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## Environmental Significance Statement:

Synthetic microfibers constitute the most significant class of microplastic pollution in the environment. A modified procedure for total suspended solids measurements, incorporating Fenton chemistry to break down natural fibers, was used to isolate and quantify synthetic microfibers in water samples from a Lake Michigan (USA) watershed. While immense amounts of microfibers are released from household laundry, wastewater treatment plants (WWTP) remove most of these microscopic pollutants. Similar numbers of synthetic microfibers were detected in surface waters above and below a watershed WWTP, and several kilometers from the effluent discharge. Shoreline water samples revealed greater numbers of synthetic microfibers, suggesting accumulation of these pollutants along the shore. Experiments using known fabrics imply under-reporting of synthetic microfibers in natural samples.

ARTICLE

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# Tracking the distribution of microfiber pollution in a southern Lake Michigan watershed through the analysis of water, sediment and air

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Microplastic waste is a worldwide problem, heavily afflicting marine and freshwater environments; the loading of this pollution in water, sediment and living organisms continues to escalate. Synthetic microfibers, resulting from the release of microscopic fibers from synthetic textiles, constitute the most prevalent type of microplastics pollution in aquatic environments. This study investigated the origin and distribution of synthetic microfibers in a representative Lake Michigan watershed in Indiana (USA) by analyzing water, sediment and air samples above and below wastewater treatment plant discharges, downstream in the watershed and water from the Lake Michigan shoreline. Synthetic microfibers were also quantified in wastewater from a local wastewater treatment plant (WWTP) and in laundry effluent. Laboratory testing of numerous fabrics suggests that Fenton oxidation, used to break down natural fibers, effectively eliminates non-polluting, natural fibers from the samples. However, the hydroxyl radical-mediated oxidation bleaches the dye from certain synthetic microfibers, which likely leads to under-reported values for these microplastics in natural samples. The data collected from the watershed samples indicate that approximately 4 billion synthetic microfibers are transported daily through the Lake Michigan tributary. Wastewater effluent is not the only source of synthetic microfibers, since surface water samples above the WWTP contained a similar load to downstream samples. Repeated sampling exhibited variability in the number of microfibers detected, substantiating the heterogeneous distribution of these pollutants and the requirement for multiple samples for a given site. The average load of synthetic microfibers from water sampled at the Lake Michigan shoreline was higher than the tributary water, suggesting the shoreline functions as a repository for the microfibers. Given the extent and potential consequences of this pollution, quantification of the ubiquitous plastic fibers can be instituted as part of the traditional total suspended solids (TSS) water quality monitoring parameter.

## Introduction

Over 8 billion metric tons of plastics have been manufactured worldwide, and since a significant amount has been made for short-term use, much has turned into garbage. The estimation is that nearly 6 billion tons, or ~75% of the produced plastic, has become plastic waste.[1] Knowledge of the global plastic waste problem continues to be augmented by studies of the small pieces of synthetic polymers or plastics, termed microplastics or nanoplastics.[2] While macroplastics are items larger than 25 mm, microplastics are usually defined as synthetic polymer pieces in the 0.1 – 5 mm size range and nanoplastics as smaller than 100 nm.[3] The ubiquitous character of plastic waste on both the macro and micro level is due to a few main factors: the enormous surge in production of the synthetic materials since the 1907 invention of Bakelite and the 1935 synthesis of nylon, and the persistent nature of plastics in the environment.[4] From car parts to artificial limbs to water bottles, packaging and clothing, the discovery and function of plastic materials continue to be vast. The largest market for plastics is packaging and the production of plastic materials is predicted to increase over the next few decades.[5] Currently, over 320 million tons of plastic are produced globally each year, with projections for a continual rise.[6]

Since plastic materials do not biodegrade on a practical timescale and a large percentage are manufactured for single use, an immense amount of plastic waste has accumulated and continues to contaminate land, air and water around the globe.[7] An estimated 8 trillion pieces are emitted daily into surface waters from wastewater treatment plants in the United States.[8] According to the study by Lebreton et al, an estimated 1.15-2.41 million tons of plastic pollution enters oceans from fresh water rivers, indicating that land-based plastic is a significant source

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of the pollution.[9] Furthermore, microplastics are classified as emerging pollutants since they are persistent chemicals in water and sediment around the globe and accumulating.[10] The weathering and breaking of synthetic polymers leads to smaller pieces classified as fragments, while other materials are purposefully manufactured in small form, such as microbeads and microfibers. Synthetic microfibers, generated in large amounts from laundry effluent, are the largest subset of microplastics pollution.[11]

