

Quantifying microplastic debris from a wastewater treatment plant in Corpus Christi, TX.

**Texas A&M University – Corpus Christi
Honors Program**

Introduction

Plastic production is increasing in countries around the world, especially in industrialized nations such as the United States. For example, in the last 60 years plastic production in Europe has increased from 1.7 to 288 Tg annually (Dris et al., 2015). According to the Bureau of Labor Statistics (2017), employment in the U.S. plastic production industry has increased by 4,000 from December 2016 to March 2017. Industrialized nations tend to produce large amounts of plastic products for a wide variety of uses. China is the largest plastic producer, followed by Europe and North America (PlasticsEurope, 2016). With increased production comes additional use and disposal of plastic products of all types and sizes. According to Pita and Castilho (2017), the increase in production and consumption of plastics has been largely responsible for greater amounts of municipal solid wastes. However, consumers do not always dispose of products in the correct way. When plastics are not disposed of properly, whether this is deliberate or accidental, it may end up as litter in the environment. Plastic pollution is a global problem and affects both terrestrial and marine environments. The United Nations Environmental Program considers it to be one of the top environmental issues facing humans today (Mason et al., 2016). Although an accurate estimate of how much marine debris is composed of plastic does not yet exist (NOAA, 2017), 75% of debris that washes ashore worldwide has been recorded as plastic (Eerkes-Medrano, Thompson, & Aldridge, 2015). But not all plastic pollution is easily observable; microplastics (<5mm) number in the trillions across our global oceans.

There are two types of microplastics: primary and secondary. Primary microplastics are materials designed for use at sizes less than 5mm, such as those found in personal care products (currently being phased out), cosmetic products, and air-blast cleaning (Dris et al., 2015). Synthetic fibers are now believed to be a major source as well. Secondary microplastics are

fragments of larger plastics and are likely the majority of plastic debris found in the surface waters. Plastics of larger sizes are broken down mainly by mechanical abrasion and the effects of natural sunlight (ultraviolet rays). They enter surface water and eventually the ocean due to their normal usage or improper disposal. Pathways for entrance into the environment include runoff from urban areas and treated effluent from wastewater treatment plants. (Auta, Emenike, & Fauziah, 2016; Ziajahromi et al., 2017). According to PlasticsEurope (2016), prevention at the source through proper waste collection and treatment combined with improved human behavior is the key to reducing plastic pollution, which can be harmful to marine environments worldwide.

Plastic debris affects biodiversity in marine environments (Gall & Thompson, 2015), causing concern for ecosystem health. Much of this concern relates to microplastic particles and fibers that are bite-sized to many aquatic organisms. Ingestion of microplastics has been reported in 267 marine species (Dris et al., 2015), including commercially important fish species, crustaceans, and seabirds. Ingestion can lead to intestinal obstruction (Ziajahromi et al., 2017) and plastics can accumulate in the stomach, which can make marine organisms feel full (pseudo-satiation), resulting in decreased food consumption (Dris et al., 2015). Microplastics can also sorb hydrophobic and persistent organic pollutants (POPs). The most common types of granulated plastics have densities that range from 1.047 to 1.372 g/cm³ (Pita & Castilho, 2017). Due to its low densities, microplastics do not settle as readily as other suspended solids in the water column. This means that when sorbed, microplastic debris can transport POPs long distances from their sources due to its low densities. The POPs can cause harm to marine organisms when ingested as the organic pollutants can be transferred into the organisms' bodies. This also depends on the type and nature of the chemical involved, size of the plastic, and the

surrounding environment (Ziajahromi et al., 2017; Bakir et al., 2014). To help reduce harm caused by microplastic pollution, the sources of the contamination must be examined. One source is through effluent released from wastewater treatment plants.

