, with 16% of materials in blanks being red. However, clear (59%) comprised the majority, while 15% were blue. Of these 329 items, 92% were cellulosic and 8% (21 items) were microplastic. The majority (86%) of microplastics were fibers, with PET, PP, PE, and PS accounting for 44% (9 fibers), 22% (4 fibers), 19% (4 fibers), and 11% (2 fibers). One particle each of PET, PP, and PVC was found in blanks. If an item in a blank and sample had identical color, shape, and plastic polymer type it was assumed that the sample was contaminated and the final concentration of materials in the sample was

reduced based on the amount from the blank. All results discussed below are for final, blank corrected data.

Coastwide Data Categorization

Of the 4,999 suspected microplastics found, 14.8% (741 items) were analyzed with the µFTIR. On average 20.2% ± 7.8% of suspected microplastics from each sample were analyzed. Results from the materials analysis are grouped into three categories: microplastic, cellulosic, and other. The materials included in the "other" category, which were not microplastic or cellulosic, are not relevant to this study and will not be discussed after this section. Microplastics are considered fully synthetic polymer materials and were the focus of this work. They include materials like PE, PP, PET, PP, etc. The spectra for these plastics are unique and often easily distinguished from the other materials sorted as suspected microplastics. Materials in the cellulosic category (cotton, linen, rayon, cellophane, etc.) are more challenging to differentiate because they have nearly identical FTIR spectra, which is that of cellulose. Differentiation is even more challenging with items from environmental samples that have undergone weathering and aging that could minimize distinguishing spectral properties of a particular cellulosic material. Despite being made from cellulose, this group of materials is sometimes referred to as "semi-synthetic" due to their chemical alterations.[23] Cotton and linen are plant-based with their chemical structure intact. However, when made into textiles they are often dyed and may have other chemicals added depending on manufacturer specifications. They may also sorb chemicals during their normal use or after entering the environment. Chemical additions to cotton and linen are not well studied from an environmental effect standpoint or with regards to how they affect material persistence. Rayon and cellophane are also cellulosic but have been chemically broken down and regenerated

into these consumer materials. Like linen and cotton, they may also have chemicals added or sorbed to them. Due to the complete chemical regeneration and chemical additions, we believe it is important that attempts are made to characterize these materials when possible. Therefore, we have reported total cellulosic materials in this report, but since they were not part of the original scope of work and we have run out of time, they have not been further sorted into specific materials. However, additional efforts may be made to further break this category down into the actual materials. Data for microplastic materials are reported both as total microplastic and for individual materials within samples, while cellulosic data is only reported at the category level.

Coastwide microplastic and cellulosic materials Texas waters

Suspected microplastics were identified as 53.8% cellulosic and 21.7% microplastic. While no significant difference was observed, Figure 3 shows that generally, bays had a higher proportion of cellulosic materials compared to what was found in rivers and the outfalls. The average for all samples was 11.1 ± 11.4 cellulosic items per liter (C L^{-1}) and 4.0 ± 2.9 microplastics per liter (MP L^{-1}). The concentrations of microplastics and cellulosic materials were not significantly different across bays, rivers, or discharges to the Gulf of Mexico. Differences were also not observed between samples collected at the surface or depth. As can be seen in Table 2, cellulosic materials had higher concentrations, but also higher standard deviations compared to the microplastics. Cellulosic materials averaged between 7.8 and 16.2 C L^{-1} with a standard deviation ranging from 3.5 and 21.8 C L^{-1}. The average for microplastics was 3.0 and 4.9 MP L^{-1}, with standard deviations ranging from 2.0 - 3.6 MP L^{-1}. This indicates that concentrations were fairly consistent across all sampling sites despite differences in land use and population densities among Texas' coastal

Table 2. Blank corrected average concentrations and standard deviations for microplastics and cellulosic materials across all samples collected.

	Microplastics MP L^{-1}	Cellulosic C L^{-1}
All Samples	4.0 ± 2.9	11.1 ± 11.4
River Surface	3.7 ± 3.7	16.2 ± 21.8
River Depth	4.3 ± 2.1	9.0 ± 8.1
Bay Surface	4.9 ± 3.3	16.0 ± 11.2
Bay Depth	3.7 ± 3.6	7.8 ± 3.5
Outfall Surface	4.2 ± 2.4	9.6 ± 8.1
Outfall Depth	3.0 ± 2.0	11.3 ± 6.7

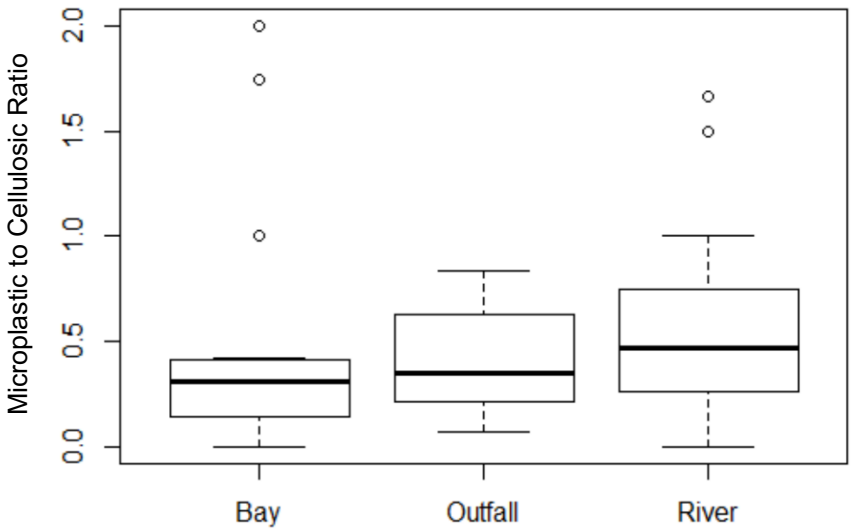

Figure 3. Box and whisker plot showing the ratio of microplastics to cellulosic materials. Lower values indicate a higher proportion of cellulosic materials

watersheds. Microplastic and cellulose concentrations observed at each site are shown in Appendix 2. The highest, although not significant, microplastic and cellulose concentrations were observed in the Rio Grande (Figure 4, Appendix 2). The broad sampling design coastwide survey was intended to assess baseline microplastics in Texas' Coastal systems. However, due to delays throughout the project due to instrument repairs and the pandemic, we were unable to

intensively sample a few of the systems as originally planned. This limits our ability to find significant differences between the systems examined and assess the influence of land use and population density. Additionally, as methods improve, higher spatial and temporal resolution sampling will enable greater sample throughput and the ability to observe significant differences between watersheds.

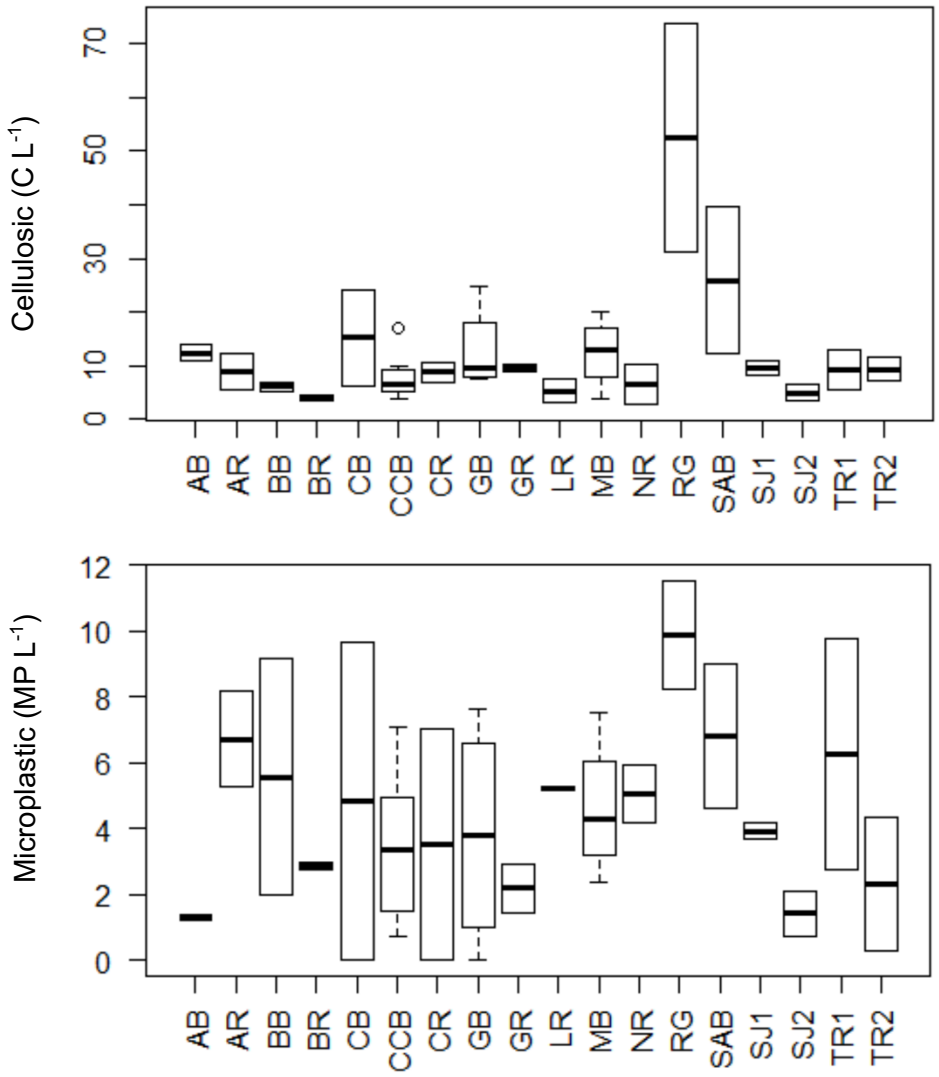

Figure 4. Concentrations of A) cellulosic and B) microplastics in bays and rivers. AB: Aransas Bay, AR: Aransas River; BB: Baffin Bay; BR: Brazos River; CB: Copano Bay; CCB: Corpus Christi Bay; CR: Colorado River; GB: Galveston Bay; GR: Guadalupe River; LR: Lavaca River; MB: Matagorda Bay: NR: Nueces River; RG: Rio Grande; SAB: San Antonio Bay; SJ1: San Jacinto River (1st sampling); SJ2: San Jacinto River (2nd sampling); TR1: Trinity River (1st sampling); TR2: Trinity River (2nd Sampling)

Microplastics in Coastal Texas

Microplastics were found in all but five of the 52 samples across the 19 sample locations. Of all the microplastic observed, polyethylene terephthalate (38.5%) and polystyrene (33.3%) were the most common, while all others accounted for <8% individual (Table 3). Polyethylene terephthalate was the dominant fiber (61%), while polystyrene accounted for 79% of particulates.

Table 3. Frequency and shape of microplastics.

	Total	Fiber	Particulate
		%	
Polyethylene terephthalate	39	61	7
Polystyrene	33	4	79
Polyethylene	8	9	7
Nylon	7	9	5
Acrylic	6	10	nd
Polypropylene	4	4	3
Poly Urethane	3	3	nd
PVC	>1	1	nd

Accounting for distribution between surface and depth; PET, PP, and PS were all almost identically distributed. Polyethylene and Nylon were found almost 50% more frequently at the surface, while acrylic and PVC were found entirely at the surface.

Loading to the Coastal Zone.

Texas' coastline stretches 367 miles with nine major riverine systems that discharge to seven coastal bays or directly to the Gulf of Mexico. Flow rates from USGS stations near river sampling sites were used to estimated discharges of microplastics and cellulosic materials into Texas' bays or the Gulf of Mexico (Table 4). These values are broad estimates of discharge amounts, therefore it is important to consider the magnitude of these values, rather than the exact numbers. There

are ~74 and 217 billion microplastics and cellulosic materials discharged to texas' bays and the Gulf of Mexico. Annually, this equates to ~27 and 79 trillion microplastic and cellulosic materials. These numbers are eye-catching but must also be presented in the context of their size and mass. Microplastics are small, therefore each particle and fiber have a proportional mass. Using a fiber with a length of 30 µm and 2.5 µm radius coupled with the density of PET (1.38 g cm^{-3}), estimates for the mass of an individual item can be calculated and scaled to water concentrations. For cellulosic materials, the same dimensions were used with the density of cellulose (1.55 g cm^{-3}). The estimated annual mass discharge of microplastics and cellulosic materials was 22 kg (49 lbs) and 73 kg (160 lbs).

Table 4. Estimates of microplastic and cellulosic items discharged annually from Texas' rivers into coastal bays or the Gulf of Mexico.

