Quantification of Microfibers from Marine Sediments from Three Locations in Southern California: An Exposed Beach (Ventura County), a Watershed (Los Angeles County), and an Enclosed Harbor (Orange County)

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ABSTRACT
Microfibers are small (<5 mm) fibers made of synthetic materials that are ubiquitous in the environment. The purpose of this observational study was to quantify the number of microfibers in marine sediments and determine which locations have the highest risk for this type of pollution. Sediment samples were taken from three locations in Southern California (Sycamore Watershed, Ventura State Beach Jetty, and Newport Beach Harbor) to determine which had the highest number of microfibers. It was hypothesized that microfibers would be found at each sample site and that the most microfibers would be found at Sycamore Watershed due to its proximity to a wastewater discharge point. The microfibers were separated from the sediment through a process of stratification and filtration and analyzed by a one-way ANOVA and Tukey s test. Per sample, there was an average of 111.5 (±99.3, n=14) microfibers found per sample at Sycamore Watershed, 59 (±17.4, n=18) at Newport Beach Harbor, and 53 (±14.4, n=18) at Ventura State Beach Jetty. A total of 3,590 microfibers were found from all three sample sites. Analysis revealed that Sycamore Watershed had significantly more microfibers than any other site (p<.05). It is likely that Sycamore Watershed had the most microfibers because of its proximity to a sewage-sludge disposal site that contains the polluted water from our washing machines. In conclusion, microfibers are polluting the sediments in harbors, open coastlines, and watersheds in California, negatively affecting the ecosystems in these areas.

KEYWORDS
Microfiber; Microplastic; Macroplastic; Marine Pollution; Synthetic Materials; Wastewater Treatment Plants; Sediments; Watershed; Harbor; Jetty

INTRODUCTION

Ocean pollution is a pressing issue that impacts all depths of the oceans, their coastliness, and the organisms that rely on the seas for food. Much of this pollution comes from plastic waste of varying sizes. As the plastic is broken into smaller pieces over time, it can become considered microplastic. Though microplastics are less than 5 mm across and smaller than macroplastics, they are still a growing and concerning type of pollution.[1, 2] Microplastic debris accumulate in natural habitats worldwide, from the North to the South poles and the bottom of the seafloor to urban beaches.[3, 4] While most microplastics come from larger pieces that have been weathered, some microplastics enter the ocean in their original state. For instance, microbeads in exfoliating scrubs are made of plastic, and once rinsed off and flushed down the drainage system, can sometimes avoid or pass through the filters in wastewater treatment facilities and end up accumulating in the environment. More than eight trillion microbeads are dumped into the water systems in the United States every day.[5, 6] The more these plastics accumulate in the ocean, the more scientists are beginning to see how they are harming the ecosystems at many levels of the trophic pyramid.

Microfibers are the most abundant category of microplastics. They are categorized as a synthetic plastic most commonly made out of polyester, acrylic, or nylon that has been shaped into a fiber used to form different styles of clothing, textiles, or non-woven textiles.[1, 2, 7-9] Today, synthetic fibers make up 14.5% of plastics produced by mass and account for almost 2/3 of fibers produced worldwide. [9] The majority of these fibers make their way into the ocean from washing machines that discharge their untreated wastewater or from wastewater treatment plants (WWTPs), where the greywater has not been properly treated, leading to the microfibers being released into the ecosystem.[2, 8-11] Browne *et al*., (2011)[10] found that a single piece of clothing can produce over 1900 microfibers from one wash cycle. In a study by Peller *et al.* (2019)[12], it was estimated that as many as eight trillion microfibers are emitted daily from WWTPs operating in the United States. Microfibers have been found worldwide but are present in higher quantities in areas with high populations of humans and runoff. [1, 10, 13]