Corpus Christi has six wastewater treatment plants (WWTPs) that treat approximately 28 million gallons daily from its population of over 324,000 (City of Corpus Christi, 2011). All wastewater treatment plants in Corpus Christi employ primary, secondary, and tertiary treatment before effluent is released into surface waters. Primary treatment involves the removal of most debris. First water flows through screens or grates to trap large objects (sticks, rocks, etc.). Then it travels through a grit chamber, which slows down the flow of water to allow the grit to fall out. Next it enters settling basins where solids settle out, which are then pumped into a separate biosolids processing area. Any oils floating on top of the water are skimmed off. Microplastics often get removed by these processes in primary treatment, but since systems are not designed for their removal, particles and fibers can still make it through. Secondary treatment in Corpus Christi consists of activated sludge treatment, which utilizes microorganisms to break down organic matter in an aeration tank. After this step, the remaining biosolids (mostly dead microbial organisms) are removed in additional settling basins. Lastly, the water undergoes tertiary treatment to reduce bacterial and viral loading, which involves UV disinfection at the Corpus Christi facilities. Once the water has been treated and disinfected, the effluent is released into the surface waters or used for irrigation. Any microplastics remaining after tertiary treatment will be released with effluent into surface waters (Mason, 2016). A study done by Lasse et al. (2017) documented the presence of microplastic debris in Texas surface waters that receive effluent from wastewater treatment plants. The objective of this study is to quantify microplastic debris entering Corpus Christi surface waters from wastewater effluent at a local treatment plant.

Methods

Samples were collected during the summer of 2016 and spring of 2017. Researchers wore closed toed shoes, long pants, nitrile gloves, and safety glasses when collecting samples.

Prior to sampling: Sample bottles (4L amber glass) were placed inside of a plastic garbage bag inside of a padded cardboard box to prevent leakage in the event of a spill during transport. Upon arrival at the wastewater treatment facility three 4L amber glass bottles were labeled with the date, location, and sample number. Bottles were carried to the effluent collection area.

During sampling: Three samples of treated wastewater effluent were collected (~2 L) from the wastewater treatment plant in 4 L amber glass bottles. The bottles were decontaminated with bleach followed by water. After cleaning, bottles were placed back in the trash bag and then into the padded cardboard box for transport back to the laboratory. A supporting researcher then cleaned the gloves of the researcher that collected samples with bleach, then water, in the same manner as the 4 L amber bottles. Gloves were then removed and the samples were transported back to the laboratory.

Post-sampling: All samples were sterilized using an autoclave in the lab of Dr. Turner upon arrival to the Texas A&M University – Corpus Christi campus. Each 4 L amber glass bottle was labeled with pressure sensitive tape prior to autoclaving. Two bottles were placed on a stainless-steel pan in the autoclave. Caps were loosened prior to starting the autoclave to allow for expansion of water during treatment. The autoclave was set to 121 °C for 30 minutes. Samples were allowed to cool for approximately 1 hr. All sterilized samples were stored at 4 °C in Dr. Conkle's laboratory prior to processing.

Processing: Sterilized samples were poured through sieve stack with the following sizes: 1000, 300, 50 μm . The total volume of the sampled effluent was measured and recorded. It was visually inspected for debris and then poured down the drain. The filtrate captured on each sieve was visually inspected for plastic debris and then the contents of each sieve were transferred into separate 400 mL beakers and covered. The samples were then dried overnight at 60 – 75 °C. The dried sample was placed under the fume hood. 20 mL Fe (II) solution, 20 mL 30% hydrogen peroxide, and a stir bar were slowly added. The sample was allowed to react for approximately five minutes (or until samples no longer bubbles). Once bubbling subsided, the solution was placed on a hot plate set to approximately 75 °C and the stirrer was turned on to gently mix the solution. When the solution began to boil, it was removed from heat until the boiling subsided. The beaker was returned to the hot plate and returned to a boil for 30 minutes. After the organic matter was digested, the filtering apparatus was set up. The solution was filtered and the plastic debris was trapped on a cellulose membrane filter. After filtering, cellulose membrane filters were placed in petri dishes and sealed, then dried. Samples were then visually inspected using a stereomicroscope. A piece of tape was placed in a second petri dish. Suspected plastics (fibers or fragments) that were recovered from the cellulose membrane filter using tweezers were placed on the piece of tape and recorded. Filters were checked by a second researcher to assure that no suspected plastics were not transferred. The suspected plastics were then tested using a hot needle to see if they were synthetic or not. The needle was heated over a Bunsen burner. Each suspected plastic was touched with the needle and its response was observed. The new number of suspected plastics was then recorded. Samples that have been analyzed are stored in a box in Dr. Conkle's laboratory.