Synthetic fabrics are materials with attractive properties such as comfort and durability, yet most fabrics shed an enormous amount of microscopic fibers.[12] Synthetic microfiber clothing and other goods are commonly comprised of polyester, nylon (polyamide), spandex, polypropylene or acrylic. The 2011 data on fabric fiber production indicates that over 60% is synthetic, which resists biodegradation.[13] Given that synthetic microfibers constitute the most prominent type of microplastics pollution, they are detected in greatest abundance in nature, and provide a vector for the transport of numerous other chemical contaminants.[14, 15] These pervasive microscopic materials have been identified in numerous aquatic organisms, but the specific health effects are just beginning to be investigated.[16, 17] In general, the research on microplastics in water points to tremendous amounts of these materials in nearly every aspect of the aquatic environment.[18-20] Current estimates from the Switzerland-based International Union for Conservation of Nature and Natural Resources are that more than a half-million metric tons of synthetic microfibers enter the ocean annually.

Many studies have substantiated the link between laundry water, wastewater treatment and synthetic microfiber pollution in waterways and sediment. Most municipal wastewater treatment effectively removes the majority of microfibers from wastewater and concentrates them in the treated sewage sludge.[21] Therefore, a major destination or reservoir for synthetic microfibers may be land, in particular farmland, where WWTP biosolids are used as fertilizer.[22] Meanwhile, the majority of published studies on synthetic microfiber contamination have focused on water contamination.[23, 24]

Since microplastics are ubiquitous pollutants in surface water, it makes sense to consider their identification and quantification as part of water quality monitoring. Moreover, the regular tracking of microplastics as part of water quality monitoring protocol can be a means to monitor and compile information on these emerging and persistent contaminants. However, methodologies for the collection, detection and quantification of these microscopic plastics are not consistent and specific; standard protocols are not yet in place.[25-27] Many water samples analyzed for microplastics have been collected in large volumes using neuston nets, which are designed for gathering of neuston, living organisms in the upper 10 cm of the water column: plankton, larval fish, etc. While these nets are effective for collecting the non-fibrous microplastics, they are not well suited for the collection of microfibers, the most abundant of the microplastics. The difficulty in collecting and isolating microfibers from the neuston net with 335 µm pore size has been shown to lead to significantly lower counts of microfibers.[28]

For an accurate assessment of synthetic microfibers, water should be collected through grab samples and filtered in the laboratory, similar to the procedure used for total suspended solids (TSS) measurements. However, it is likely that sampling low volumes of water can lead to a high standard deviation due to the heterogeneous distribution of microfibers in the environment. Water samples for TSS analysis are collected on the 100-1000 mL volume scale and filter papers of pore size less than a micron are used to collect the suspended solids. Therefore, microplastics in water are inherently collected in the sampling for TSS. For microplastics isolation, a few alterations to the TSS standardized procedures can be made to enable the quantification of microfibers, namely more durable filter paper, clean techniques and field and lab blanks. By instituting these changes and implementing a few additional processing steps to remove natural materials, including cotton and other natural fabric fibers, the amount of synthetic microfibers can be assessed and added to water quality data.

The reported microfiber data offer an understanding of the origin and distribution of microfiber pollution in the studied watershed. It constitutes part of a larger set of traditional water quality parameters and an effort to more effectively describe the water quality of the Northwest Indiana Lake Michigan tributary, Salt Creek, given the universal plastic pollution problem. Current investigations do correlate the presence of microplastics with lower water quality.[29] The recurrent detection of synthetic microfibers in the environment should warrant the inclusion of this parameter in surface water quality monitoring protocol, given the ever rising amount of plastic pollution in the natural environment. This investigation also corroborates on the challenges associated with quantifying the synthetic microfiber content of natural waters and sediment samples.

## Experimental

### Sampling locations

Salt Creek (Porter County, Indiana, USA) and its tributaries flow to the Little Calumet River in Northwest Indiana, and then to Burns Waterway, which discharges into Lake Michigan. The Salt Creek watershed, draining 20,061 ha (49,572 acres) of land via abundant streams, is subjected to a wide array of nonpoint source pollution in addition to point sources, such as wastewater treatment plants. Water, air and sediment samples were collected along with several traditional water quality parameters from May 2018 through October 2018 from four sites along Salt Creek, labelled 1-4 (Figure 1); site 1 is the most upstream location from Lake Michigan. Samples sites for this study were selected to obtain creek water from above and below the Valparaiso, IN wastewater treatment plant (Elden Kuehl Pollution Control Facility), which is a Class IV Advanced Single Stage Air Activated Sludge Wastewater Treatment Plant and from different treatment levels at the plant. Samples were also retrieved immediately below a second, smaller wastewater treatment plant and approximately 8 km downstream at a public park, Imagination Glen – site 4, in the city of Portage. The locations of the WWTPs are shown in Figure 1. Lake Michigan shoreline water samples were collected at Porter Beach in Porter, Indiana.