	Microplastics		Cellulosic Materials	
System	**MP Day^{-1}**	**MP Yr^{-1}**	**C Day^{-1}**	**C Yr^{-1}**
Trinity River	27,523,978,200	10,046,252,043,000	40,735,487,736	14,868,453,023,640
San Jacinto River	358,533,457	130,864,711,613	880,449,248	321,363,975,352
Brazos	7,251,650,790	2,646,852,538,262	10,432,199,382	3,807,752,774,342
Colorado River	11,902,591,462	4,344,445,883,484	30,266,589,717	11,047,305,246,574
Lavaca River	14,757,746	5,386,577,095	14,899,647	5,438,371,106
Guadalupe River	4,488,977,351	1,638,476,733,200	19,384,220,380	7,075,240,438,817
Aransas River	85,544,524	31,223,751,350	112,158,376	40,937,807,325
Nueces River	1,139,150,177	415,789,814,555	1,488,790,330	543,408,470,507
Rio Grande	21,532,558,625	7,859,383,898,280	114,296,561,189	41,718,244,833,798
Total	*74,297,742,331*	*27,118,675,950,838*	*217,611,356,004*	*79,428,144,941,462*

Among rivers, discharge amounts of microplastics and cellulosic materials are a function of concentration and discharge volumes. The trinity river, which has the highest discharge (Appendix 3) and the highest population density was the largest contributor of microplastics to

coastal Texas (10 trillion), but a distant second in cellulosic materials (14.8 trillion). The Rio Grande was first in cellulosic materials (41.7 trillion) and second in microplastics (7.8 trillion).

Nueces Bay

Suspected microplastics found in samples from the Nueces River and Bay contained 63% fibers, 27% particulates, and 10% films, which is similar to the coastwide survey. Additionally, the most common color among these materials was also clear. After chemical analysis using µFTIR, samples microplastic concentrations in samples ranged from non-detect to >30 MP L^{-1} (Table 5). The highest microplastic concentrations were observed in the Nueces River at Hazel Bazemore Park (Figure 1 in Appendix 4). Cellulosic concentrations were lower in this study than microplastics, which contrasts the coastwide survey. However, both microplastic and cellulosic concentrations were generally similar to the coastwide survey, except in the Nueces River. The microplastic polymers found at all sites and the highest concentrations were polyester (0.3 to 2.4 MP L^{-1}) and polystyrene (0.4 to 11.5 MP L^{-1}) (Table 6).

Table 5. Concentrations of microplastic and cellulosic materials in the Nueces River at Hazel Bazemore Park (HBP) and the 6 sites within Nueces Bay for samples collected in August 2019 and 2020.

Sites	HBP	NB1	NB2	NB3	NB4	NB5	NB6
	MP or C L^{-1}						
Fully Synthetic							
Particulates	16.3 ± 10.1	4.4 ± 5.1	6.8 ± 0.6	1.6 ± 0	1.2 ± 1.7	1.6 ± 2.3	2.8 ± 1.9
Fibers	27.5 ± 11.1	23.2 ± 21.5	13.6 ± 11.3	7.2 ± 4.5	1.6 ± 0	8.8 ± 9.1	6 ± 3.8
Films	1.3 ± 0.5	5.2 ± 2.8	6.4 ± 1.1	0.4 ± 0.6	0.8 ± 0	1.2 ± 1.7	1.8 ± 0.4
Cellulosic							
Particulates	3.7 ± 3.8	4.8 ± 4.5	1.2 ± 1.7	2 ± 0.6	nd	2.8 ± 2.8	0.4 ± 0.5
Fibers	22.1 ± 2	16.4 ± 15.3	16 ± 17	11.6 ± 2.8	4.4 ± 2.8	11.2 ± 13.6	6.8 ± 3.7
Films	nd	1.6 ± 2.3	1.2 ± 1.7	0.4 ± 0.6	nd	0.8 ± 1.1	0.2 ± 0.4

Table 6. Concentrations of microplastic in the Nueces River at Hazel Bazemore Park (HBP) and the 6 sites within Nueces Bay for samples collected in August 2019 and 2020.

Fully Synthetic	HBP	NB1	NB2	NB3	NB4	NB5	NB6
				MP L^{-1}			
Acrylic	nd	nd	1.2 ± 0.6	0.4 ± 0.6	nd	0.4 ± 0.6	nd
Polyester	2.4 ± 0.8	1.6 ± 2.3	2.4 ± 3.4	1.2 ± 0.6	0.3 ± 0.5	0.8 ± 1.1	0.4 ± 0.8
Polyethylene terephthalate	0.3 ± 0.5	nd	0.8 ± 1.1	0.8 ± 0	nd	0.8 ± 0	nd
Polyetherurethane	nd	1.6 ± 1.1	nd	nd	nd	nd	nd
Polyethylene	2.1 ± 2.4	nd	0.8 ± 1.1	nd	nd	1.2 ± 1.7	0.8 ± 1.6
Polyhexyl acrylate	nd	nd	nd	nd	nd	nd	0.2 ± 0.4
Polypropylene	2.4 ± 2.1	nd	4.8 ± 1.1	nd	nd	nd	0.2 ± 0.4
Polystyrene	11.5 ± 7.4	5.2 ± 5.1	7.6 ± 2.8	0.4 ± 0.6	1.3 ± 2.3	1.6 ± 2.3	0.6 ± 0.4
Rubber	nd	nd	nd	nd	0.3 ± 0.5	nd	nd
Acrylonitrile Butadiene Styrene	1.9 ± 1.8	1.2 ± 0.6	1.6 ± 1.1	nd	nd	nd	nd
Nylon	1.3 ± 2.3	nd	0.4 ± 0.6	0.4 ± 0.6	nd	0.8 ± 1.1	0.8 ± 0.7
Olefin	0.5 ± 0.5	0.8 ± 0	1.2 ± 0.6	nd	nd	nd	1 ± 0.8
Kodel	0.3 ± 0.5	nd	nd	nd	nd	nd	nd

DISCUSSION

This study worked to identify the loading of microplastics to the Gulf of Mexico through Texas waterways. Microplastics were found throughout the state, at both surface and depth of the water bodies sampled. Fibers were the most prevalent fiber shape and clear was the most frequent color. The hypothesis that microplastics will be found in the coastal stretches of all the river samples, as well as the bays and their discharge to the Gulf of Mexico, was correct. While some of the samples did not have microplastics at either the surface or the depth, other samples from those watersheds and bays did have microplastics. No significance was identified between the coastal systems examined and therefore the influence of land use and population density on microplastic loads was not assessed.

The morphology distribution from this research was comparable to the values from Lake Superior that found 70% of microplastics as fibers (Minor et al., 2020). Similarly, the Northwest

Mediterranean Sea also reported fibers to be the predominant microplastic, as well as PET being the primary material type (Lefebvre et al., 2019). However, their total microplastic concentration was lower (0.00023 ± .00020 MP L^{-1}). Research on the Thames River in England showed that polyethylene and polypropylene were the most common material types, but as with the Mediterranean Sea, the concentration was much lower at .0248 MP·L^{-1} (Rowley et al., 2020). A study geographically to this one, along the Southeastern coast of the United States, also found PET to be the most common microplastic sampled (Yu et al., 2017).

With there being no significant difference between the watershed values, population densities in the respective watersheds were not compared, though, more robust sampling could allow for significant differences to be found and population densities to be addressed. Another variable that could be addressed with more robust sampling would be land usage. The coastal bend area has high agricultural activity, especially cotton. Between harvesting and transportation, this activity could artificially increase the per liter volume of cellulosic materials in watersheds where cotton production and transport are high. Other variables that could be addressed include dams on different river systems, as well as international regulations and influence along the Rio Grande.

One important note is that the current and widely accepted methods for processing microplastic samples require visual identification of suspected microplastics by researchers using a microscope. Researchers are trained to recognize suspected microplastics by shape, color, malleability, and a few other context clues. They also use a sorting key to help focus on items of interest. Once sorted, the filtered samples are re-checked by another researcher at a rate of 1 per 20 samples. This ensures that samples were screened to similar standards. However, these

visual methods of sorting microscopic materials are likely producing a conservative amount of suspected microplastics. Subsequently, the results of this work are also believed to be conservative, rather than an overestimation of the microplastics and cellulosic materials found in the samples collected.

This study provides a coastwide baseline microplastic and semi-synthetic cellulosic material concentrations in Texas. As we learn more about the presence, fate, and impacts of microplastics and semi-synthetic materials, including those described above as cellulosic, this data will help to understand the role of these materials in coastal ecosystems. The number of microplastics and semi-synthetic cellulosic materials that enter Texas' coastal bays and the Gulf of Mexico annually is in the 10s of trillions although their combined mass is ~95 kg (209 lbs). The total mass seems small, while the total numbers are eye-catching. These values provide context for the problem, but ultimately the most important aspect to consider is the potential impacts of these materials on ecosystems, aquatic organisms, and humans. Those impacts are beyond the scope of this work but given their small size and numbers in the trillions, microplastics are likely to interact with aquatic organisms. Additional sampling, particularly more intensive sampling to understand individual systems or compare systems would provide more information for decisions makers, but much of the future focus of microplastics research in Texas should focus on understanding organismal exposures and quantification of effects.

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APPENDIX 1. MICROPLASTIC SORTING KEY

Microplastic key used to aid visual sorting of fully and semi-synthetic microplastic materials. Key developed by the Rochman Lab at the University of Toronto.

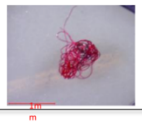
MICROPLASTICS CATEGORIES

FIBERS

- Equally thick throughout (can differentiate from fragment with this and that it can be bend)
- Strand/string-like and any colour (can change colour halfway through fiber due to bleaching)
- 3 dimensional bending (straight indicates biological origin so those don't count)
- Ends: flat, pointed, or can also be fraying at the end

FIBER BUNDLES

- 20+ individual fibers that cannot be teased apart (tightly wound, separation would cause breakage)
- Consistent appearance



FRAGMENTS

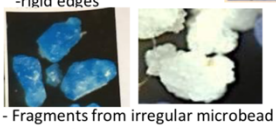
- Irregular shapes (can be rounded, subrounded, subangular, angular)
- Have rigid edges
- Hard, unable to compress
- Includes black materials including: soft black-tar like material, "rubbery" material
- Can be any colour, or multicoloured

Examples:

- Long fragments that change thickness throughout

- Fragments from commercial activity – appear twisted and curled because they have been shaven off, may look like melted material/droplets

-rigid edges

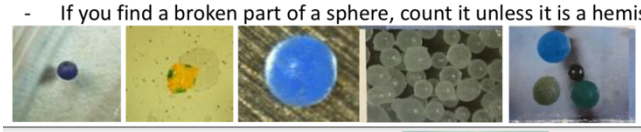


- Fragments from irregular microbead from product – asteroid-like shape with bumpy nodule pocketed edges, usually is shiny

MICROPLASTICS CATEGORIES

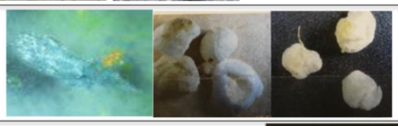
SPHERES

- Perfectly spherical in shape (size range is from 100um to 2mm)
- Smooth and sometimes glossy surface
- If you find a broken part of a sphere, count it unless it is a hemisphere (half sphere)



FOAM

- Soft, compressible, cloud like shape
- Normally whitish colour or opaque



FILM

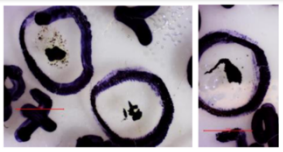
- Thin, flat, flexible sheets, can fold or crease
- Usually clear or somewhat clear
- Check with tweezers to see if it breaks easily (then its not film)

PELLETS

- Similar to spheres, but bigger (3 – 5mm), rounded or cylindrical

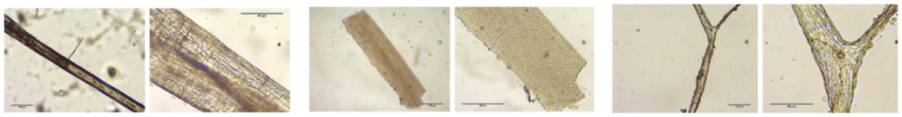
RUBBER

- Often black
- Stretchy, resistant to breakage
- Often cone-shaped or S-shaped (tire dust)



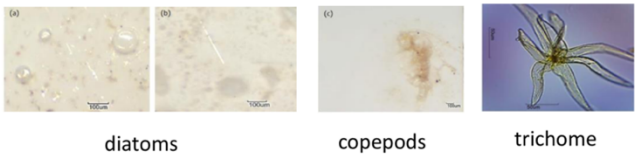
2

Not Microplastic



Algae

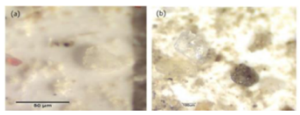
Very brittle, breaks apart when prodded



diatoms copepods trichome

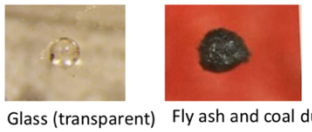
Animals, Animal Parts, Shells

brittle, usually breaks apart when prodded, may be in form of geometrical shape, or spring back when prodded