Results

Due to biosafety permitting, sampling was halted from August 2016 to March 2017. The results are highly preliminary as only three samples in this data set have been hot needle tested and checked by more than one researcher. Figure 1 shows the concentration of suspected microplastics per liter for each sampling date. Most plastics were found in the smaller sieve sizes (300 and 50 μm) than the 1000 μm category (Figure 1). Error bars have been added to show standard deviation within the sample sets. Figure 2 shows the distribution of fibers and fragments/beads in each sample per liter of effluent. The average concentration of fibers for all samples is 9.33 ± 11.53 particles/L, while the concentration for beads is 1.56 ± 2.32 particles/L. Synthetic fibers were more prevalent than microbeads or fragments in wastewater effluent; synthetic fibers made up 85.3% of all recovered plastics (Figure 2). In the samples that were hot needle tested, no microbeads or fragments were found.

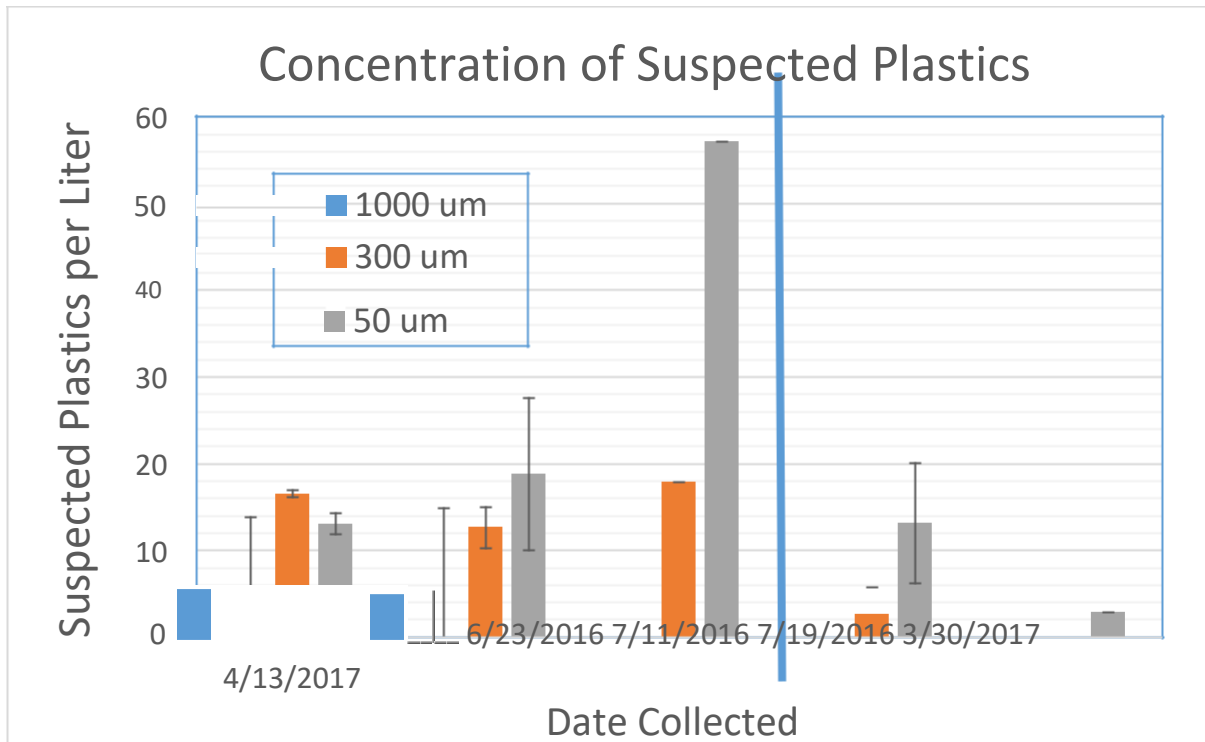


Figure 1. Concentrations of suspected microplastics in effluent samples.