Creek water samples were collected a total of eight times and sediment was collected on five different dates. For each sampling, four replicates of water were collected and two or three sediment replicates. Throughout the summer and fall months of the reported study, the weather was mostly dry and void of any heavy precipitation events. Lake Michigan water samples were taken along the shoreline, the swash zone, where sediment is exchanged between water and land and when the lake water was calm.

INSERT FIGURE 1 HERE

**Sample collections**

For the determination of total suspended solids (TSS) and quantification of synthetic microfibers in water, the sample volume was 500 mL. Prior to the sampling, bottles were thoroughly rinsed several times with laboratory deionized water to prevent microfiber contamination. At two locations, the water samples were retrieved using a water sampling pole with attached bottle, which was rinsed three times with the surface water before it was poured into collection bottles. At the other locations, the water was collected directly into the sampling bottles after rinsing with creek water. Blank samples (field blanks) containing deionized water were opened and held near the sampling bottles during the field collections. Water from the local wastewater treatment plant was collected in a similar manner. Laundry effluent samples were collected directly from local households.

Sediment samples were taken from the bed of the creek at the four sampling locations; however, during the dry summer months, this sediment was not covered by the creek water. A garden spade was rinsed with deionized water and then used to dig up the sediment sample. All sediment samples were collected in excess and field blanks were not taken on the sediment samples since the top layer of the sediment was discarded. Only the undisturbed sediment within the sample was used for analysis in the laboratory.

Air samplers were placed on remote location with the sampling sites. Each sampler was constructed with a 12 V DC battery powering a small pump. The pump was connected in line with a 25mm diameter, 0.2 µm pore teflon filter disc at an average flow rate of 3 L/min. Battery life varied from 2-3 days depending on conditions. Some samplers were placed nearby sampling sites within the same air mass under AC power for as long as a three week period of continuous operation.

**Laboratory procedures**

The water samples were refrigerated immediately after collection and then filtered within 48 hours of collection in a laminar flow hood to eliminate external microfiber contamination. To determine total suspended solids, EPA method 160.2[30] was followed, but slightly modified by substituting a nylon filter disc. The mass of the 47 mm, 0.45 µm nylon filter disc was determined using an analytical balance, and then placed in a covered glass petri dish. In the laminar flow hood, the water sample was vacuum filtered, and the filter funnel was thoroughly rinsed with deionized water. The filter disk was carefully transferred back into the glass petri dish and then set in an oven at approximately 102°C overnight. The mass of the dried filter paper and contents was recorded, and the filter paper was placed back into the covered glass petri dish.

The following steps were also performed in the laminar flow hood. For the isolation of synthetic microfibers, the filter paper with the collected solids was transferred to a 40 mL glass centrifuge tube and covered with foil. Blank centrifuge tubes constituted laboratory controls. Hydrogen peroxide, iron (II) chloride solution and 1 M HCl were added to the centrifuge tube to create the Fenton reagent,[31, 32] which generates hydroxyl radicals ($OH^{\bullet}$) according to the following reaction. These radicals effectively oxidize natural organic materials, including cotton, wool and other natural fibers. Synthetic fibers are not readily broken down with the Fenton reagent.

$$Fe^{2+}(aq) + H_2O_2 \rightarrow Fe^{3+}(aq) + OH^{\bullet} + OH^{-}(aq)$$

The reaction mixture was heated to approximately 80°C and typically reached a maximum reactivity several minutes after the addition of the reagents. The total reaction time was 30-45 minutes; the filter paper was then rinsed with deionized water and removed from the centrifuge tube. To ensure that microscopic fibers were not left behind on the filter paper, the rinsed filter paper was periodically viewed with the stereomicroscope. No microfibers were detected on the rinsed filter papers. The liquid mixture in the centrifuge tube was filtered onto a clean 0.45 µm nylon filter paper, followed by thorough rinsing of the centrifuge tube and filter funnel with deionized water. The filter paper was transferred back to the glass petri dish, covered and viewed under a stereomicroscope. The synthetic microfibers were identified, counted and photographed. Water samples collected from the wastewater treatment plant (raw influent, primary-treated and effluent samples) and from laundry effluent were filtered, processed and analyzed in the same manner, except that smaller sample volumes were required for these more highly concentrated samples.

For each sediment sample, the top layer of sediment was removed and a portion of the sediment was transferred to beakers in the lab and lightly covered with foil. The beakers were placed in an oven set around 100°C for 2-3 days until the contents were dry. Approximately 30 g of the dried sample was transferred into a 250 mL beaker, the exact mass of sediment was recorded and the beaker was covered. A $ZnCl_2$ solution of density 1.4 g/mL was prepared, since most synthetic microfibers float or suspend in this solution. A volume of 40-50 mL of the zinc chloride solution was added to each sediment sample and a lab blank was prepared with the solution. Each mixture was stirred for 30 minutes on a stir plate with a magnetic stir bar to ensure thorough mixing of the sediment with the solution; the mixture was left overnight to settle out the heavier particles.