Salt Crystals and Sand

Salt crystal break apart when prodded and sand will also break and sound like breaking glass



Glass (transparent) Fly ash and coal dust

Spherical like objects

3

Sources: Paul Helm, Keenan Munno, MERI Guide 2017, Chelsea Rochman's Lab

APPENDIX 2. CONCENTRATION OF MICROPLASTIC & CELLULOSIC MATERIALS

River concentrations of microplastics and cellulosic materials

Site	Type	Depth	Microplastic MP L^{-1}	Cellulosic C L^{-1}
Guadalupe River	River	Surface	1.5	10.2
Guadalupe River	River	Depth	2.9	8.8
Nueces River	River	Surface	5.9	10.4
Nueces River	River	Depth	4.2	2.8
Trinity River 1	River	Surface	9.8	13.0
Trinity River 1	River	Depth	2.7	5.5
Trinity River 2	River	Surface	0.3 ± 0.6	7.2 ± 2
Trinity River 2	River	Depth	4.3	11.5
Rio Grande	River	Surface	11.6	73.9
Rio Grande	River	Depth	8.2	31.2
San Jacinto 1	River	Surface	3.7	11.0
San Jacinto 1	River	Depth	4.2	8.4
San Jacinto 2	River	Surface	2.1 ± 0.5	6.4 ± 3.9
San Jacinto 2	River	Depth	0.7	3.6
Brazos River	River	Surface	2.7	3.7
Brazos River	River	Depth	3.0	4.5
Lavaca River	River	Surface	5.3	7.4
Lavaca River	River	Depth	5.1	3.1
Aransas River	River	Surface	8.2	5.4
Aransas River	River	Depth	5.3	12.3
Colorado River	River	Surface	0.0	10.8
Colorado River	River	Depth	7.0	7.0

Bay concentrations of microplastics and cellulosic materials

Site	Type	Depth	Microplastic MP L^{-1}	Cellulosic C L^{-1}
Baffin Bay	Bay	Surface	2.0	6.9
Baffin Bay	Bay	Depth	9.2	5.2
San Antonio Bay	Bay	Surface	9.0	39.7
San Antonio Bay	Bay	Depth	4.6	12.3
Aransas Bay	Bay	Surface	1.2	10.9
Aransas Bay	Bay	Depth	1.4	13.9
Copano Bay	Bay	Surface	9.6	24.1
Copano Bay	Bay	Depth	0.0	6.4
Matagorda Bay	Bay	Surface	4.5	13.6
Matagorda Bay	Bay	Depth	7.5	3.8
Corpus Christi Bay 1	Bay	Surface	7.1	17.0
Corpus Christi Bay 1	Bay	Depth	6.0	6.0
Corpus Christi Bay 2	Bay	Surface	3.4	8.4
Corpus Christi Bay 2	Bay	Depth	1.2	7.1
Galveston Bay	Bay	Surface	2.0	7.5
Galveston Bay	Bay	Depth	0.0	8.1

Bay outfall concentrations of microplastics and cellulosic materials

Site	Type	Depth	Microplastic MP L^{-1}	Cellulosic C L^{-1}
Matagorda Bay	Outfall	Surface	4.1	12.2
Matagorda Bay	Outfall	Depth	2.4	20.2
Corpus Christi Bay 1	Outfall	Surface	1.8	4.9
Corpus Christi Bay 1	Outfall	Depth	3.3	4.0
Corpus Christi Bay 2	Outfall	Surface	3.9 ± 2.3	5.2 ± 2.1
Corpus Christi Bay 2	Outfall	Depth	0.7	10.1
Galveston Bay	Outfall	Surface	7.7	24.9
Galveston Bay	Outfall	Depth	5.6	11.1

APPENDIX 3. RIVER FLOW RATES

Rivers and flow rates at time of sample and yearly average in cubic feet per second (CFS)

System	Flow rate at time of sample (CFS)	Average flow rate (CFS)
Trinity River	1,800.0	14,000.0
San Jacinto River	37.1	149.2
Brazos	1,040.0	4,261.0
Colorado River	1,390.0	934.1
Lavaca River	1.2	59.1
Guadalupe River	834.0	922.0
Aransas River	5.2	11.7
Nueces River	92.2	106.6
Rio Grande	889.0	1,100.0

APPENDIX 4. SUB-REPORT ON STUDY OF NUECES BAY.

Final Report

Quantifying Microplastic (Particles and Fibers) Loading to the Texas's Coastal Bays and Estuaries

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ABSTRACT

Every year, about 300 million tons of plastics are produced globally, which leads to the generation of massive amounts of plastic waste, a portion of which enters the environment through aquatic pathways such as bays, creeks, and rivers. Plastic particles are categorized by size rather than shape. A category of plastic particles are microplastics (< 4.75 mm). Within microplastics, microfibers, a subcategory of microplastics, are a result of repeated washing of clothing made of polyester, acrylic, nylon, and rayon. Since little is known about the environmental impacts produced by microfibers, there are no current regulations or guidelines. In the last three decades, microplastics have been the primary focus on numerous field studies in global water systems (i.e., bays, rivers, oceans). To understand the extent of microplastic pollution and to mitigate microplastics impacts in the South Texas region, this study quantified and characterized the microplastics (particles, films, and fibers) in Nueces Bay. It was the first microplastics pollution study conducted in the region. Water samples were collected at various locations in Nueces Bay. Samples were analyzed for microplastic concentrations and properties such as size, shape, and color and plastic polymer types. Potential factors affecting the loadings and concentration levels of microplastics observed have been examined. Understanding the abundance and characteristics of microplastics loading in Nueces Bay will help local authorities, researchers, and regulators to

gain insights on the extent of microplastics pollution and their potential effect on the local fishing industry and marine life and to look for ways to mitigate their impacts.

1. INTRODUCTION

Plastics are known as materials that are easy to manipulate, durable, lightweight and inexpensive to manufacture (McEachern et al., 2019). When marine litter such as plastics enter global water systems, a series of issues will occur as the plastics (microplastics) begin to slowly break down. Microplastics (MPs) can be easily transported by water, wind, and air due to their sizes (Auta et al., 2017). Microplastics have been found in the deep sea, ocean basins, coastal sediment, beaches, estuaries, and bays (McEachern et al., 2019). As MPs circulate through global water systems, their potential effects on marine and human life have created a new global environmental concern. In recent studies, researchers have reported an increase in global MPs concentration and a need to identify microplastic pollution sources, thus, studying the impacts of MPs on humans and marine life has increased (Auta et al., 2017). To manage the increasing microplastic pollution and the toxicity associated with MPs and to protect our water resources, human health, and marine life in global water systems, it is important to study MPs pollution in water.

Although there have been dozens of studies about microplastic toxicity on birds, fish, mussels, and oysters, toxicity effects on marine life and birds are relatively unknown or not fully understood (Wang et al., 2019). However, the present evidence suggests that MPs are toxic to marine life (McEachern et al., 2019). MPs cause various health problems in fishes and birds such as mechanical damage and digestive tract blockage (Wang et al., 2019). Furthermore, if a single celled marine organism like ciliate ingests a hazardous substance like a microplastic, this will create a potential pathway and any toxic chemicals absorbed by the microplastic will be ingested

by a larger marine organism (Wright and Kelly, 2017). Fish, whales, and birds are especially susceptible to MPs due to mistaking them for natural prey or as a source of food (McEachern et al., 2019, Wang et al., 2019). An abundance of MPs or their accumulation within marine organisms can cause physical harm, starvation or even death due to toxicity, internal abrasions, and blockages of the digestive tract (Wright et al., 2013).

Barboza et al. (2020) studied bisphenols A (commonly known as BPAs) in fish found in the Atlantic Ocean and they reported that MPs may be source of bisphenols in fish. A concentration range of 5-302 ng/g dry weight of BPA found in fish livers was reported (Barboza et al., 2020). This concentration is higher than the daily intake, hazard index and hazard quotient established by the European Food Safety Authority (Barboza et al., 2020). Barboza et al. (2020) suggested that more research is required to determine if fish can be contaminated from MPs and if there is a food safety issue.

Marine life ingests MPs due to their small sizes. According to Pico and Barcelo (2019) and Hartman et al., (2015), plastics can be classified into four groups based on their sizes (i.e., nanoplastics, microplastics, mesoplastics, and macroplastics). Nanoplastics range from 1 nanometer to 1 micrometer (Pico and Barcelo, 2019). Microplastics have an upper size limit of less than 4.75 mm (Eriksen et al., 2013). Mesoplastics can range from 1 millimeter to 2 centimeters (Pico and Barcelo, 2019). Macroplastics are larger than 2 centimeters (Pico and Barcelo, 2019).

The growing interest in MPs has led to a variety of studies that are mainly focused on the harmful effects in marine life, identification of microplastics and methodology reviews. During the last three decades, studies on the sources and abundance of MPs and comparisons of different methodologies have been conducted. Studies conducted by McEachern et al. (2019), Akdogan and Guven (2019), and Keisling et al. (2020) indicated that most current research work do not fully

consider the effects of spatial distribution, which can affect the concentration of MPs found in a particular area (McEachern et al., 2019). According to McEachern et al. (2019), most studies are an "one-time effort". McEachern et al. (2019) conducted the first MP study of Tampa Bay and investigated the temporal variations of MPs. They found during intense rainfall events, the highest MPs concentrations were recorded. Keisling et al. (2020) indicated that MPs spatial distribution patterns will not differ if population density increases or decreases. Microplastic spatial distribution between environmental compartments (e.g., soil vegetation, indoor dust, animals, and humans) can be shaped by physical processes (i.e., wind, tides, surface runoff and flooding) which are changing due to climatic forces (Akdogan and Guven, 2019, National Research Council, 2000). Thus, McEachern et al. (2019) suggested that spatial or temporal patterns of MPs distribution should be considered in microplastic studies.

There are several sources that contribute to MPs pollution such as wastewater discharge and human activities. Due to constant human activities such as tourism, fishing, recreational and maritime use, the microplastic pollution is expected to increase (Andrady, 2011, Yu et al., 2018, Retama et al., 2016, McEachern et al., 2019). It is also estimated that litters from land-based sources (i.e., beaches and fishing piers) contribute about 80% of plastic debris (Andrady, 2011). Wastewater discharged MPs are considered a primary source of microplastic pollution (Yu et al., 2018). In addition, it was also found that MPs released in Cuba entered the Gulf of Mexico, where they circulated for a long period due to smaller eddies in the Gulf of Mexico (Yu et al., 2018).

Located on the Gulf Coast, Corpus Christi is known as the city on the bay. In 2016, Corpus Christi had a population of 325,733. The climate in Corpus Christi is mild, but very warm and humid during the summer months with a southeast wind. The yearly average precipitation in Corpus Christi is 31.7 inches. The Corpus Christi Bay is located along the Gulf of Mexico and

entirely in Nueces County. The Nueces River feeds directly into Nueces Bay which is a saltwater estuary. Nueces Bay has an average depth of two to three feet (NOAA, 2020). The Port of Corpus Christi, the fourth largest port in Texas, is conveniently connected to the ship channel and the Gulf of Mexico. In addition to the port, Corpus Christi's economy relies heavily on oil and gas refineries such as Flint Hills, CITGO, and Valero, fishing, and tourism to the various beaches along the coast, such as Padre Island and Mustang Island.

In a study conducted by Yu et al. (2018), sand samples from the Padre Island National Seashore located in Corpus Christi, Texas, were examined for MPs and the data was applied to a Regional Ocean Modeling System (ROMS). The model was used to predict the spatiotemporal distribution of potential MPs with a primary focus on coastal cities and the transport of MPs (Yu et al., 2018). The field samples slowed that Padre Island National Seashore had an average fiber length of 1.25 cm (Yu et al., 2018). The study also indicated that the spatiotemporal distribution of MPs in coastal regions such as Nueces Bay is an important factor to consider in microplastic research (Yu et al., 2018).

In addition to determining the sources (i.e., primary or secondary) of microplastic pollution, the effects of MPs on marine life (i.e., toxicity) are equally important to microplastic research and analysis. Despite the numerous studies of MPs conducted in various water systems around the United States, there are not any MPs studies focusing on the Gulf of Mexico specifically Nueces Bay. Due to their comparable size to planktonic organisms, MPs are easily digested by filter and deposit feeders, detritivores and planktivores (Browne et al., 2008; Graham and Thompson, 2007; Murray and Cowie, 2011; Thompson et al., 2004). An abundance of MPs or their accumulation within marine organisms can cause physical harm, starvation or even death due to toxicity, internal

abrasions, and blockages of the digestive tract (Wright et al., 2013). Furthermore, harmful chemical additives found in MPs could be transferred to marine life (Thompson, 2015).