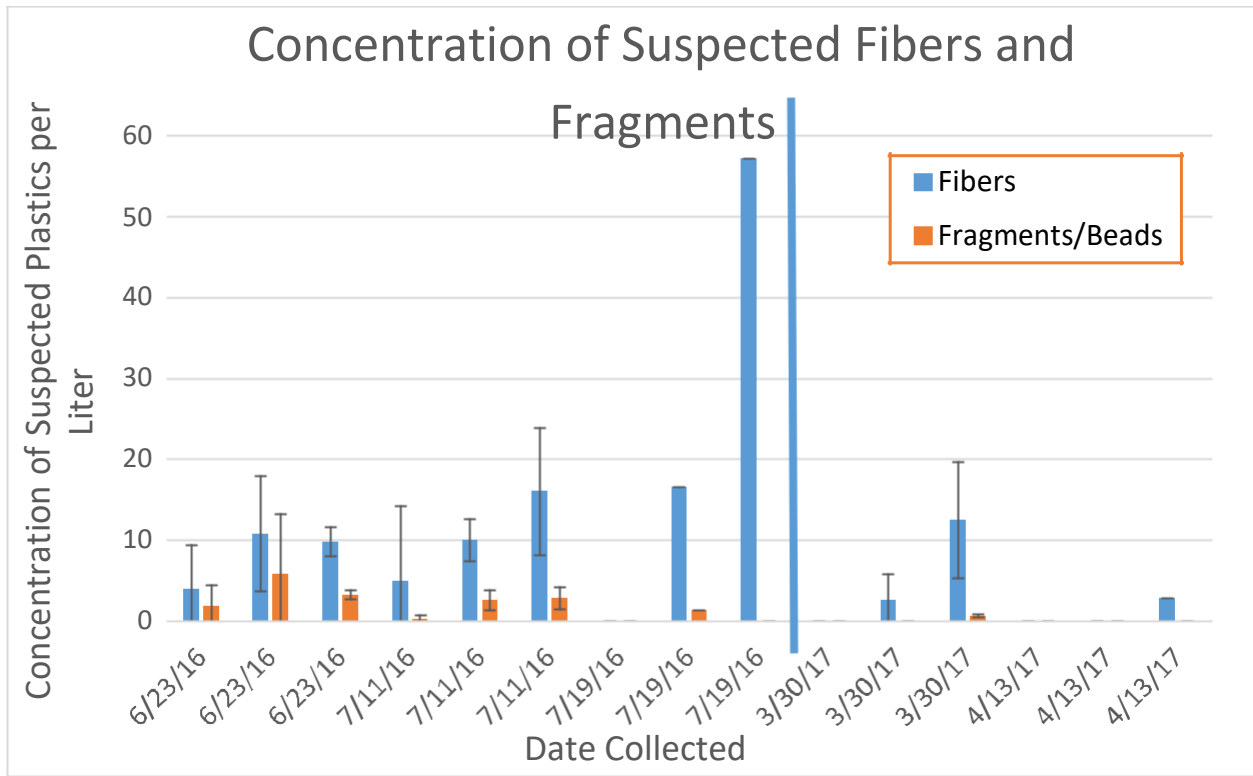


Figure 2. Concentration of fibers and fragments/beads. Sampling was halted from August 2016 to March 2017 due to biosafety permitting.

Figure 3 compares the type and colors of all microplastics found in the samples, separated by size class. There was a greater proportion of clear and blue plastics than any other color. 77.5% of all suspected plastics recovered were clear or blue microplastics (Figure 3). Blue fibers were found in all samples in the 50 μm size class (Figure 3a). Green and red beads were the least recorded type of microplastic; each only made up .009% of the total fragments/beads found. Lab controls were left uncovered for 1 week in five locations around the laboratory. Locations 1 and 2 are the areas where samples were processed. Lab controls did contain varying amounts of fibers, so there was possible introduction of fibers to analyzed samples (Table 1). On

average, Location 1 had the lowest amount of contamination by fibers. 47.6% of fibers found were clear and 35.4% were blue.

Table 1. Laboratory Controls. Controls were left uncovered for one week.