The liquid portion of the zinc chloride mixture was decanted into a filter funnel containing a 47 mm, 5.0 µm nylon filter disc in the laminar fume hood. Since observed microfibers were longer than 100 µm, filter paper of pore size 5.0 µm and smaller effectively collected the pollutants. After the liquid mixture was transferred and filtered, the filter paper and contents were placed into a 40 mL glass centrifuge tube and subjected to at least one round of Fenton oxidation, similar to the TSS filters. The isolation and visualization of synthetic microfibers proceeded in the same manner as the TSS samples.

### Microfiber standards and visualization

Polyester microfibers were spectroscopically verified using both Raman and IR techniques at the initial stages of the study. The analyzed fibers were also clearly visualized using a stereomicroscope; while this limits the detection of microfibers to approximately 100 microns (length) and larger, the stereomicroscope is widely utilized to identify microfibers in this type of work.[26] Therefore, synthetic microfibers were identified and counted using a stereomicroscope for the reliable detection of microfibers greater than 100 microns.

Several samples of natural and synthetic fabrics constituted microfiber standards. The tested natural fibers were cotton, wool, and linen and cotton-like hairs from the seeds of eastern cottonwood (*Populus deltoids*) collected in the field. Several different synthetic/semi-synthetic fabrics of different colors were analyzed: polyester (polyethylene terephthalate or PET), acrylic (polyacrylonitrile), nylon (polyamides), rayon and modal (regenerated cellulose). Small swatches (~ 1 cm x 1 cm, 40-60 mg) of each fabric were placed in covered 500 mL glass jars containing deionized water. A magnetic stirring bar was added and the mixture was stirred for a minimum of 30 minutes. A specified volume of each freshly shaken mixture, usually 10-30 mL, was filtered onto a 47 mm, 0.45 µm nylon filter disk. The filters were viewed under the microscope and the number, color and shape of fibers were counted and recorded.

The laboratory processing procedures were performed using the standard fabrics to assess the selectivity of the chemical oxidation and the accuracy of the quantification of MFs in the field samples. To effectively break down natural fibers, as to avoid counting these non-pollutant fibers, Fenton oxidation was employed on the standards. The highly oxidative hydroxyl radicals created by the Fenton reagent oxidatively decompose numerous organic compounds.[34,35] The list of several of the fabric materials and their colors used as standards, or positive controls, are listed in Table 1, along with the data and observations for the outcome of the Fenton reagent processing. Many of the chosen standards were polyester fabrics from clothing, blankets or stuffed animals, in line with the most common and manufactured fabrics, which release microfibers.[36, 37] At least four repetitions of each of the fabric fibers were completed. The microfiber counts for blue polyester, purple acrylic and the cotton were consistent, and provide full validation of the laboratory processing steps. However, the processing of other fabrics/colors showed variation in the recovery of the MFs post-Fenton reaction.

INSERT TABLE 1 HERE

For the processed red polyester fabric, a close analysis of the magnified filter containing the microfibers showed numerous colorless microfibers similar in size to the red ones. For the black rayon samples, the color was nearly entirely removed from the microfibers as a result of the reaction with hydroxyl radicals; however, the colorless microfibers were still readily visible under the microscope, shown in Figure 2. This indicates that the hydroxyl radicals reacted with the black dye, but did not affect the integrity of the polymer, as expected. The gold polyester microfibers were notably thinner fibers when viewed before the Fenton reagent processing, and the color was difficult to verify under the microscope. After the oxidative processing, these microfibers looked similar, or slightly lighter in tone. Additional experiments were performed where humic acid and bicarbonate were added to simulate real world water contents. No differences were observed with these samples, substantiating no loss of effectiveness of the Fenton chemistry under these conditions.

INSERT figure 2 here

### Statistical Analysis

Data presented in figures 3 and 4 were graphed and analyzed with Graph Pad Prism 7 (San Diego, CA) using a one-way ANOVA with multiple comparisons with a significance level set at 0.05.