Natural fabrics are made from natural materials like silk, cotton, wool, and linen. As technology has advanced, new synthetic fabrics have been created to improve upon the durability of natural fabrics and lower the manufacturing cost. These synthetic fabrics have garnered global usage for their inexpensive, versatile, and durable nature, but are also negatively impacting our marine environment. Synthetic textiles are a danger due to the same factors that make them marketable: they are resistant, lightweight, and long lasting. Their durability means that the synthetic threads that make up the clothing are not biodegradable and will persist in an environment for decades. The thread's lightweight nature allows them to be carried hundreds if not thousands of miles from their point of origin and their diversity in shapes makes them available to be ingested by numerous aquatic organisms.[5, 10, 11, 14, 15] Ingestion of these items is a growing concern for organisms at all levels of the food chain. If ingested, the plastic thread can lead to starvation by blocking or damaging the stomach and lessening feeding. Organisms that ingest plastic have also been found to have lower energy reserves, decreased ability to remove bacteria, and are more susceptible to oxidative stress.[15] This also becomes a problem where trophic-transfer occurs, as when small organisms such as zooplankton ingest synthetic threads, the pollutants are transferred up the food chain, and if enough are accumulated, it could harm a predator higher on the trophic pyramid. [6, 11, 16] Synthetic materials are not only dangerous for these factors, but also for the fact that they can leach many chemicals into the surrounding environment. For instance, flame retardants such as triphenyl phosphate (TPhP), AZO dyes, PFCs (Perfluorocarbon), and formaldehydes can be added to clothing to make them stain and wrinkle resistant as well as waterproof. These additives have been found to be cancerous and in some cases linked to hormone disruption in humans.[17] Chemical additives play a great role in modifying physical or chemical properties of plastic materials.[18] Plastic additives include plasticizers such as dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), diisobutyl phthalate (DiBP), bis (2-ethylhexyl) phthalate (DEHP), and other phthalates to promote the plasticity and flexibility of the material.[19] These same chemicals leach into marine ecosystems following a machine wash cycle and, along with the fibers, can threaten the health of marine organisms.

These increased quantities of microfibers are a danger to all marine organisms, especially to filter feeders and other organisms at the bottom of the food chain. Due to their long, thin shape, they have a higher probability of becoming entangled in an animal's digestive tract, and if the digestive system is blocked long enough, the fiber can leach chemicals into the animal, causing disruptions in its endocrine system and hormone regulation.[5, 6] It has been noted that in zooplankton, ingestion of microplastics and fibers has had negative repercussions for the organism's growth, sexual development, and mortality.[6] Over 200 marine and aquatic species, from filter feeders to crabs and fish, have been documented to have ingested microplastics.[16] Microfibers are extremely widespread, having been found in a study by Browne *et al.* (2011)[10] on six continents and 18 sites worldwide. Microfibers have been documented in beach sediments and throughout the water column from the shallows to the deep sea, 5000 m below the surface.[20] This type of pollution is an issue especially in the deep sea because the organisms rely on detritus from the euphotic zone in the form of 'marine snow 'for the majority of their nutrients. These microfibers are the same size as much of that detritus and are being readily ingested by those organisms despite the fact that the point of origin of the plastic was thousands of meters above them . [15] In Cape Town, South Africa, Ryan *et al.* (2020)[14] found microfibers in 26 of their 30 total sediment cores sampled from a public beach. They also noted that microfibers were in the overwhelming majority of particulate pollutants that were found, accounting for 99.7% of microplastics on the study beach.