Location	Data Set	Date Collected	Fiber Color					Total
			Clear	Blue	Red	Black	Green	
1	7/5/2016	7/12/2016	1	0	0	2	0	3
2	7/5/2016	7/12/2016	11	1	0	3	0	15
3	7/5/2016	7/12/2016	8	5	0	0	0	13
4	7/5/2016	7/12/2016	4	0	1	3	0	8
5	7/5/2016	7/12/2016	n/a	n/a	n/a	n/a	n/a	0
1	7/14/2016	7/21/2016	7	0	1	2	0	10
2	7/14/2016	7/21/2016	15	16	2	8	0	41
3	7/14/2016	7/21/2016	14	20	5	6	2	47
4	7/14/2016	7/21/2016	5	1	0	2	0	8
5	7/14/2016	7/21/2016	6	7	1	0	0	14
1	7/22/2016	7/29/2016	3	3	0	0	0	6
2	7/22/2016	7/29/2016	9	11	0	0	0	20
3	7/22/2016	7/29/2016	9	17	0	0	0	26
4	7/22/2016	7/29/2016	12	15	0	1	0	28
5	7/22/2016	7/29/2016	15	11	0	0	0	26
1	8/3/2016	8/10/2016	7	2	2	2	0	13
2	8/3/2016	8/10/2016	17	5	4	7	0	33
3	8/3/2016	8/10/2016	23	16	1	4	1	45
4	8/3/2016	8/10/2016	11	4	2	1	0	18
5	8/3/2016	8/10/2016	10	5	0	4	0	19

c)

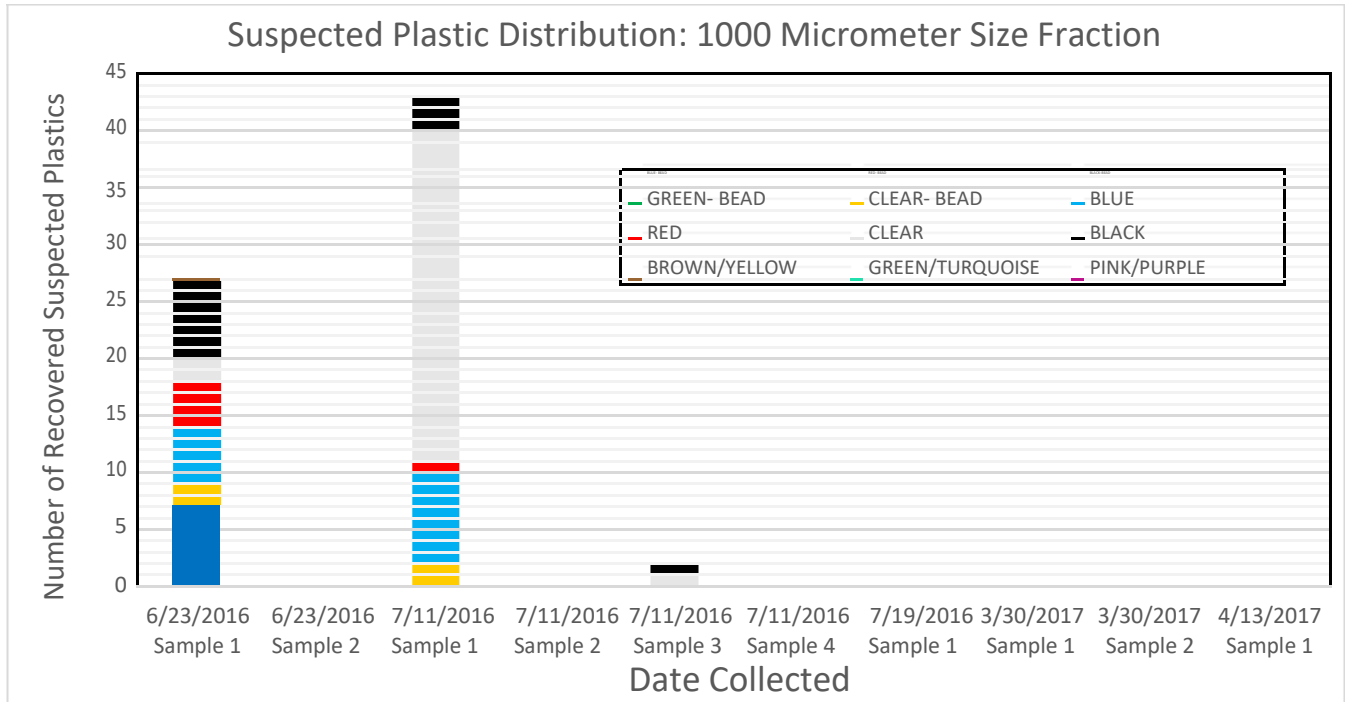


Figure 3. Color distribution of suspected plastics in the a) 50 μm , b), 300 μm , and c) 1000 μm categories.