## Results and discussion

### Watershed and lakeshore samples

The majority of the water and sediment samples collected from the four selected sampling sites in the Salt Creek watershed contained one or more synthetic microfibers. From each watershed location, 32 surface water samples and 12 sediment samples were analyzed from May through October of 2018. All data reported in the figures, tables and text correspond to the number of synthetic microfibers after the subtraction of MFs in blanks. Approximately half of the field and lab blanks were free of microfibers. Contaminated blanks contained 1-3 MFs/500 mL water, even though clean lab and field procedures were followed, similar to other reported studies.[33]

Since wastewater treatment plants are likely the most significant source of microfiber pollution to the watershed, the sampling sites were chosen with reference to the two WWTPs. The average number of synthetic microfibers per 4L of water from sampling site 1, the first bar in Figure 3, (10 ± 2) represents the surface water above the wastewater treatment plants. It is important to note that even though site 1 is above the largest, local WWTP, it receives run-off from farm fields. Sites 2-4 in Figure 3 represent water samples from the other three watershed sites, which are located below the WWTPs. Site 1-3 are in close proximity to the WWTPs, less than 1 km, while site 4 is about 8 km downstream (Figure 1).

The number of synthetic microfibers detected in the water sampled above the WWTP was statistically the same as the other creek locations, sites 2 and 4, downstream from WWTP effluent, in the range of ~8-10 MFs/4L. The highest average number of synthetic microfibers (~13 MFs/4L) was from the sampling area directly below the discharge of the smaller WWTP (South Haven), site 3, though there was no statistical difference between this site and the other three sites. These data are shown in Figure 3.

The water sampled from Salt Creek is part of the greater Lake Michigan watershed and this tributary is one of many that contributes to the load of plastic pollution in Lake Michigan. A total of 15 lakeshore water samples were collected and analyzed to compare the synthetic microfiber pollution in the tributaries to the shoreline. Lake water samples were collected on three different sampling dates from two sites along the shoreline in Porter, Indiana, approximately 60 meters apart. The load of synthetic microfibers was in the range of 20 - 40 per 4L, with an average of 26/ 4L, statistically different from the average number of MF in watershed samples from sites 1, 2 and 4 (Figure 3). The higher load of synthetic microfibers from the shoreline water of Lake Michigan is possibly due to the swash action of the water and the ability of the sand to harbor particulates. Microfibers can be trapped in the sand and accumulate over time. The swash action may move some of the suspended materials/debris along the beach, in a type of cross-shore exchange.

INSERT FIGURE 3 HERE

Since the creek water above the WWTPs contained a similar amount of microfiber pollution in comparison to the downstream sites, air samples were taken at each of the sampling sites to determine the significance of the air in the source and distribution of microfiber pollution. While several thousand liters of air were sampled, no processed air samples exceeded even background blank levels of microfibers. Optical detection was applied as with the water and soil samples, which limits the size of fibers detected to approximately 100 µm or larger. These results may be consistent with attenuation of microfibers in the air mass by foliage near ground level. Overall, it was concluded that microfiber pollution was not transported through the air in the watershed to any significant extent.

The average number of synthetic MFs identified in the sediment samples from the three locations near the WWTPs was similar at ~41, 32, and 46 microfibers (MFs) per kilogram of sediment (dry mass), shown in Figure 4. For the sediment samples collected several kilometers downstream from the WWTPs, site 4, the number of synthetic microfibers was higher, nearly double (~68 MFs/kg dry sediment) in comparison to the samples in the closer proximity to the WWTPs. Despite the difference in the average number of MF/kg dry sediment, a one-way ANOVA indicated no statistical difference between the MF abundance at the four sites. Even though the density of most synthetic microfibers is greater than water: polyester is 1.37 g/mL, acrylic is 1.16 g/mL and nylon is 1.15 g/mL, microfiber pollution is transported by the surface water and settles to some degree in the sediment. As these particulates travel, other chemicals and particulates may adsorb or cling to the microfibers and change the density.

INSERT FIGURE 4 HERE

The distribution of the microfibers in water and sediment is heterogeneous and requires several samples to realize an average. For the sampled water from site 3, the average number of microfibers was 13 MF/ 4L and showed the greatest variation, 0-31MF/ 4 L. The samples for the study were collected during the 2018 spring, summer and early fall months and no correlation existed between number of microfibers and season. While it is possible that the quantitative average and variation is somewhat dependent on weather conditions, the data does not support weather-dependent variations of microfiber pollution. Differences in the make-up of the sediment particles were not analyzed, but are another factor that should be considered and analyzed for understanding the role of the sediment in the distribution of MFs. Given the discharge of water from Salt Creek (~ 1000 CFS), the data suggests that approximately 50,000 synthetic fibers are carried through the watershed each second, which translates to roughly 4 billion plastic microfibers transported each day.