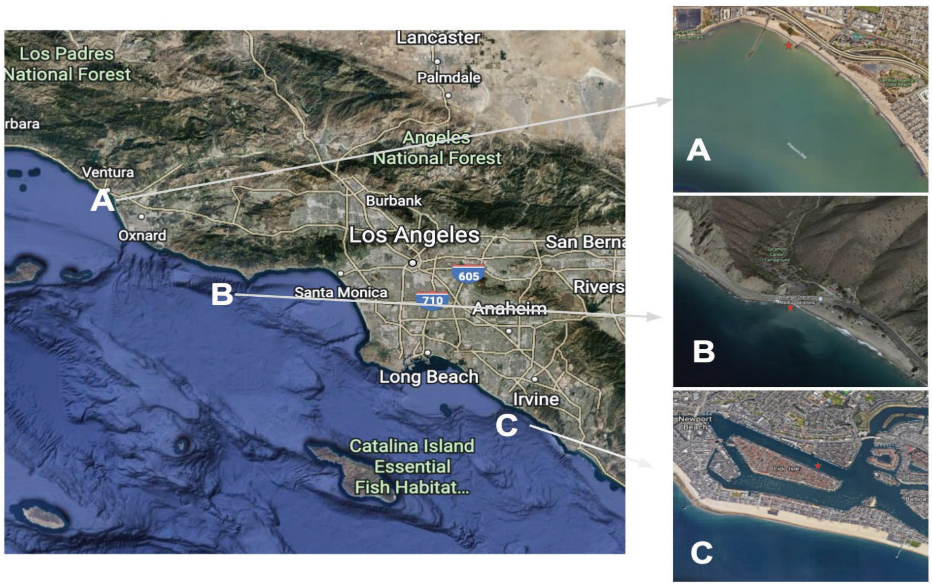
The purpose of this observational study is to quantify microfibers found at different locations to determine which has the most and discuss possible reasons why some locations may be more polluted than others. The locations sampled in Southern California are Sycamore Watershed, Ventura State Beach Jetty, and Newport Harbor. These three locations were selected because they experience different degrees of wave action and polluted runoff due to their geographical location and environment makeup. Sycamore Watershed in Los Angeles County receives wastewater runoff from WWTPs in Southern Ventura County. Ventura State Beach Jetty in Ventura County is located at an exposed coastline that receives high amounts of wave action and water circulation. Newport Harbor in Orange County is the largest small-boat harbor on the west coast and is a stagnant location with little wave action.

This study will help to fill a local knowledge gap about levels of microfiber pollution in Southern California and to compare sediment microfiber quantities from very different marine environments to understand if there are differences in microfiber abundance between sediments from an exposed beach, a watershed and an enclosed urban harbor. If the results are significant, it could be worth looking into the local WWTPs or runoff sewers to determine if there is a large amount of pollution caused by dumping or improper water treatment. It is expected that there will be microfibers found at all three locations. It is also expected that Sycamore Watershed will have the highest number of microfibers, as it directly receives wastewater contaminated with microfibers from inland wastewater treatment plants.

METHODS AND PROCEDURES

Sample collection

Sand samples were collected from Sycamore Watershed, Ventura State Beach Jetty, and Newport Harbor at the hightide water line. 14 samples were taken from Sycamore Watershed, 18 from Ventura State Beach Jetty, and 18 from Newport Harbor. Samples were collected from Sycamore Watershed in summer 2021 and from Ventura Jetty and Newport Harbor in summer 2020. There were 14 samples taken from Sycamore Watershed due to time restraints. The sediment from Ventura Jetty and Newport Harbor was collected on the same day and as such, the same number of samples were taken. All of the sediment samples were collected by hand every few feet along the hightide water line and placed into new, previously unopened 1-gallon Ziploc bags that were immediately closed and remained closed until the samples were processed in the lab.



Source: Aerial Image" Southern California Coast." 34.31338°N -118.92202°W. Google Earth.

Figure 1. Location of samples taken from southern California (A: Ventura State Beach Jetty, B: Sycamore Watershed, C: Newport Harbor).

Source: Data Basin WasteWater Treatment Plants, California, *USA"* Conservation Biology Institute.

Figure 2. Map of the Wastewater Treatment Plants in Southern California with Sample Sites Labeled (A: Ventura State Beach Jetty, B: Sycamore Watershed, C: Newport Harbor).

Sample preparation

Instant seawater was made and kept in a closed system in the lab to avoid being contaminated by microfibers and maintained at a salinity of 35 ppm. 100 mL of dry sand was mixed with 200 mL instant seawater in a beaker. The mixture was stirred, suspending the microfibers in the seawater mixture, and allowed to settle so the denser sediment would fall to the bottom. During the stratification process, the mixture was covered with saran wrap to ensure no microfibers fell into the beaker from the air. To avoid potential contamination, cotton lab coats were worn, the mixtures were covered to avoid microfiber fallout from the air, and the samples were only uncovered for brief periods when they were being actively filtered or observed.