Corpus Christi is a well-known fishing destination along the Gulf of Mexico. In Corpus Christi and surrounding areas, there are around 18 piers and, there are two piers along Nueces Bay. According to the Economic Impacts of Recreational Fishing in the Corpus Christi Bay System (Ropicki et al., 2016), the recreational fishing impacts were estimated around $33.8 million contributing to Texas GDP and $59.4 million in output.

This project addressed the lack of MPs research in the region. It represents the first measurement of MPs in Nueces Bay. The study focused on quantifying the abundance and characterizing the properties of MPs such as sizes, shapes, chemical composition, and polymer types in samples collected at various locations. The findings reported here could be used as a reference for future microplastic studies and MPs modeling efforts in the region. Particularly, the chemical characteristics of MPs obtained provide insights on regional MPs pollution.

2. MATERIALS AND METHODS

2.1 Study Site

The Nueces Estuary consists of Nueces, Corpus Christi and Oso Bays and spans 106,990 acres (Schoenbaechler and Guthrie, 2011). Surrounding cities include Corpus Christi and Portland. Nueces Bay was selected as study site (Figure 1). Nueces River is a source of freshwater inflow to the Nueces Bay (Schoenbaechler and Guthrie, 2011). Nueces Bay is a western extension of Corpus Christi Bay and is located north of Corpus Christi on the San Patricio County line (Leatherwood, 2010). In addition to the port located in the Nueces Bay, Corpus Christi's economy relies heavily on oil and gas refineries (Flint Hills, CITGO, and Valero), and fishing and tourism to the various beaches along the coast, such as Padre Island and Mustang Island. Corpus Christi Bay has been

studied by several authors including Peters et al. (2018) and Waddell et al. (2020). However, Nueces Bay has not been studied for MPs.

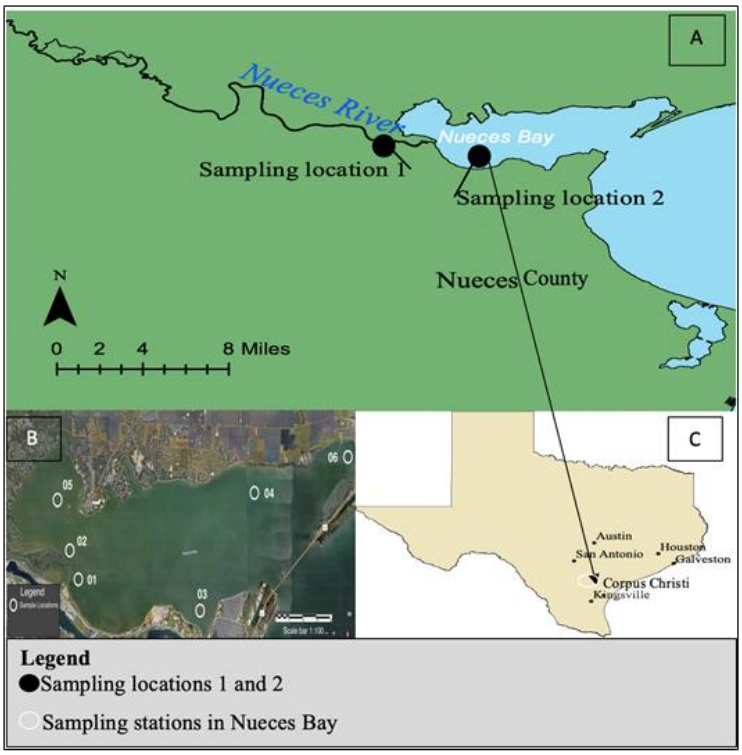

Figure 1. Study site and sampling locations. A) An overview of sampling locations 1 and 2, B) six randomly selected sampling stations for discrete water sample collection in Nueces Bay (NB), and C) sampling locations in relation to Texas' major cities.

2.2 Sampling locations

Nueces River was sampled at Hazel Bazemore Park (HBP) (Coordinates: 27.8666 and -97.6396) located in Calallen, Texas (Sampling location 1 shown in Figure 1A). Calallen is a northwest district of Corpus Christi, Texas. Nueces River at HBP was sampled on May 17, 2019. Sampling conditions were pristine: sunny and around 80 degrees. According to the weather tower at Corpus Christi International Airport, precipitation (i.e., 0.01 inch of rainfall during a three-hour period) did occur in the week prior to sampling.

Nueces Bay (NB) was sampled at six stations (NB1-NB6) on August 28, 2019 and August 6, 2020 (Figure 1B). Sampling conditions were pristine: sunny and around 80 degrees. Precipitation of 0.01 inches over an hour occurred on August 25, 2019 prior to the sampling trip on August 28, 2019 and precipitation of 0.08 inches over a three hour period on August 1, 2020 prior to the sampling trip on August 6, 2020. University of Texas at Austin-Marine Science Institute collected the samples on August 6, 2020 due to COVID-19 safety protocols.

2.3 Sample Collection

On May 17, 2019, the preliminary sampling took place at Nueces River at HBP. A total of six samples (i.e., six replicates) were collected using 1.25 Liter amber glass jars and the grab and scoop (bulk) method (Wang and Wang, 2018). During the August 28, 2019 sampling trip, a total of 16 water samples were collected in NB using amber glass jars (six 1.25 Liter and ten 0.95 Liter) and the grab and scoop (bulk) method with 6 ft Swing Sampler. Prior to sample collection, the amber glass jars were triple rinsed with micro-90 (Aldrich), a cleaning solution, to reduce the possibility of contamination. Duplicate water samples were collected at NB1, NB2 and NB5 and triplicate samples were collected at NB3, NB4 and NB6. In the August 6, 2020 sampling trip, a total of 11 water samples were collected using six 0.95 Liter amber glass jars and five 1.25 L Qorpak wide-mouth amber glass jars and a Van Dorn Sampler (McEachern et al., 2019). At each sampling station, duplicates were collected. The Qorpak wide-mouth amber jars used were purchased from Fisher Scientific. In addition to the water samples, the water quality data including pH, specific conductivity, salinity, turbidity, dissolved oxygen (%), DO (mg/L) and water temperature were also collected at each sampling station in NB (i.e., NB1-NB6) using a YSI 6600 Multiparameter Water Quality Sondes during both sampling trips to NB.

After sampling, each sampling jar was placed on ice and transported to Texas A&M University-Corpus Christi (TAMUCC) for processing and analysis of the samples. Any sample that was not processed immediately was placed in a refrigerator until the samples were processed.

2.4 Sample Processing and Analysis

Samples collected were filtered using a vacuum filtration system following method of McEachern et al. (2019), Kanhai et al. (2017) and Retama et al. (2016). The vacuum filtration system consisted of a stainless-steel funnel, a clamp to secure the funnel to the 1000 mL Erlenmeyer flask, and a pump. Before filtration, the vacuum filtration system was triple rinsed with micro-90 (Aldrich), a cleaning solution, followed by DI water rinse to remove any remaining cleaning solution, to reduce the possibility of contamination. During filtration, water samples collected were filtered through 0.45 micrometer with a 47 mm diameter MCE cell membrane filters. Thus, the MPs analyzed in this study have a lower limit of 0.45 micrometers.

For every 75 ml of the samples filtered through the membrane, a new filter was used. DI water was used to flush the filtration system and the resulting filters (flush filters) were examined for any potential microplastics that could be stuck to the filtration system. After filtration, each of the filters including the flush filters was placed in individual sterile petri dishes and dried out at 60 °C for 24 hours. Each cell membrane filter including the flush filters was then carefully and methodically placed under an optical microscope (Meiji Techno 5895 Rue Ferrari, San Jose, CA 95138 USA) to examine any potential MPs (Akarsu et al., 2019). When a potential microplastic was found, the suspected microplastic was placed using a stainless-steel single end probe and a tweezer in a small petri dish with ethanol (Gniadek and Dabrowska, 2019). Potential MPs were selected based on the Guide to Microplastic Identification by Hidalgo-Ruz et al. (2012) and best

judgement. Characteristics including shape (i.e., fibers, films, and particulates) and color of the potential microplastic were documented.

To confirm the material composition for potential MPs, each individual potential MPs was carefully placed, using a tweezer and a probe, onto an aluminum EZ-spot micro mount plate used for Thermo Nicolet 380 FTIR spectrometer with a germanium crystal (Thermo Fisher Scientific 168 Third Avenue, Waltham, MA, 02451) for attenuated total reflectance or absorbance (ATR) analysis. Prior to each analysis, the background of each sample was measured. Two FTIR runs, with an average of 100 scans per run, were conducted for each sample analysis if the percentage match is greater than 70% in each run (Piperagkas et al., 2019); otherwise, three FTIR runs were conducted for each sample analysis. The percentage match represents the similarity (i.e., shape, size, and type of peaks of each FTIR spectrum) between known FTIR spectra found in common FTIR reference/libraries/database and the spectra of the potential MPs being analyzed (Waddell et al., 2020; Li et al., 2006).

Based on quantification assessment methods of Mendoza and Balcer (2019) and Ramírez-Álvarez et al. (2020), around 20% to 30% of potential MPs found were analyzed using the FTIR. Natural or organic particles were also analyzed using FTIR in this project. However, any materials confirmed by the FTIR as organic or natural materials were excluded from further calculation of microplastic concentrations. The number of potential MPs was determined by documenting the color and shape of each potential microplastic found in each filter. At the end of processing, the total number of potential MPs from each sampling trip was determined.

2.5 Data Analysis

To examine the variations of the MP concentrations among the sampling stations and between the sampling trips, Analysis of Variance (ANOVA) was employed. All data were tested for

normality and homogeneous variances before the ANOVA analyses. The normality tests were conducted using IBM SPSS Statistics (IBM Corporation). The homogeneous variances tests were carried out using Minitab 15 (Minitab Inc.) The ANOVA analysis was conducted for those datasets that passed both the normality and homogeneous variances tests in Excel. A level of significance of 0.05 was used in all statistical analyses.

2.6 Quality Control Measures

Airborne fibers are a common source of contamination during the processing of samples for MP analysis (Mendoza and Balcer, 2019). To avoid contamination prior to field sampling, each amber glass jar was washed with micro-90 (Aldrich), a cleaning solution, and tripled rinsed with warm to hot DI water and a new pair of gloves was used during washing for each jar. To avoid plastic contamination in the lab, bright red cotton lab coats, masks and gloves were worn during the sample processing (filtration and sorting processes). The amber glass jars were thoroughly rinsed with 250 mL DI water twice after filtration was complete to avoid cross contamination. The vacuum filtration system was also thoroughly rinsed with 250 mL DI water twice between samples to avoid cross contamination.

During the filtration and sorting process, an unused filter was used as a blank between samples for quality control to account for any contamination within the lab including the MPs that might be stuck to the stainless-steel funnel of the filtration system. These blank filters were sorted and would have been analyzed using the FTIR but the FTIR broke down and requiring maintenance prior to analysis. Preliminary results showed approximately 24 potential MPs found in 6 blank filters obtained when processing the samples collected at HBP. For the samples collected on August 28, 2019 in NB, a total of 16 blank filters were examined and approximately 80 potential MPs were found. Thus, additional preventative measures such as triple rinsing amber glasses jars,

filtering DI water to account for any potential MPs, and dusting and wiping down the lab daily were utilized when processing the samples collected in NB on the August 6, 2020 sampling trip. After implementing the additional preventative measures, the blank filters had approximately 33 potential MPs in 15 blank filters, thus a substantial improvement was achieved. Blank filters were not analyzed using the FTIR due to FTIR needing to be repaired and requiring maintenance.

3. RESULTS

3.1 Conventional Water Quality Parameters

The water quality parameters measured at 6 inches from the surface of the water column at each sampling station in NB are shown in Table 1. NB4 is the deepest station and had a depth of 1.7 meters. The pH ranged from 8.0 to 8.2. The water temperature varied slightly between the two sampling trips. Turbidity ranged from 11.0 to 25.8 NTU. The dissolved oxygen varied from 5.6 mg/L to 6.1 mg/L. Salinity ranged from 27.0 ± 4.1 to 31.8 ± 0.2. Specific conductivity ranged from 42.5 ± 5.0 to 49.2 ±1.0. Between the two sampling trips of NB, turbidity varied the most. Very similar water temperatures, pHs and depth measurements were observed. Specific conductivity and salinity varied slightly. Among the sampling stations, NB4 had the second highest specific conductivity measurement and the deepest water depth in Nueces Bay. NB1, NB2, and NB5 had very similar salinity measurements. NB5 had the highest dissolved oxygen reading. NB6 had the highest turbidity, pH and salinity reading.