### Sources of microfiber pollution: laundry and WWTP water samples

As part of the analysis of the synthetic microfiber distribution study, a number of laundry effluent and WWTP samples were collected and chemically processed. These samples were analyzed to assess the amount of synthetic microfibers 1) generated in local household laundry, 2) flowing into the wastewater treatment plant and 3) discharged to surface waters (watershed) after the treatment process. The laundry effluent water and the WWTP influent were highly concentrated with MFs; therefore, several small volumes of water were filtered (typically 10-100 mL) and tallied. The average number of synthetic MFs experimentally determined for actual laundry effluent was approximately 5000 /4L. Since a large load of laundry from an efficient washing machine discharges about 57 L of water, these measurements correspond to about 20,000 synthetic MFs/load. Much larger numbers of microfibers released from laundry effluent of polyester garments have been reported, in the range of 600,000 to 6 million microfibers for a 5 kg load of polyester clothes.[41, 42]

Two samples of WWTP influent were collected, processed and analyzed; for one sample, 440 synthetic MFs/4L were determined for the raw sewage; a much larger number was determined for the second sample, 2200 MFs/4 L. According to a report by the Water Research Foundation, 17% of domestic water use is for washing clothes.[43] Using the number of MFs determined in our laundry effluent, the predicted load of synthetic MFs originating from household wastewater and received by the WWTP is approximately 800 MFs/4 L. This value is similar to the amount determined from our two raw sewage samples (average = 1300 MFs/4L). Wastewater does not originate only from households, but laundry water is expected to be a major source of microfibers. The data from this study and others strongly suggest additional significant sources and reservoirs of synthetic microfibers, which travel through the watershed and contribute to MF loads in surface water.[21, 44]

The treatment steps of most WWTPs remove the vast majority of small particles in wastewater, including microfibers.[45, 46] Samples of primary treated wastewater were analyzed and an average of 310 synthetic MFs/4L were identified. For the final treated effluent, approximately 12 synthetic MFs /4L were identified, indicating greater than 97% removal of MFs from the overall treatment. In line with other reported studies, the local WWTP discharges only a small fraction of the MFs that enter the treatment plant. The treatment process sequesters the major load of MFs into the biosolids or sludge. This analysis of the WWTP effluent also correlates with the watershed data, which showed similar amounts of synthetic MFs in surface water collected both above and below the WWTPs (Figure 3). The farm fields that drain into the watershed, some of which are fertilized using biosolids, are possible contributors to the microfiber pollution in the watershed.

Figure 5 summarizes the transport of synthetic MFs from household laundry discharges through the Valparaiso WWTP, where a drastic reduction in MFs occurs according to the samples studied in our lab. Clearly, the fate of the MFs in the biosolids is a significant unknown, in terms of transport and distribution of synthetic microfibers. The figure also depicts the loads of MFs in the creek water and the Lake Michigan shoreline water. While the lake is a huge depository for a wide variety of waste which rely on natural processes, persistent plastic waste is expected to accumulate and should be considered a significant threat to the ecosystem and all who rely on it.

INSERT FIGURE 5 HERE

### Experimental methodology limitations

The data from both the processed natural samples and the standard synthetic fabric fibers lead to the conclusion that the synthetic microfibers quantitatively determined for the natural water and sediment samples are limited by a few constraints. For the natural samples, there are wide variations in the number of detected synthetic microfibers (distribution), according to the experimental findings (Figures 3 and 4). The data suggest that once the microscopic synthetic MFs are released into the environment, they distribute in a heterogeneous manner. Some collect in reservoirs, such as the sediment, while the pollution travels via the tributary into the lake environment. Multiple samples were taken at the selected study locations for the determination of an average value to minimize the variability associated with the uneven distribution; a few samples were dismissed as outliers, where clumps of microfibers were collected.

The second significant limitation was that our work with synthetic fibers (standards) indicated an inability to consistently recover 100% of the oxidatively processed fibers. In order to quantify only synthetic microfibers, laboratory procedures must either utilize an oxidation step to remove non-polluting microfibers,[38] or sophisticated instrumentation to verify the polymeric properties. The loss of color from dyes susceptible to oxidation likely leads to lower counts of MFs in natural samples. It is noteworthy that the microfibers from the blue polyester fabric standard tested in our laboratory (blue fleece jacket) were recovered nearly completely each time. This seems to correspond to other reported studies which have suggested that blue microfibers are the most recovered and detected microfibers.[39] We suggest that dye stability and natural weathering influence the quantification of synthetic microfibers in natural samples and present a significant limitation to the accurate count of the contaminants.

The third difficulty encountered in this study was the inability to create samples free of natural debris for microscopic observations for the identification of synthetic microfibers. While most of the organic and inorganic substances from the natural samples were removed in the laboratory processing procedures, some natural materials remain and can mask synthetic fibers. Figure 6 shows representative microscope images of processed water and sediment samples. Colored synthetic microfibers are readily detected, but colorless fibers are more difficult to observe and may not be counted.