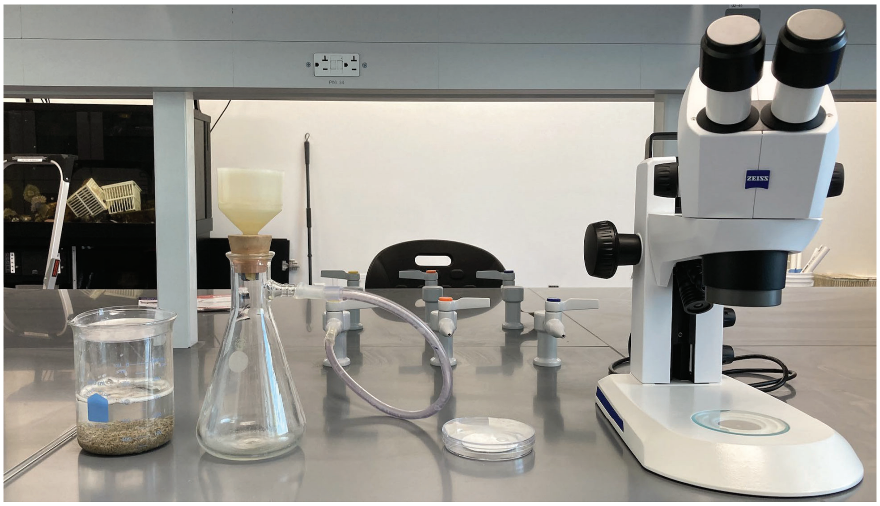


Figure 3. The process by which microfibers were stratified out from sediment, filtered, and observed.

Filtration

Once the sediment in each sample settled, the seawater solution above the sediment layer was poured off and filtered through a Buchner Funnel filtration system that used a 5 µm cellulose nitrate filter paper to catch the microfibers from the mixture. The process was completed a total of six times for samples from Sycamore Watershed and five times for samples from Ventura Jetty and Newport Harbor. The samples from Sycamore Watershed still had microfibers present after five washes had been done, unlike most of the samples from Ventura and Newport, and thus a sixth wash was performed to obtain a more accurate count of the microfibers in each sample. Following the sixth wash, there were zero microfibers observed in the majority of samples, and it was decided that a seventh wash was not needed.

Observation and validation of microfibers

The filter paper was released from the filtration system and placed into a petri dish for examination. The filter paper remained covered in a petri dish to reduce contamination until it was placed under a dissecting microscope. The filter papers were analyzed under a Nikon Stereo dissecting microscope, and the number and color of the microfibers present were noted. Microfibers were counted based on their overall shape and texture, which was consistent with microfibers observed by the lab in previous years. The microfibers generally were smooth, one solid color, and had twisted shapes. Lab blanks were used as a control to determine approximately how much microfiber contamination was in the lab. 20 samples were processed without sediment using the prior methodology. 200 mL of instant seawater was stirred, allowed to settle, and passed through a filter paper. One microfiber was found in one sample, and as such, it is likely that there was very little airborne microfiber contamination in the sediment samples that were processed.

Data analysis

The data were analyzed using Microsoft Excel. The program was used to determine the sums and averages of microfibers found at all three locations based on the number of microfibers per sample. Excel was also used to conduct a one-way analysis of variance (ANOVA) to determine if a difference existed in the means of all three locations sampled. Due to the significance of the data, a post-hoc Tukey's test was conducted using the website Astatsa to determine which specific locations had a significantly different number of microfibers from each other. Results were significant at $p<.05$.

RESULTS

There were microfibers present in sediment samples from each of the three locations (Sycamore Watershed, Ventura State Beach Jetty, and Newport Harbor). Black, red, green, and other colors, such as purple or blue microfibers **(Figure 4)** were all analyzed under a dissecting microscope. A total of 3,590 microfibers were found. 1,561 of those microfibers were found in Sycamore Watershed, accounting for 43.5% of all microfibers observed. There were 1,062 microfibers found in Newport Harbor and 967 fibers in Ventura State Beach Jetty **(Figure 5).**