Table 1. Conventional Water Quality Parameters Recorded on August 28,2019 and August 6, 2020 in NB.

Parameters	Sampling Stations in Nueces Bay					
	NB1	**NB2**	**NB3**	**NB4**	**NB5**	**NB6**
Temperature (C°)	29.7± 0.2	29.8± 0.2	30.3 ± 0.6	30.2 ± 0.0	29. 8 ± 0.1	30.3 ± 0.0
Water Depth (m)	1.1 ± 0.2	1.0 ± 0.0	1.3 ± 0.1	1.7 ± 0.0	0.9 ± 0.3	1.0 ± 0.1
Specific Conductivity (mS)	43.1 ± 4.1	42.2 ± 5.8	47.0 ± 0.3	49.0 ± 0.3	42.5 ± 5.0	49.2 ± 1.0
pH	8.1 ± 0.2	8.1 ± 0.1	8.1 ± 0.1	8.0 ± 0.1	8.1 ± 0.1	8.2 ± 0.1
Salinity	27.6 ± 2.9	27.0 ± 4.1	30.4 ± 0.2	31.8 ± 0.2	27.2 ± 3.5	32.0 ± 0.7
DO (mg/L)	5.6 ± 0.4	5.7 ± 0.3	6.0 ± 0.3	6.0 ± 0.2	6.1 ± 0.1	6.0 ± 0.4
Turbidity (NTU)	14.2 ± 0.4	16.2 ± 0.9	11.0 ± 4.9	19.1 ± 10.8	13.2 ± 1.2	25.8 ± 1.0

Note: Values reported represent the mean ± one standard deviation of measurements conducted on August 28, 2019 and August 06, 2020 at each sampling station in Nueces Bay.

3.2 Concentrations of MPs measured in Nueces Bay

3.2.1 MP concentrations by color

Figure 2 shows the concentration (#/L) of MPs for different colors found at HBP (sample location 1) and at each sampling station in NB prior to FTIR confirmation. The most abundant colors found in the May 17, 2019 sampling trip at HBP were clear, brown, metallic and black (Figure 2A). Clear particles concentration was 34.59 ± 26.78 #/L, metallic particles were 35.87 ± 46.99 #/L, and black particles were 32.67 ± 22.60 #/L. For both sampling trips conducted in NB, clear MPs had the highest concentration among all six sampling stations (NB1-NB6) and purple, opaque, metallic and orange had the lowest concentrations.

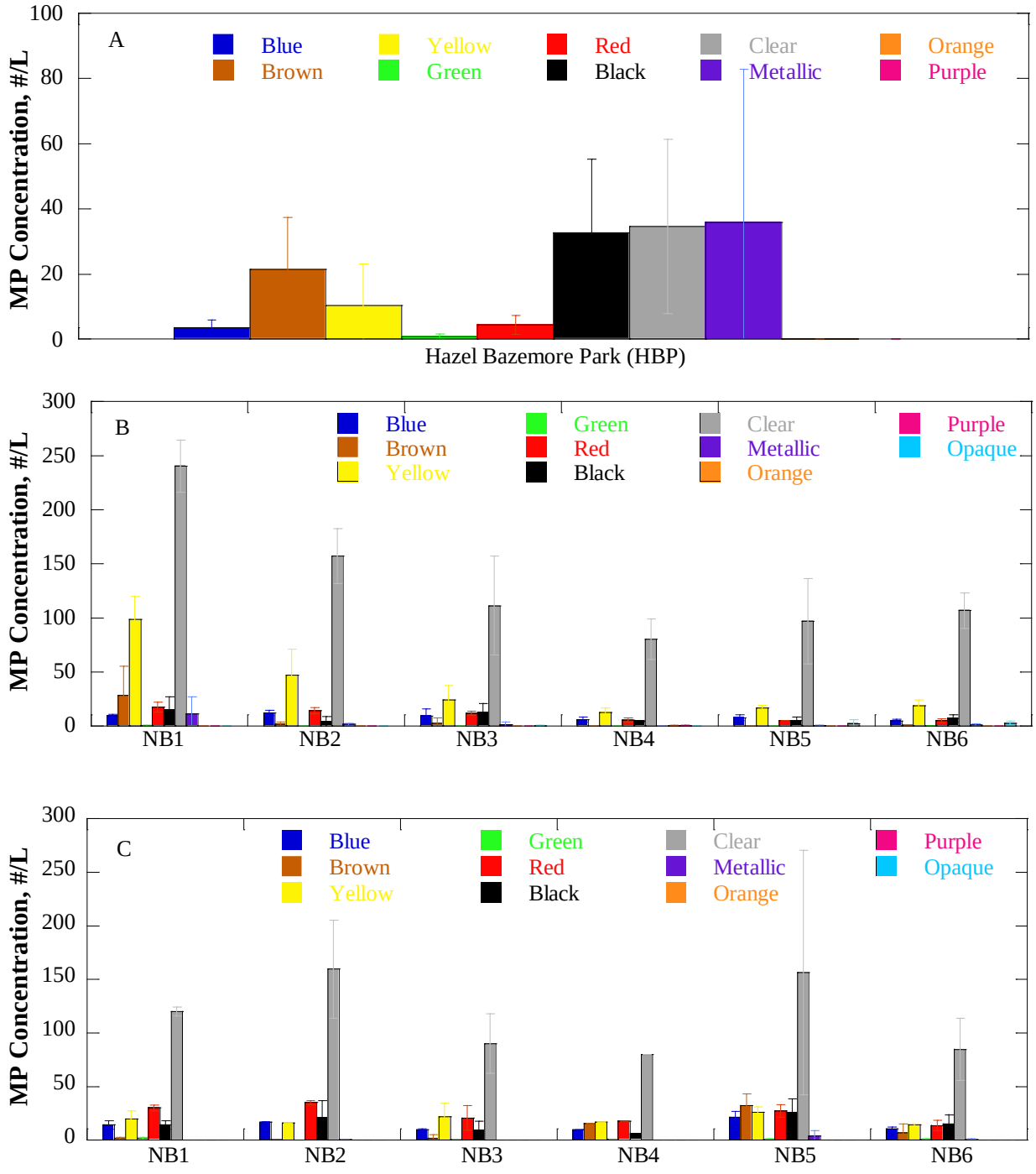

Figure 2. Concentration (#/L) of MPs by color, A) from HBP sampled on May 17, 2019, B) from NB sampled on August 28, 2019 and C) from NB sampled on August 06, 2020. For Figure A, error bars represent ± one standard deviation of 6 measurements at the sampling location. For Figures B and C, error bars represent ± one standard deviation of 2-3 measurements at each sampling station except NB4, where only one measurement was obtained.

The most abundant colors found in the August 28, 2019 sampling trip in NB were clear, yellow and blue (Figure 2B). Clear particles ranged from 80 ± 18.68 #/L to 240.40 ± 24.32 #/L. At NB1, clear particles had a highest concentration of 240.40 #/L. Yellow particles ranged from 12.53 ± 3.78 #/L to 98.40 ± 21.50 #/L. The most abundant colors found in the August 06, 2020 sampling trip in NB were clear, red, yellow and blue (Figure 2C). Clear particles ranged from 80.00 #/L (collected at NB4 with no standard deviation) to 159.60 ± 45.82 #/L and NB2 had the highest concentration. Red MPs ranged from 13.62 ± 4.56 #/L to 34.80 ± 2.83 #/L. Yellow MPs ranged from 14.17 ± 5.21 #/L to 25.79 ± 11.89 #/L and blue particles ranged from 9.94 #/L (collected at NB4 with no standard deviation) to 21.58 ± 5.21 #/L. At NB2 and NB5, clear particles had a similar concentration of 159.60 #/L and 156.32 #/L, respectively. Overall, clear particles had the highest concentration found in NB. Metallic particles were abundant at HBP but were scarcely found at NB.

3.2.2 MP concentrations by shape

Figure 3A shows HBP had the highest concentration of fibers (262.24 ± 235.67 #/L) and films (68.643 ± 136.55 #/L). During the August 28, 2019 sampling trip, NB2 had the highest concentrations for fibers (159.60 #/L) (Figure 3B). However, NB1 had a very similar fiber concentration of (152.80 #/L) and highest particulate concentration of 232.80 #/L. NB4 had the lowest concentration for fibers, particulates, and films. Overall, fiber concentration ranged from 86.93± 18.80 #/L to 159.60 ± 66.19 #/L, particulate concentration ranged from 10.40 ±3.67 #/L to 232.80 ± 27.15 #/L, and film concentration ranged from 11.47 ± 6.00 #/L to 34.80 ± 0.57 #/L. During the August 06, 2020 sampling trip, NB2 had the highest concentrations of MPs found in

NB with a highest fiber concentration of 136.00 ± 5.70 #/L (Figure 3C). NB6 had the lowest concentration for particulates. NB3 had the lowest concentration for films (8.34 ± 1.61 #/L).

Between the two sampling trips conduced in NB, fibers ranged from 60.40 ±18.70 #/L to 159.60 ± 66.19 #/L. Film concentration ranged from 8.30 ± 1.60 #/L to 34.8 ± 0.60 #/L. Particulate concentration ranged from 10.40 ±3.67 #/L to 232.80 ± 27.15 #/L. Fibers were the most abundant shape found in NB and HBP. NB3 and NB5 had very similar fiber concentrations during the August 28, 2019 sampling trip. NB1 and NB2 had very similar particulate and film concentration during the August 06, 2020 sampling trip. However, the fiber concentration range between sampling trips of NB and HBP varied greatly.

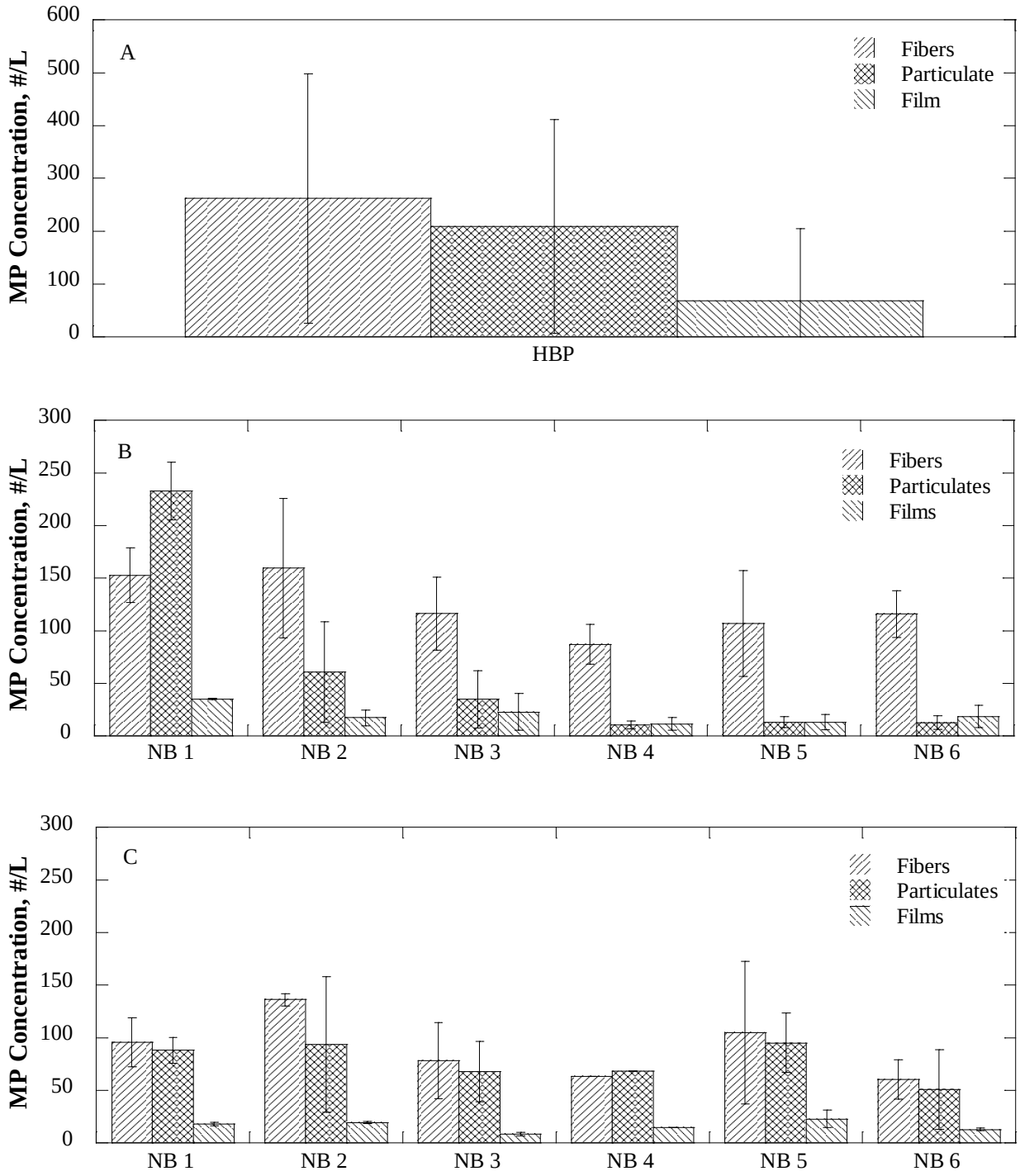

Figure 3. Concentration of MPs by shape from A) HBP sampling trip on May 17, 2019, B) NB sampling trip on August 28, 2019, and C) NB sampling trip on August 06, 2020. Error bars represent ± one standard deviation of 6 measurements at HBP and 2-3 measurements at each sampling station in NB.