INSERT FIGURE 6 HERE

For colorless fibers or fibers chemically stripped of colorant, stereomicroscopic detection and quantification of microfibers is subjective. The experimental count of synthetic microfibers in our processed natural samples is most likely lower than the actual number. The oxidative processing and microscopic visualization used in this study are commonly used in sample prep and visualization for the study of microplastic pollution in surface waters since they are relatively inexpensive and do not require extensive training.[40] In order to achieve a higher level of accuracy in the analysis of natural water and sediment samples, further optimizations of the laboratory procedures are required.

### Synthetic microfibers and traditional water quality parameters

The determination of synthetic microfibers was part of a larger water quality monitoring project, and the MF data was compared to other water quality parameters. Since these plastic pollutants were reported as part of the total suspended solids (TSS) data, synthetic MF were graphed with the TSS data; no obvious correlation was noted from this relatively small dataset. Given the heterogeneity of the MF distribution in the water, it is likely that no correlation will exist between these pollutants and other water quality parameters such as turbidity, dissolved oxygen or other dissolved substances. However, additional data should be collected and assessed under different weather conditions before making this determination.

## Conclusions

As far as the authors know, typical water quality monitoring protocol does not currently include the quantification of microplastics. Studies involving the quantification of microplastics in surface water have been investigated separate from other water quality indicators. Given the discharge of massive amounts of synthetic microfibers into natural waters, this types of pollution will continue to accumulate in watersheds for the foreseeable future. We suggest this type of pollution can be monitored regularly and we demonstrated the use of the common water quality parameter of total suspended solids (TSS) as a straightforward means to collect this important data. Synthetic microfibers greater than 100 μm are readily viewed under a stereomicroscope following minor TSS procedural modifications: nylon filter paper, clean techniques and oxidative processing to remove natural materials. However, the reported data carry the noted limitations associated with the oxidative processing and stereomicroscopic detection. For more accurate and reliable data, the scientific community needs to establish systematic methods for the quantification of microplastics and synthetic microfibers.

Synthetic microfibers were frequently detected in the studied surface water and sediment of the Lake Michigan watershed of Northwest Indiana and the water along the shoreline. The investigation revealed a heterogeneous distribution of the plastic particulates in water and sediment. A statistically higher load of the plastic pollution was detected in the water sampled along the lakeshore. The lake ecosystem is likely the final destination for this type of persistent pollution. The flow of nearly 4 billion microfibers daily in this watershed is expected to increase as the production of synthetic textiles meets the demand of a growing population. The health consequences of these pollutants are largely unknown. Early investigations into the effects of microplastics in the ecosystem indicate wide consumption by aquatic organisms and harm to numerous organisms.[47-50] It is important to continue studying the sources, distribution and fate of synthetic microfibers to assess the cumulating amounts and effects in surface water and the ecosystem.

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### Conflicts of interest

There are no conflicts of interest to declare.

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Figure 1. Map of sampled area and wastewater treatment facilities. Sampled locations are indicated by overlaid arrows and wastewater treatment facilities are outlined in orange circles. Site 1 is located above the WWTPs, sites 2 and 3 are directly below WWTP discharge, and site 4 is 8 km downstream. Top, right insert shows the entire Great Lakes region, with the study area denoted by the star.

Figure 2. Black rayon (left side) and green polyester (right side) microfibers, before (top) and after (bottom) chemical oxidation. The black color is mostly not observed after the Fenton oxidation, as noted in the lower left photograph, while the green color remains intact (lower far right photograph).