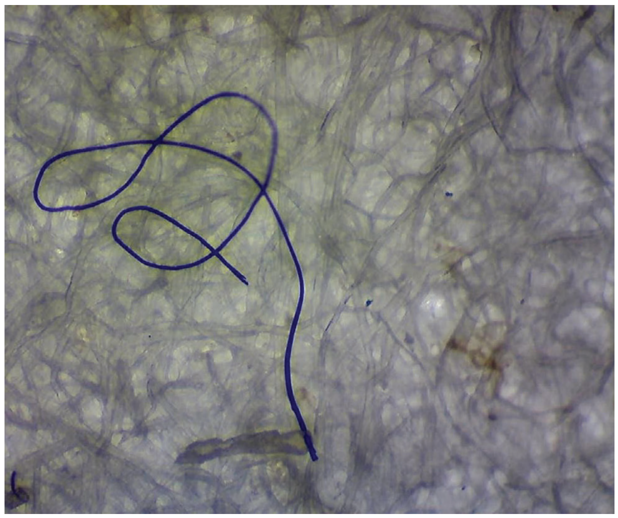


Figure 4. A blue microfiber under a dissecting microscope.

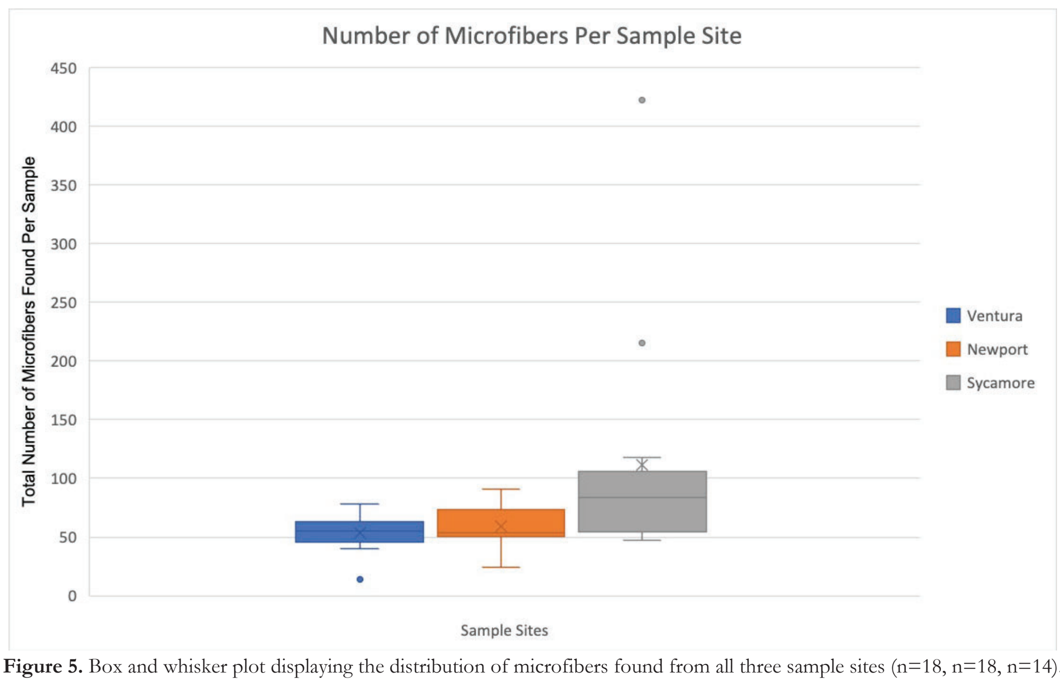


Figure 5. Box and whisker plot displaying the distribution of microfibers found from all three sample sites (n=18, n=18, n=14).

Sycamore Watershed had the highest average number of microfibers per sample, with 111.5 fibers per sample. Newport Harbor had the second-highest average number of microfibers per sample with 59 fibers per sample. Ventura State Beach Jetty had an average of 53.7 microfibers per sample.