One weakness to the method used here is the potential for false detection of plastic materials. This is partially overcome by the hot needle test, which has been performed on some of the samples presented here. However advanced techniques like micro-Fourier transform infrared (micro-FTIR) spectroscopy would improve accuracy and confidence. Our lab expects to acquire this instrument during the summer and will further verify these sample results. Effluent samples from additional wastewater treatment plants have also been collected and will continue to be collected in the coming months to develop a better understanding of wastewater discharge of plastic debris.

Discussion

Multiple studies have documented the passage of microplastics through wastewater treatment plants (Mason et al., 2016; Baltic Marine, 2014; Magnusson & Wahlberg, 2014; Carr et al., 2016, Murphy et al., 2016; Martin & Eizhvertina, 2014). All studies noted microplastic counts within the effluent, although the counts vary between the studies. Baltic Marine (2014), Magnusson & Wahlberg (2014), and Murphy et al. (2016) sampled at only one facility, as was done with the present study. The studies done at more than one facility have been large-scale studies where anywhere from 500 to 232,000 L were processed (Mason et al., 2016; Carr et al., 2016; Martin & Eizhvertina, 2014). The average concentration of fibers and particles in this study (9.33 ± 11.53 and 1.56 ± 2.32 particles/L, respectively) is consistent with those found in other studies, which range from .004 to 32 microfibers and .004 to 7 microparticles. This makes sense because of the institution of the Microbead-Free Waters Act of 2015. This legislation banned the use of microbeads in manufactured products by July 2017 (Lasse et al., 2017). Many companies have phased out the use of microbeads in their products to comply with the law, so this may be why low amounts of microbeads/fragments are being recorded. Fibers are released each time synthetic clothing is washed, so this is why fibers have been recorded in all studies in higher numbers than particles.

Previous studies (Mason et al., 2016; Magnusson & Wahlberg, 2014) only classify sizes in two groups: $\sim 100\text{-}350\ \mu\text{m}$ and $>350\ \mu\text{m}$, so there is very little size data to compare the $1000\ \mu\text{m}$ size class to in this study. Very few large ($>1000\ \mu\text{m}$) particles/fibers were found; this is because the larger sizes are most likely filtered out by wastewater treatment plants during the primary treatment process. They are most likely caught on the screens/grates when the wastewater enters the facility. In this study, there were no beads/fragments found in the 1000 or

300 µm size class in the samples that have been hot needle tested. This may mean that there was false identification of plastics in the samples taken in the summer of 2016, because those samples have not been hot needle tested.

Murphy et al. (2016) showed the color distribution of all the microplastics observed in the samples. They found that red, blue and green microplastics were the most numerous. The present study, in contrast, found that blue and clear were the most numerous (77.5%) and red and green particles had the lowest counts (<2%). These differences may be due to a variety of factors such as location, the population that is served, time of day, and daily flow variations (Murphy et al., 2016). The Conkle lab will be conducting studies in the future to determine the extent of temporal variation in microplastic loading.

The majority of previous studies utilized a microscope for visual identification of microplastics (Mason et al., 2016; Baltic Marine, 2014; Magnusson & Wahlberg, 2014; Martin & Eizhvertina, 2014), so the method used in this study is comparable to that of the literature. However, according to a case study on methodology of plastic identification, only 1.4% of particles visually resembling microplastics were of synthetic origin (Loder & Gerdts, 2015). Advanced techniques like micro-Fourier transform infrared (micro-FTIR) spectroscopy would improve accuracy and confidence. The Conkle lab will be acquiring a micro-FTIR later in 2017, so these preliminary results will be further verified.