3.3 Percentage of MPs observed

A total of 3378 potential MPs (without FTIR confirmation) from HBP were examined based on color and shape. Clear fibers, black fibers and black particulate and brown particulates were the most common (Figure 4) at HBP. The most common colors found were clear, black, brown, and yellow. During the trip of August 28, 2019 to NB, clear fibers, clear particulates and yellow particulates were the most commonly found from the six sampling stations (Figure 4). The most common colors found in NB from the August 28, 2019 sampling trip were clear, yellow, and red. For the samples collected in NB on August 06, 2020, a total of 2528 potential MPs were identified from the six sampling stations. Among these potential MPs, clear fibers (41.2% of the samples analyzed), clear particulates (19.4% of samples analyzed), clear films (9.9% of the samples analyzed), and red particulates (7.5% of the samples analyzed) were the most common (Figure 4). The most common colors found in NB from the August 06, 2020 sampling trip were clear, yellow, red, and black.

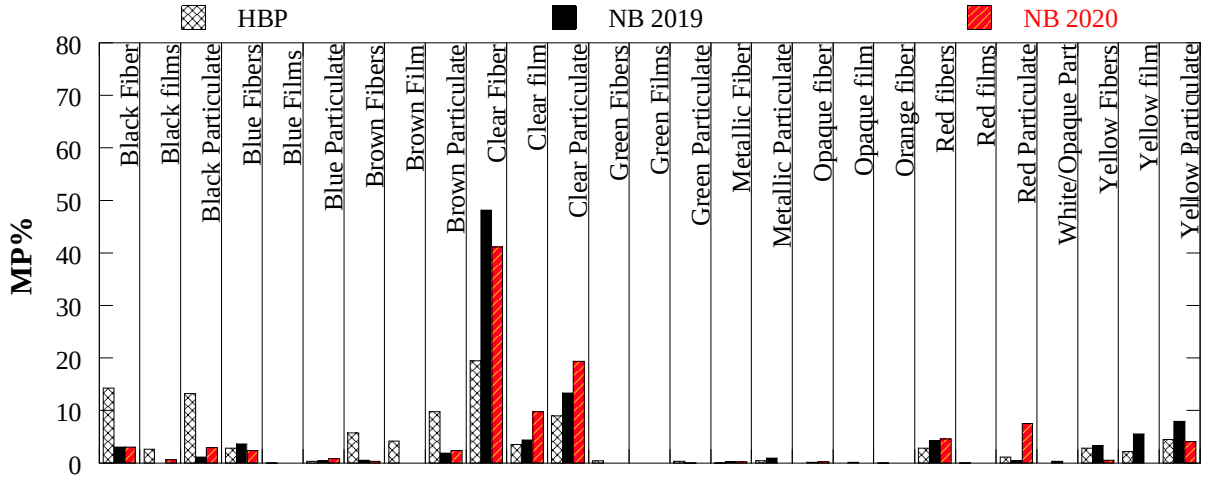

Figure 4. %MP identified without FTIR confirmation for samples collected at HBP on May 17, 2019 and NB on August 28, 2019 and August 06, 2020.

3.4 MPs confirmed by FTIR and their synthetic types

A total of 446 samples (12% of 3772) collected on August 28, 2019 in NB were analyzed by the FTIR. A total of 244 (7.25% of 3378) potential MPs identified (without FTIR confirmation) from HBP were processed based on color and shape. Each potential microplastic was characterized by color, shape, synthetic type, and the % match. The percentage match was obtained by comparing a known sample's spectrum found in common FTIR reference or sample libraries/database to the unknown potential sample's spectrum. Based on similarities between the two spectra, the % match was determined.

Figure 5 shows two examples of MPs (red participate and clear fiber) photographed during the FTIR analysis. The two MPs analyzed were less than 80 μm in length. The red particulate (Figure 5A) was from NB2. FTIR analysis indicates that this red particulate was polyethylene. The clear fiber from NB2 and shown in Figures 5B and 5C was an anthropogenic material such as rayon.

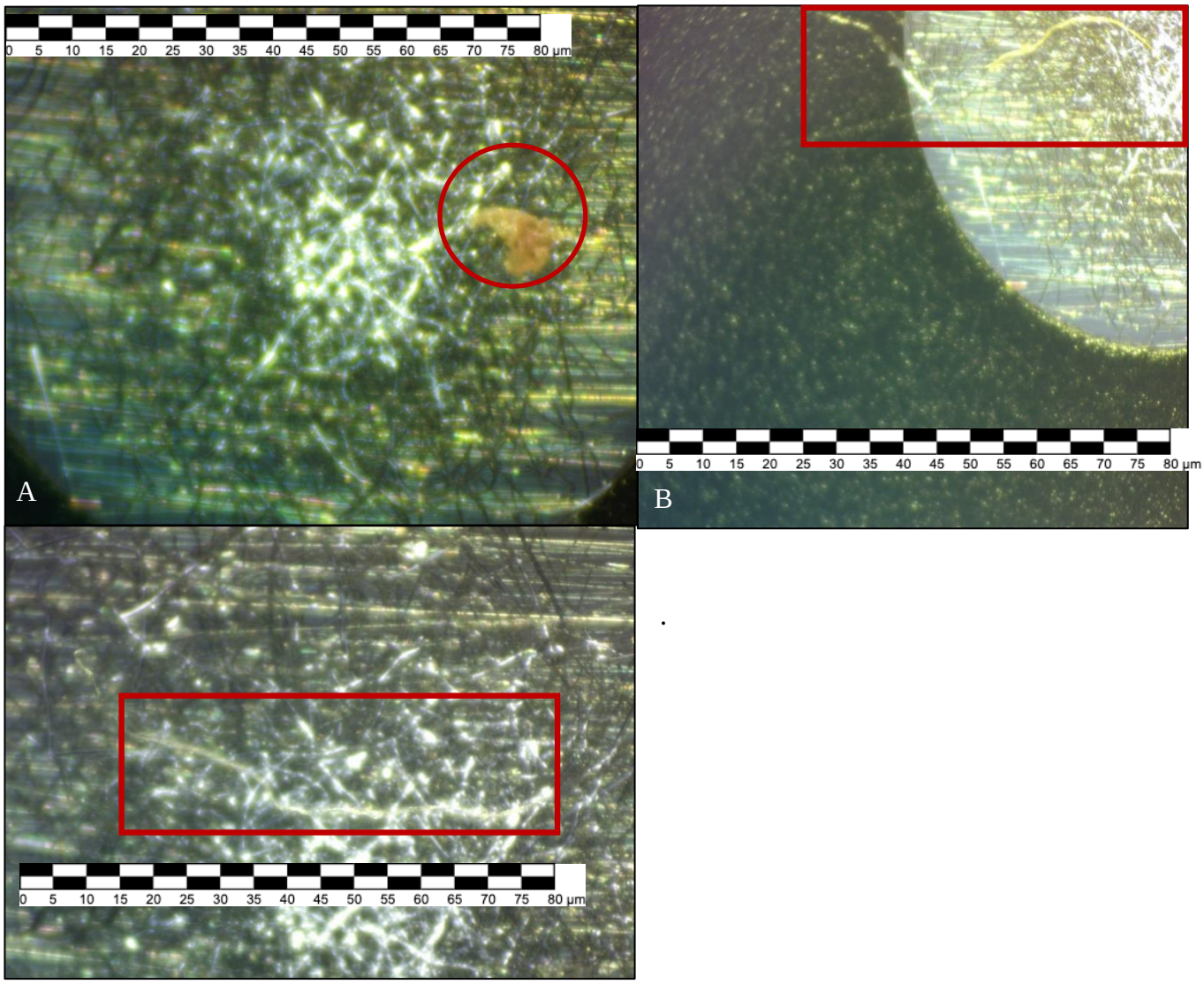

Figure 5. MPs found in NB during sampling trip of August 28, 2019. A) Red particulate prior to FTIR Analysis found at NB2. The FTIR identified it as polyethylene. B-C) Alternative view of a clear fiber from NB2 prior to FTIR Analysis. The FTIR identified it as rayon.

Figure 6 shows example FTIR Spectra. The unknown microplastic material (top spectrum, from an unknown sample collected in Nueces River at HBP) was compared to the spectra of potential microplastic materials such as cotton blend and rayon. Based on this comparison, the unknown material was identified as cotton-rayon blend which was composed of 33% cotton, 49% rayon and 2% elastan. The cotton-rayon blend was one of the most commonly identified

microplastic materials at HBP. The FTIR reference library indicates the unknown samples had an 83.45% match to a cotton-rayon blend standard and a 78% match to rayon. All three spectra had similar peaks and dips.

In addition to the FTIR reference library, a random selection of microplastic materials was also uploaded to SLoPP and SLoPP-E. SLoPP was created by Rochman Lab based in Toronto. The SLoPP and SLoPP-E reference library was used to confirm the validity of OMNIC, a reference software created by ThermoFisher Scientific. All FTIR and OMNIC confirmed plastic materials (i.e., polystyrene, polypropylene, polyacrylonitrile butadiene styrene, polyetheryrethane, and polyethylene) were validated by the SLoPP and SLoPP-E reference library. However, anthropogenic materials such as rayon, linen, and wool were more difficult to validate by OMINIC and SLoPP and SLoPP-E reference libraries due to the manufacturing processes (i.e., blends of cotton/linen in clothing) of these materials.

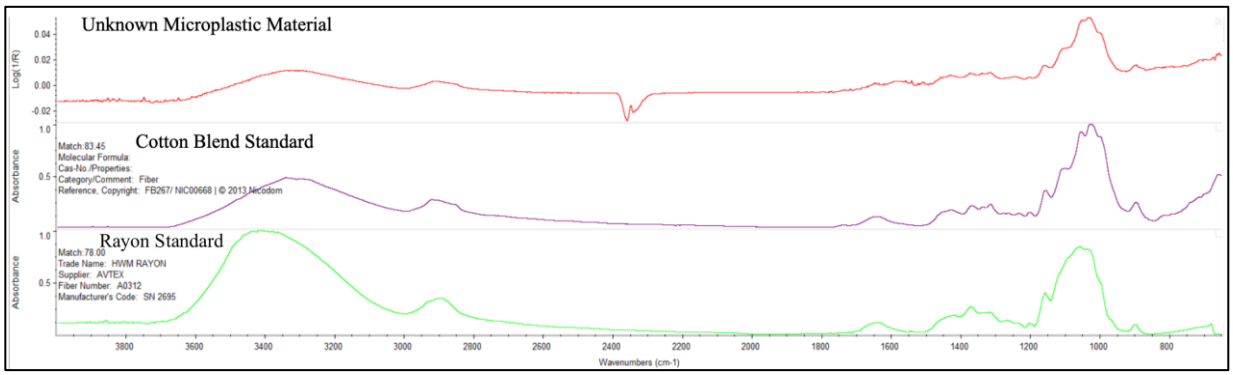

Figure 6. Example FTIR Spectra.

Figure 7A shows the concentration of MPs confirmed by the FTIR from HBP. Clear particles had the highest concentration of 34.40 ± 5.25 #/L. Metallic MPs were found abundantly prior to FTIR analysis (Figure 2A); however, none of the metallic samples were confirmed by FTIR. Black particles, which were found abundant prior to FTIR analysis only had a concentration of 2.40 ± 0.80 #/L confirmed by FTIR. In NB, clear particles had the highest concentration among

all six sampling stations (Figure 7B). At NB1 and NB2, clear particles had a similar concentration of 35.60 #/L and 35.20 #/L, respectively. Yellow particles had a concentration range of 2.00-5.20 #/L among all six sampling stations. The FTIR did not confirm any purple, opaque, metallic and orange potential MPs. Figure 7C shows NB2 had the highest concentrations of fibers and films with concentrations of 30.40 #/L and 8.80 #/L, respectively. NB1 had the highest particulate concentration of 14.80 #/L. NB4 had the lowest concentration for fibers, films, and particulates.