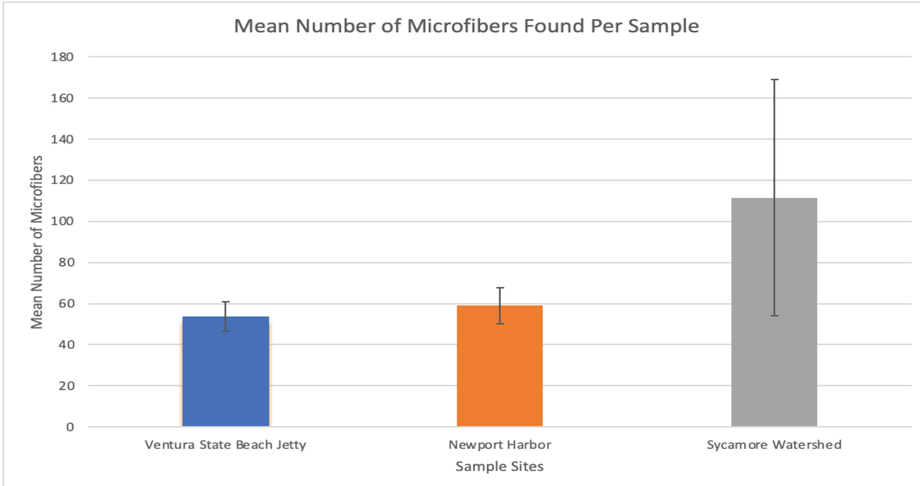


Figure 5. Bar graph depicting the mean number of microfibers found per sample at the Ventura State Beach Jetty (53.7 ±14.4, n=18), Newport Harbor (59 ± 17.4, n=18), and Sycamore Watershed (111.5 ±99.3, n=14) sample sites. (Error bars represent 95% confidence interval).

A one-way ANOVA revealed that there was a significant difference in the mean number of microfibers found in at least two sample sites (DF (2, 47) = 5.3099, p=0.0083) **(Table 1)**. A Tukey's post-hoc test was done to determine which locations differed from each other and found that Sycamore Watershed had significantly more microfibers than the other locations (p<.05) **(Table 2)**. Ventura State Beach Jetty and Newport Harbor were not different from each other.

Data Summary				
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**
Group 1	18	53.7222	14.3519	3.3828
Group 2	18	59	17.4356	4.1096
Group 3	14	111.5	99.2555	26.5271

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	2	30896.9034	15448.4517	5.3099	0.0083
Within Groups	47	136741.1177	2909.3855		
Total:	49	167638.0211			

Table 1. A one-way ANOVA was performed to determine if sample means differed between groups (Group 1: Ventura State Beach Jetty, Group 2: Newport Harbor, Group 3: Sycamore Watershed).

Treatments Pair	Tukey HSD Q Statistic	Tukey HSD p-value
Sycamore vs Ventura	4.2511	.011
Sycamore vs Newport	3.8628	.024
Ventura vs Newport	.4151	.90

Table 2. Results of the Tukey's post-hoc test done following the ANOVA to determine differences in means between all three sample sites.

DISCUSSION

Microfibers were abundant at all three sample sites, with Sycamore Watershed having significantly more microfibers than the other locations. Field investigations have shown that microplastic abundance in sediments is correlated to the amount of human activity in the surrounding environment.[21] There are multiple factors that influence microfiber contamination levels, such as proximity to sources of effluent discharge, nearby human activity, and geography, which must all be taken into account when looking at the global problem that is plastic pollution.[1, 10] It is likely that Sycamore Watershed had the most microfibers due to the fact that wastewater from washing machines runs through this sediment, which traps microfibers that have detached from clothing. Newport Harbor had the second greatest amount of microfibers, possibly because the stagnant water allows microfibers from pollution in the harbor to settle to the sediment. Ventura State Beach Jetty has the highest amount of wave action of the three locations, as it is an exposed beach and is subject to constant water circulation which could carry the microfibers out to sea, beyond the high tide line where samples were taken. Microfibers are present in multiple locations bordered by the Pacific Ocean, further supporting the hypothesis that microfibers are ubiquitous.

Sediment samples were also collected from Oahu, HI (n=24), as well as Bundegi Exmouth (n=3) and Turquoise Bay (n=2) in Western Australia by Dr. Huvard s previous research students and shipped to our lab. The samples were processed the same way in summer 2021, but since the collection methods were different, it was decided that they could not be directly compared to the Southern California samples. Even so, the data collected supports the idea that microfibers are ubiquitous in the marine environment, as fibers were found in Hawaii at an average of 16.4 (±10.2) per sample. Bundegi Exmouth had an average of 20.7 (±1.5) and Turquoise Bay an average of 34.5 (±2.1) microfibers per sample. Hawaii could have had a smaller average number of microfibers per sample than all three locations in Southern California due to having a less dense population compared to the sample sites in California, where over 20 million people live along the southern coastline.