Conclusion

Plastic pollution is a problem that will continue to face humans for years to come, and it is something that should be considered a priority. Microplastic debris is a form of pollution that isn't as easily seen as others, but it is still a threat to marine organisms and the health of marine

ecosystems. This research showed that wastewater effluent is a source of microplastics and that the loads vary between sampling dates. Due to the ban on microbeads enacted in the United States, companies have been phasing them out of their personal care products (toothpastes, facial cleansers, etc.). In July of this year, it will be illegal to have microbeads in any wash-off products. Because of this, there were very few microbeads found in the effluent samples. The majority of the microplastics found were synthetic fibers; this is a source of pollution that needs to be further researched. Clear and blue microplastics were the majority (77.5%) of suspected plastics. The data presented will be further analyzed using a micro-FTIR, which will increase the accuracy of microplastic identification. Samples from other wastewater treatment plants in the city will give a broader scope to the project and will help give us a better understanding of the quantities of microplastic debris entering surface waters through effluent.

References

- Auta, H.S., Emenike, C.U., & Fauziah, S.H. (2016). Review article: Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environmental International*. 10.1016/j.envint.2017.02.013
- Bakir, A., Rowland, S.J., & Thompson, R.C. (2014). Transport of persistent organic pollutants by microplastic estuarine conditions. *Estuarine, Coastal and Shelf Science*, 140, 14-21. <https://doi.org.manowar.tamucc.edu/10.1016/j.ecss.2014.01.004>
- Bureau of Labor Statistics. (2017). *Plastics and rubber products manufacturing: NAICS 326*. Retrieved from <https://www.bls.gov/iag/tgs/iag326.htm#iag326emp1.f.p>
- Carr, S.A., Liu, J., & Tesoro, A.G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.*, 91, 174-182.
- City of Corpus Christi. (2011). *Wastewater service area*. Retrieved from <http://www.cctexas.com/government/wastewater/service-area/index>
- Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., & Tassin, B. (2015). Microplastic contamination in an urban area: a case study in Greater Paris. *Environ. Chem.*, 12, 592-599. <http://dx.doi.org/10.1071/EN14167>
- Eerkes-Medrano, D., Thompson, R.C., & Aldridge, D.C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritization of research needs. *Water Research*, 75, 63-82. <http://dx.doi.org/10.1016/j.watres.2015.02.012>

Lasse, S., Mauricio, J., Thompson, W.A., Karnjanapiboonwong, A., Kasumba, J., Subbiah, S. ...

Anderson, T.A. (2017). Microplastics in a freshwater environment receiving treated wastewater effluent. *Integrated Environmental Assessment and Management*, 13, 528-532. doi: 10.1002/ieam.1915

Loder, M., & Gerdt, G. (2015). Methodology used for the detection and identification of microplastics—A critical appraisal. *Marine Anthropogenic Litter*. doi: 10.1007/978-3-319-16510-3_8

Magnusson, K., & Wahlberg, C. (2014). *Screening of microplastic particles in and downstream of a wastewater treatment plant*. Swedish Environmental Research Institute: Stockholm, Sweden.

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J. . . . Rogers, D.L. (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environmental Pollution*, 218, 1045-1054.
<http://dx.doi.org/10.1016/j.envpol.2016.08.056>

Murphy, F., Ewan, C., Carbonnier, F., & Quinn, B. (2016). Wastewater treatment works as a source of microplastics in the aquatic environment. *Environmental Science and Technology*, 50(11), 5800-5808.

NOAA. (2017). Marine debris program: Plastics. Retrieved from <https://marinedebris.noaa.gov/info/plastic.html>

Pita, F., & Castilho, A. (2017). Separation of plastics by froth flotation. The role of size, shape and density of the particles. *Waste Management*, 60, 91-99.
<https://doi.org.manowar.tamucc.edu/10.1016/j.wasman.2016.07.041>

PlasticsEurope. (2016). *Plastics - the facts 2016: an analysis of European plastics production, demand and waste data*. Retrieved from

<http://www.plasticseurope.org/Document/plastics---the-facts-2016-15787.aspx?FolID=2>

Ziajahromi, S., Neale, P.A., Rintoul, L., & Leusch, F. (2017). Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. *Water Research*, *112*, 93-99.

<http://doi.org.manowar.tamucc.edu/10.1016/j.watres.2017.01.042>