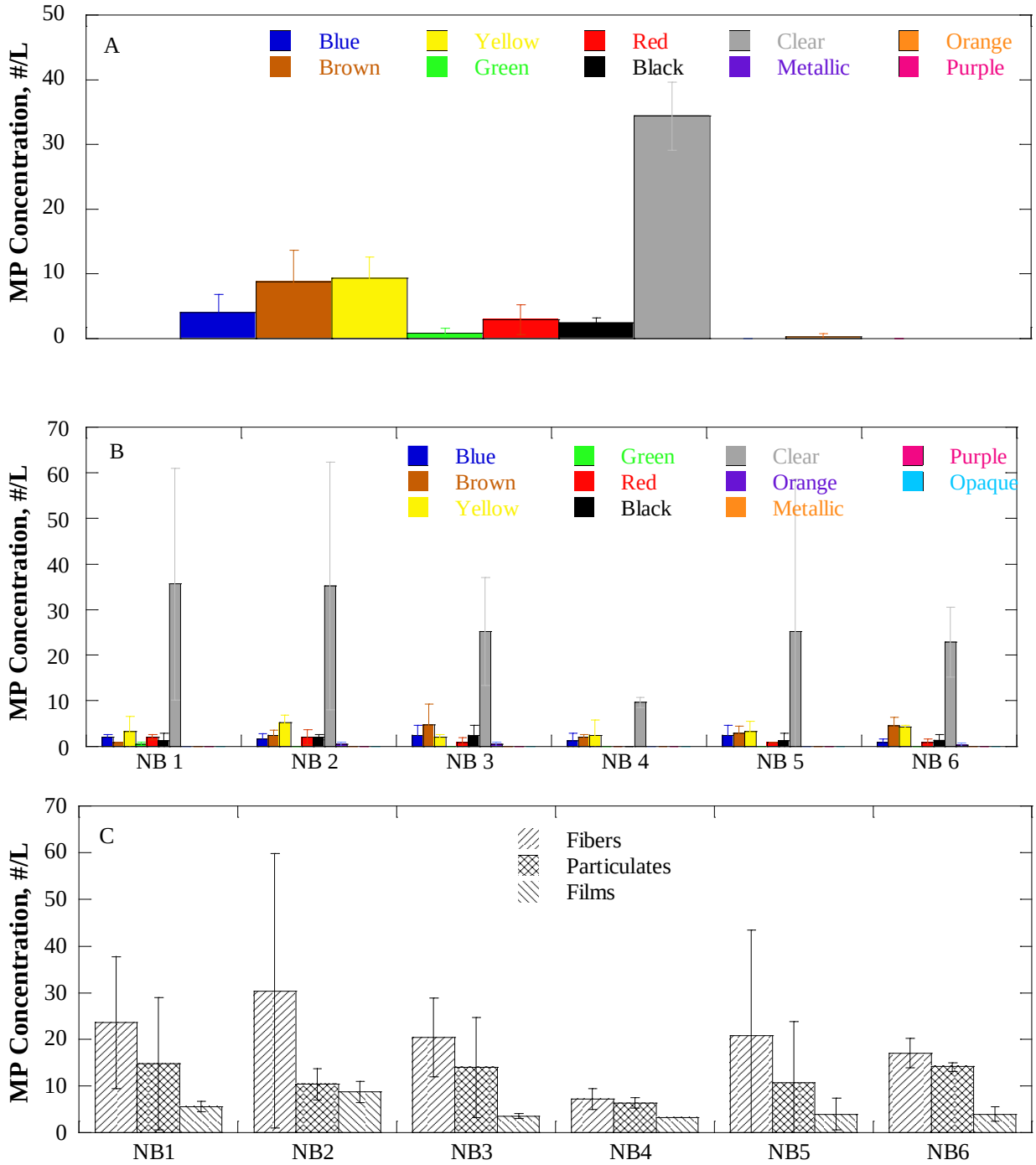

Figure 7. Concentration (#/L) of MPs confirmed by FTIR. A) Results for samples collected on May 17, 2019 at HBP, B) results (by color) for samples collected on August 28, 2019 in NB, and C) results (by shape type) for samples collected on August 28, 2019 in NB. Error bars represent ± one standard deviation of 2 to 3 measurements at each sampling station in NB and 6 measurements at HBP.

Figure 8 shows the percentage abundance distribution of MPs classified based on their shape and color from each sampling trip and after FTIR analysis. At HBP prior to FTIR analysis, clear fibers, black fibers, black particulates, and brown particulates were the most abundant (Figure 4). After FTIR analysis, clear fibers, clear particulates, yellow particulates and brown particulates were the most abundant (Figure 8). Of the 244 MP identified by the FTIR from HBP, 35% were clear fibers, the most abundant type found at HBP and 15% were clear particulates (Figure 8).

Of the 446 MPs identified from the August 28, 2019 sampling trip in NB, clear fibers, clear particulates, and yellow particulates were the most common. Red, blue, yellow, and black fibers were also confirmed by the FTIR. Between the two locations (HBP and NB), clear fibers were the abundant type of microplastic found followed by clear and yellow particulates and red fibers.

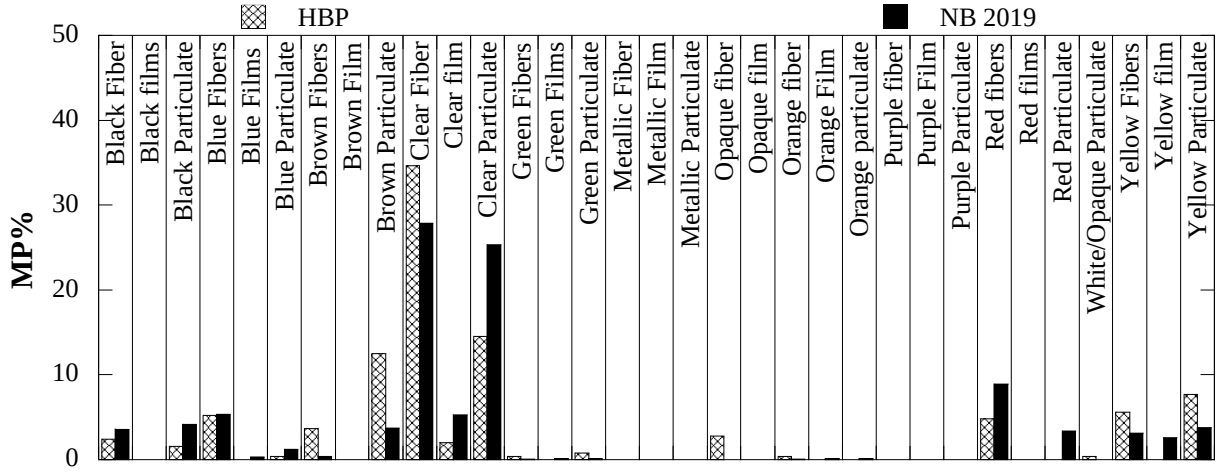

Figure 8. % MPs by shape and color confirmed by FTIR for the samples collected at HBP on May 17, 2019 and NB on August 28, 2019.

Figure 9 shows the concentrations of microplastic synthetic types for the samples collected. HBP had the highest concentration of fully synthetic fibers and fully synthetic particulates. NB2 had the highest concentration of fully synthetic films. NB4 had the lowest concentration of fully synthetic particulates (1.20 #/L) and fully synthetic fibers (1.60 #/L). NB3 had the lowest

concentration of fully synthetic films (0.30 #/L). For semi-synthetic materials, HBP had the highest concentration of semi-synthetic fibers of 22.13 #/L. NB4 had the lowest concentration of semi-synthetic fibers (4.40 #/L) and no semi-synthetic particulates and films were confirmed at NB4. From the August 28, 2019 sampling trip in NB, fully synthetic fibers and semi-synthetic fibers were the most common in NB. The least common in NB was semi-synthetic films (Figure 9).

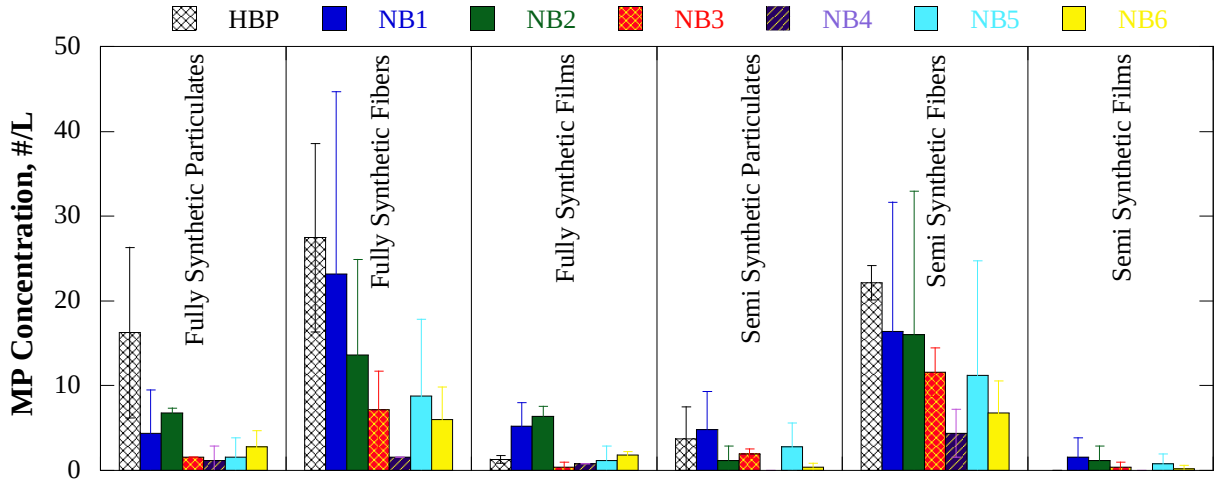

Figure 9. Concentrations of microplastic material types identified by FTIR for the samples collected at HBP and NB. Error bars represent ± one standard deviation of 6 measurements taken at HBP on May 17, 2019 and 2- 3 measurements taken at each sampling station at NB on August 28, 2019.

Of the 3,378 potential MPs identified (without FTIR confirmation) from HBP, 244 were identified using the FTIR. Among these samples, 49 were confirmed as cotton-rayon blend or a cotton-based material, 43 as polystyrene, 45 as rayon, and 18 as cellulose nitrate. Cotton-rayon blend (an anthropogenic material) had the highest concentration at HBP (Figure 10). Rayon (12.00 #/L) had the second highest concentration followed by polystyrene (11.47 #/L). Please note the anthropogenic particles (cotton based or cellulosic) such as cotton-rayon blend, rayon, and burlap

were categorized as semi-synthetic here. Plastic polymers (fully synthetic) such as polystyrene, polyester, polyacrylonitrile butadiene styrene, polyetheryrethane, olefin, poly(cis/trans cyclohexane diol terephthalate), polypropylene, nylon, acrylic, polyethylene terephthalate and polyethylene were found throughout HBP on May 17, 2019 and NB in August 28, 2019 sampling trips.

Among the samples analyzed by the FTIR (446 out of 3772 samples collected in NB on August 28, 2019), over 30 types of materials were identified among the six sampling stations (Figure 10). Among these samples, 37 were natural/organic materials, which were excluded from the FTIR analysis. Results of the remaining 409 samples analyzed show that 78 samples were cotton-rayon blend or a cotton-based material and 31 were polystyrene. The cotton-based materials presented as clear fibers and clear particulates. Cotton-rayon blend identified as an anthropogenic material had the highest concentration of 8.80 #/L found at NB1 and NB2 (Figure 10). Cotton-Rayon blend presented as red, clear, blue, black colors and as fiber, film, and particulate shapes. Polystyrene presented as several different colors such as brown, yellow, and clear and shapes such as films, fibers and particles. For example, polystyrene presented as clear film or clear particulate. Polystyrene had the second highest concentration of 7.60 #/L found at NB2. NB3 and NB4 had the lowest average concentrations (0.19 #/L and 0.28 #/L, respectively) of plastic materials (i.e., linen, methyl glucose dioleate, polyhexyl acrylate, and wool found. Overall, after FTIR confirmation, NB2 was the most diversified in terms of microplastic materials found. The microplastic materials found at NB2 included nine different types of plastic materials including: polystyrene, polyster, nylon, polyethylene, poly acrylonitrile butadiene styrene, poly(cis/trans cyclohexanediol terephthalate, acrylic, PET, olefin and burlap.

Overall, after FTIR analysis, the following microplastic materials were found in HBP and NB1-NB6: cotton-rayon blend, cellulose, cellophane, linen, polyester, polyethylene, rayon, polypropylene, PET and polystyrene. Cotton-rayon blend was the most abundant throughout HBP and NB1-NB6. The most abundant plastic material found throughout HBP and NB1-NB6 was polystyrene. Anthropogenic materials such as rayon, cellulose, filter material were also abundant throughout HBP and NB1-NB-6.