As the global demand for plastic increases, the number of all living organisms ingesting microplastics will too. Microfibers detrimentally affect marine ecosystems and could do the same for human health. Although in current literature there is limited data on the toxicity of microplastics in humans, it is likely that microplastics absorb monomers, additives, and persistent organic pollutants (POPs), which have been linked to a disruption in humans 'gut biomes.[22] With microfibers prevailing in many environments from the Arctic to terrestrial environments and the deep seafloor, microfiber pollution is truly a global issue.[23, 24] A standardized methodology for determining the number or concentration of microfibers in a given sample location would lead to results that could be shared with labs worldwide and in turn aid in determining the toxicological risk microplastics pose to filter feeders, humans, and other organisms. Long-term studies looking at seasonal variability in microfiber concentrations would also be useful to determine the impact that tides and wave action have on microfiber deposition on beaches. It is important to study the levels of microfiber pollution so that mitigation strategies and cleanup efforts can be implemented to help curb microfiber pollution, especially in the most heavily impacted areas.

Solutions include improving globally based pollution prevention, introducing microorganisms to degrade polymers and additives, and reducing plastic use. Recent work indicates that certain microorganisms may be capable of degrading petroleum-based synthetic polymers. For example, Halle *et al.* (2020)[20] observed that the gut bacteria of mealworms can slowly break down polystyrene, and although residues remain, this could be a way to reduce the amount of plastic in our environment. Yoshida *et al.* (2015)[25] reported that bacteria exposed to polyethylene terephthalate at a recycling site produced enzymes that could degrade it to its basic monomers. This reveals that steps can be taken within the scientific community to help decrease the impact of plastic pollution.

The differences in techniques of isolating and identifying microfibers make it difficult to compare the levels of pollution between studies. The creation of a more uniform and effective method should be developed in the future to ensure consistency. While our process was simpler than that completed by other studies, it was unique in that it gave us an easily reportable and exact number of microfibers found at each location. Many studies, like the one performed by Claessens *et al.* (2011)[26] reported microfiber pollution as concentration instead of number. They used a dry sand density of 1.6 g/cm^3 to find the concentration of microfibers at a given location and compared those values to determine how polluted a location was. The number of microfibers recorded in our study might be slightly overestimated due to airborne fibers in the laboratory, water supplies, and materials that could have contaminated the samples. We measured approximately 100 mL of sediment per sample, but as it was not precise, we did not feel comfortable converting our data to concentrations and chose to report our data as averages so as to not over or underestimate the true number of microfibers found. As our data were collected on individual days, they represent a snapshot of microfiber pollution at these locations on these given days. Further studies should look at long-term microfiber pollution and how it could be influenced by seasonal or tidal changes. Despite the limitations of this study, microfibers were observed in large numbers in every sample taken from all three locations, indicating widespread microfiber pollution at these sites. Our data also still indicate that Sycamore Watershed was significantly more polluted than the other locations, with both the highest average number of fibers and the highest total microfiber count of the three sites.

CONCLUSIONS

The results of this observational study of microfiber quantities in sand sediments from three different locations support both of our hypotheses. There were microfibers found at all three sites and by using an ANOVA and Tukey's post-hoc test, it was determined that Sycamore Watershed had the highest average number of microfibers per sample. To our knowledge, this is the first observational study documenting microfiber pollution in sediment from Sycamore Watershed, Ventura State Beach Jetty, and Newport Harbor. This study shows that microfiber pollution is a pressing issue for land masses bordered by the Pacific Ocean and that better pollution prevention methods are needed.

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PRESS SUMMARY

Microfibers are a prevalent pollutant in our oceans and are disrupting aquatic organisms and human health worldwide. These microscopic pieces of plastic, typically defined as less than 5 mm in length, are a type of debris that originate from synthetic clothing and textiles, such as polyester and nylon. Often through the process of machine washing, fiber fragments are expelled into the wastewater and can travel long distances before eventually being deposited into the environment. It is important to figure out which locations are heavily impacted by microfiber pollution so that remediation strategies can be developed and implemented. In this study, we quantified the number of microfibers in marine sediments from a harbor, jetty, and watershed in Southern California. We discovered microfibers at all of the sample sites examined, indicating widespread microfiber pollution in these locations.