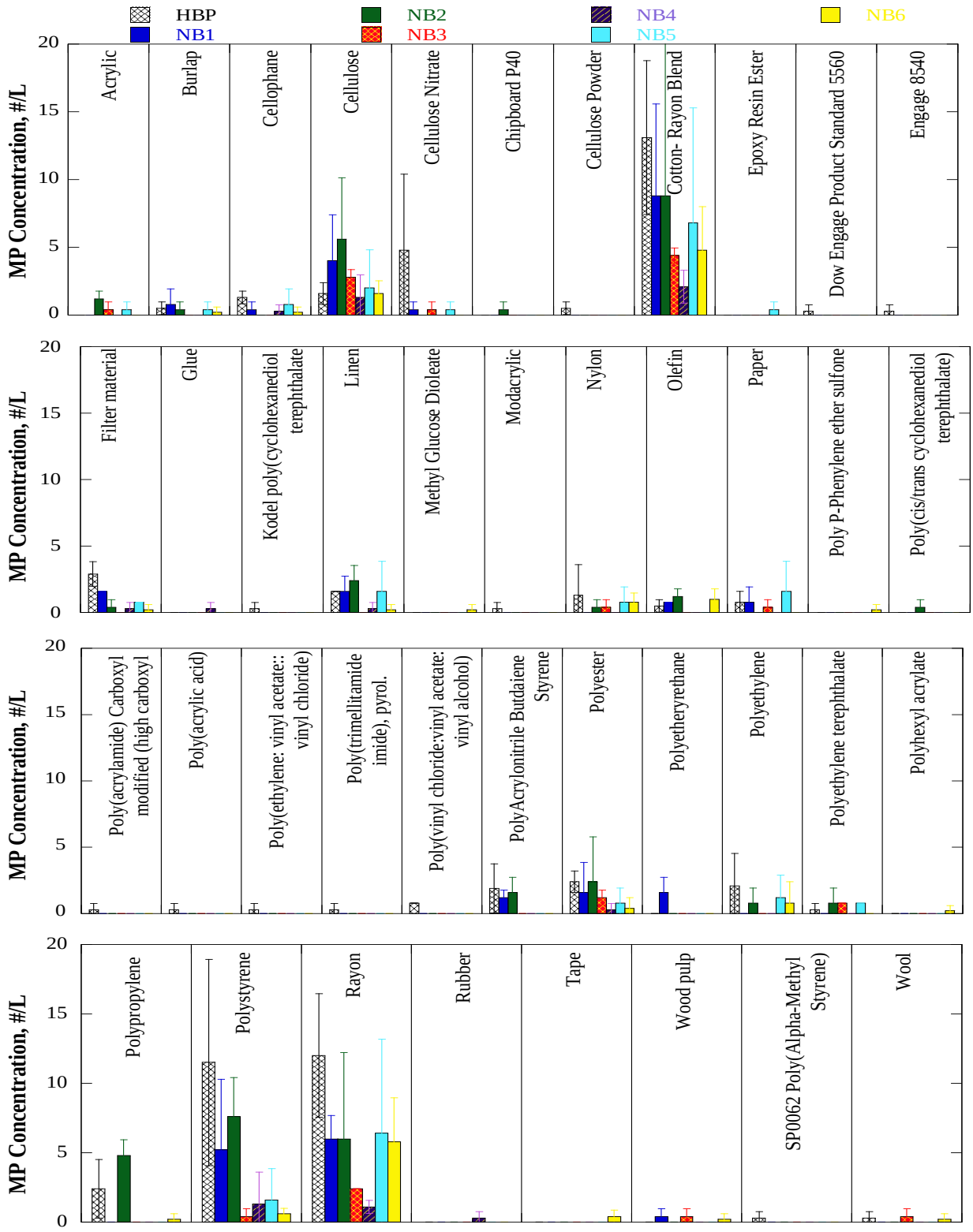

Figure 10. FTIR confirmed MP materials.

3.5 Statistical analysis on variations of MP concentrations observed

For the data collected on August 28, 2019 in NB, the variations of MP concentrations for each color type identified among the six sampling stations were evaluated. Data of six colors (blue, red, black, metallic, brown, and yellow) passed the normality test. After the normality test, the groups passed the test for equal variance (i.e., data collected at NB3-NB6) were analyzed using ANOVA single factor test. The resulting ANOVA results are 0.90 (F-value) and 3.10 (F-crit value) (Table 2) indicating there is not a significant difference among the average MP concentrations of colors found in NB among locations of NB3-NB6.

When comparing the differences among sampling stations by shape, data collected from NB4 did not pass the normality test, thus were excluded from the ANOVA analysis. The remaining data collected from NB1-NB4 and NB6 and consisted of fibers, films and particulates all passed the equal variance test, thus were tested using ANOVA. The ANOVA results (Table 2) are 1.00 (F-value) and 3.48 (F-crit value) indicating there is not a significant difference between the average MP concentrations of shape types found in NB among locations of NB1-NB4 and NB6.

When comparing the differences among locations by FTIR confirmed colors, datasets collected at locations of NB1-NB4 and NB6 for colors of blue, red, black, brown, yellow, orange, green passed the normality and equal variance tests and thus were tested using ANOVA single factor analysis. The ANOVA results (Table 2) are .55 (F-value) and 2.48 (F-crit value) indicating there is not a significant difference between the average MP concentrations of FTIR confirmed colors found in NB among locations of NB1-NB4 and NB6.

When comparing the differences among locations by FTIR confirmed materials, the variations of MP concentrations for each confirmed MP material type identified after FTIR analysis were evaluated. Datasets collected at all six sampling stations in NB and confirmed for

Cotton Rayon Blend, Rayon, Polystyrene, and Cellulose passed the normality and equal variance tests and thus were analyzed using ANOVA single factor test. The ANOVA results (Table 2) are 0.97 (F-value) and 2.77 (F-crit value) indicating there is not a significant difference between the average MP concentrations of FTIR confirmed microplastic materials found in NB among sampling stations studied.

Similarly, the variation of MP concentrations for each color were evaluated among sampling stations from the August 6, 2020 sampling trip. Data collected at all six stations passed the normality and equal variance tests for colors of blue, brown, yellow, green, red, black, and orange. ANOVA results of 0.31 (F-value) and 2.43 (F-crit value) indicate there is not a significant difference among the average MP concentrations of colors found in NB among sampling stations. For MP concentrations categorized by shape (fibers, films and particulates), data collected from NB1-NB4 and NB 6 passed both the normality and equal variances tests. The ANOVA results for these datasets (Table 2) are 0.47 (F-value) and 3.11 (F-crit value) indicating there is not a significant difference between the average concentrations of MPs by shapes found in NB among stations of NB1-NB4 and NB6.

Table 2. ANOVA analysis results.

Difference among sampling stations in NB		**Datasets analyzed based on results of normality and equal variance tests**	**ANOVA Results, F-Value (F-crit Value)**
Aug. 28, 2019	by color	NB3-NB6 for blue, red, black, metallic, brown, and yellow	0.90 (3.10)
	by shape type	NB1, NB2, NB3, and NB5, NB6 for fibers, film and particulates	1.09 (3.48)
	by FTIR confirmed colors	NB1-NB4, NB6 for blue, red, black, brown, yellow, orange, and green	0.55 (2.48)
	by microplastic materials types	NB1-NB6 for Cotton Rayon Blend, Rayon, Polystyrene, Cellulose	0.97 (2.77)
Aug. 06, 2020	by color	NB1-NB6 for blue, brown, yellow, green, red, black, and orange	0.31 (2.43)
	by shape type	NB1-Nb4, NB6 For fibers, films and particulates	0.47 (3.11)
Difference between the two sampling trips in NB by MP shapes			
Comparison of Aug. 28 2019 & Aug. 06, 2020	by Fiber	NB-Aug 28, 2019 & NB Aug 06 2020, for fibers	4.17 (4.96)
	by Film	NB-Aug 28, 2019 & NB Aug 06 2020, for films	0.84 (4.96)
	By Particulate	N/A	N/A
Difference between the two sampling trips in NB by MP colors			
Comparison of Aug. 28 2019 & Aug. 06, 2020	for blue MPs	NB-Aug 28, 2019 & NB Aug 06 2020, for blue MPs	6.52 (4.96)
	for red MPs	NB-Aug 28, 2019 & NB Aug 06 2020, for red MPs	12.91 (4.96)
	for black MPs	NB-Aug 28, 2019 & NB Aug 06 2020, for blackMPs	4.07 (4.96)
	for clear MPs	NB-Aug 28, 2019 & NB Aug 06 2020, for clear MPs	0.36 (4.96)

When comparing the differences between the two sampling trips, the data collected at the six locations were tested. The fiber MP concentrations collected during the two sampling trips passed normality and the equal variances tests, thus were analyzed using the ANOVA single factor

analysis. The ANOVA results are 4.17 (F-value) and 4.96 (F-crit value) indicating there is a significant difference in the average concentrations of fibers found between the two sampling trips in Nueces Bay. Similarly, analyses were conducted for film MP concentration and the ANOVA results are 0.84 (F-value) and 4.96 (F-crit value) indicating there is not a significant difference between the average concentrations of films found in Nueces Bay. The data for the particulate concentrations of MPs did not pass the normality test, thus the ANOVA test was not conduced for the particulate MP concentrations.

The MP concentrations for different colors collected during the two sampling trips were analyzed using ANOVA test as well following the same procedure described above. The results indicate a significant difference between the average concentrations of blue and red MPs found in Nueces Bay (Table 2).

3.6 Comparison with results from other studies

The findings obtained from this study were compared with those reported in the literature and results are shown in Table 3. HBP and Monterey Bay had a similar percentage of fiber particles of 34-35%. The most common microplastic shape type in NB was fibers which is similar to the findings of Tampa Bay. The most common color in Nueces Bay was clear, which is similar to that of Sanggou Bay. Chesapeake Bay and Nueces Bay had similar findings for particulate MPs. Nueces Bay found several types of plastics such as polyethylene, polystyrene, polypropylene, and anthropogenic materials such as cotton or cellulose, which is similar to Chesapeake Bay, Monterey, and Sanggou Bay. Nueces Bay average MP concentrations ranged from 1.60 #/L to 23.20 #/L, which is similar to microplastic concentration of 21.21 #/L reported in Sanggou Bay (Xia et al., 2021). However, the microplastic concentration in Nueces Bay was found a magnitude

higher than those found in Tampa Bay (0.0045 #/L), Chesapeake Bay (0.0012 #/L), and Monterey Bay (0.0032 #/L) (McEachern et al. 2019, Bikker et al. 2020, and Kashiwabara et al. 2021).

Table 3. Comparisons with results from other studies.

Location		Tampa Bay[1]	Chesapeake Bay[2]	Monterey Bay[3]	Sanggou Bay[4]	Nueces Bay[5]
Prior to FTIR Analysis	Fiber Particle (%)	76%	18%	35%	~10%	63%
	Particulate /Fragment (%)	17%	32%	65%	~55%	27%
	Film	Not Mentioned	Not Mentioned	Not Mentioned	Varied by sampling site	10%
	Foam (%)	Scarce	13%	N/A	0%	N/A
FTIR Results	Common Color	Not Mentioned	Black, Blue, Brown, Clear, Green, Grey, Orange, Purple, Red, Tan, White, Yellow	Not Mentioned	Clear	Clear
FTIR Results	MP materials found	N/A Focused on shape type and abundance.	Polyethylene (32%), polypropylene (13%), polystyrene (9%) and anthropogenic (10%) particles.	Polypropylene and polyethylene	Polyethylene, Polystyrene, Polypropylene, cellulose materials	Cellulose Material, Polystyrene, Polyester, Polypropylene
	Concentration (#/L)	0.0045	0.0012	0.0032	21.21	23.20

[1] McEachern et al. 2019; [2] Bikker et al. 2020; [3] Kashiwabara et al. 2021; [4] Xia et al. 2021; [5] This study

4. CONCLUSION

This study demonstrated there is much to learn about MPs research from discrete water sampling methods to processing methods. Semi-synthetic and fully synthetic fibers were the most

abundant in NB and at HBP. Fibers were the most abundant shape in both sampling trips of NB and HBP. The microplastic materials confirmed by the FTIR were the following: rayon, linen, cellulose, polyester, polyethylene, polypropylene, olefin, petroleum wax and polyetheryrethane. The most common color found in NB and HBP was clear. Using the data from this study, a baseline can be provided for future microplastic studies to characterize, monitor and mitigate the microplastic pollution. As microplastic research continues to advance, the shape, chemical composition and color could provide details on the source of plastic pollution in water systems around the world.

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