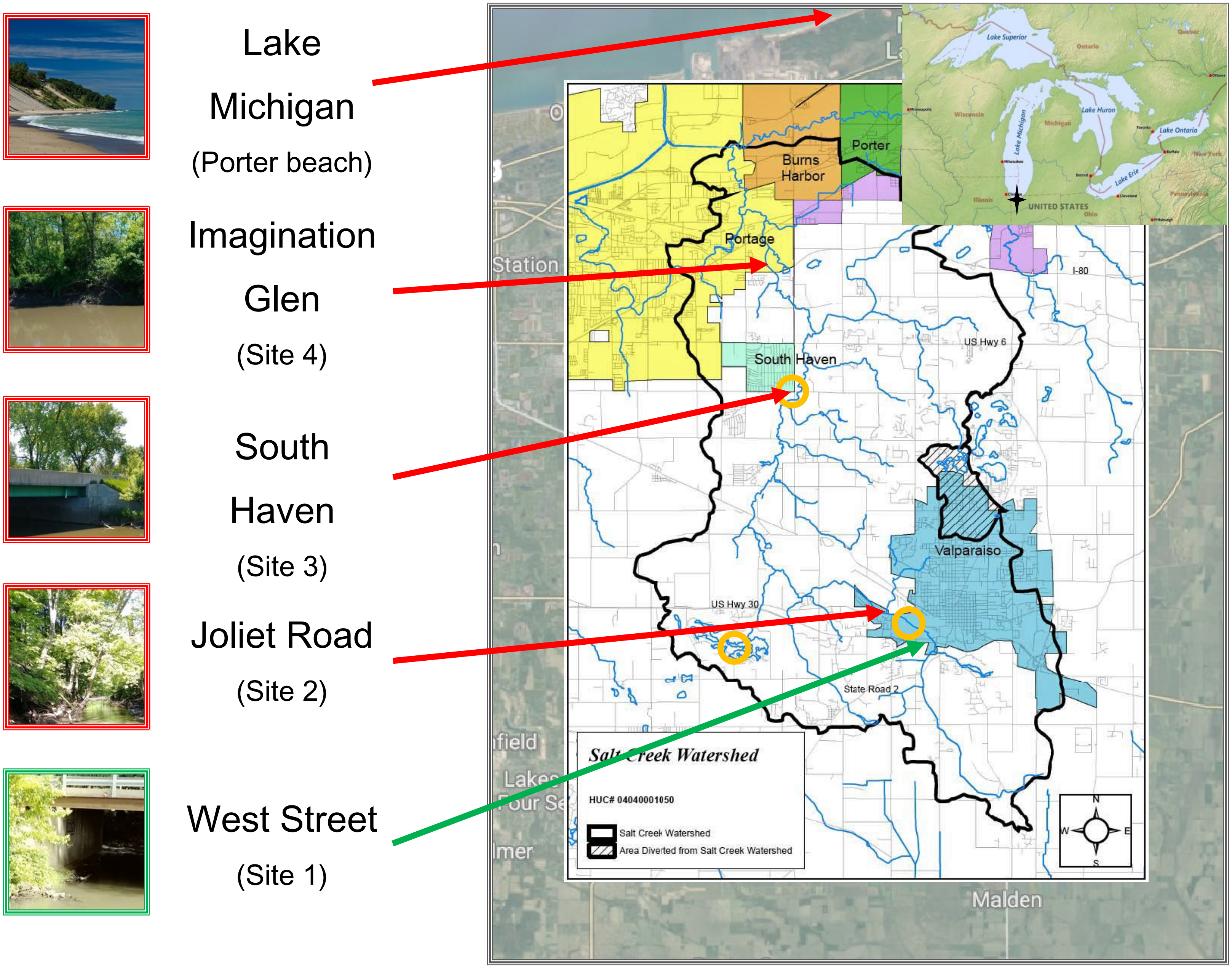
Figure 3. Average Synthetic Microfiber (MF) Abundance in Water per 4L ± SEM from each of the four watershed locations, WWTP effluent, and Lake Michigan shoreline. Site 1 is located above the WWTP while sites 2, 3 and 4 are below WWTPs. Sample number (n) = 34, 12, 32, 30, 28 and 15 from left to right. A one-way ANOVA with a Tukey's post-hoc test indicated the MF abundance in Lake Michigan was statistically higher (*) than sites 1, 2 and 4 ($p<0.001$) but not different from site 3 ($p=0.056$) or the WWTP ($p=0.106$).

Figure 4. Average Synthetic microfiber abundance (per kg dry mass) ± SEM from each of the four watershed locations. Site 1 is located above the WWTP and the other sites are below WWTP's. Sample size (n) for the above data was 12, 11, 11 and 12 from site 1 through 4. A one-way ANOVA indicated no statistical difference between sites.

Figure 5. The number of synthetic microfibers isolated per 4 L of household water, wastewater influent to post-treatment effluent from the Valparaiso Wastewater Treatment plant and then through the watershed to the Lake Michigan shoreline.

Figure 6. Photographs of magnified filters containing synthetic microfibers from processed sediment (left) and total suspended solids (TSS) from a water sample.

Table 1. Efficiency of recovery of standard fibers (known fabrics) tested using the Fenton reagent.


Lake Michigan
(Porter beach)
Imagination Glen
(Site 4)
South Haven
(Site 3)
Joliet Road
(Site 2)
West Street
(Site 1)
Burns Harbor
Porter
Portage
South Haven
Valparaiso
US Hwy 6
I-80
US Hwy 30
State Road 2
Station
Malden
Lake Superior
Lake Michigan
Lake Huron
Lake Ontario
Lake Erie
Ontario
Quebec
Wisconsin
Michigan
Illinois
Ohio
Pennsylvania
New York
UNITED STATES
Salt Creek Watershed
HUC# 04040001050
Salt Creek Watershed
Area Diverted from Salt Creek Watershed
N
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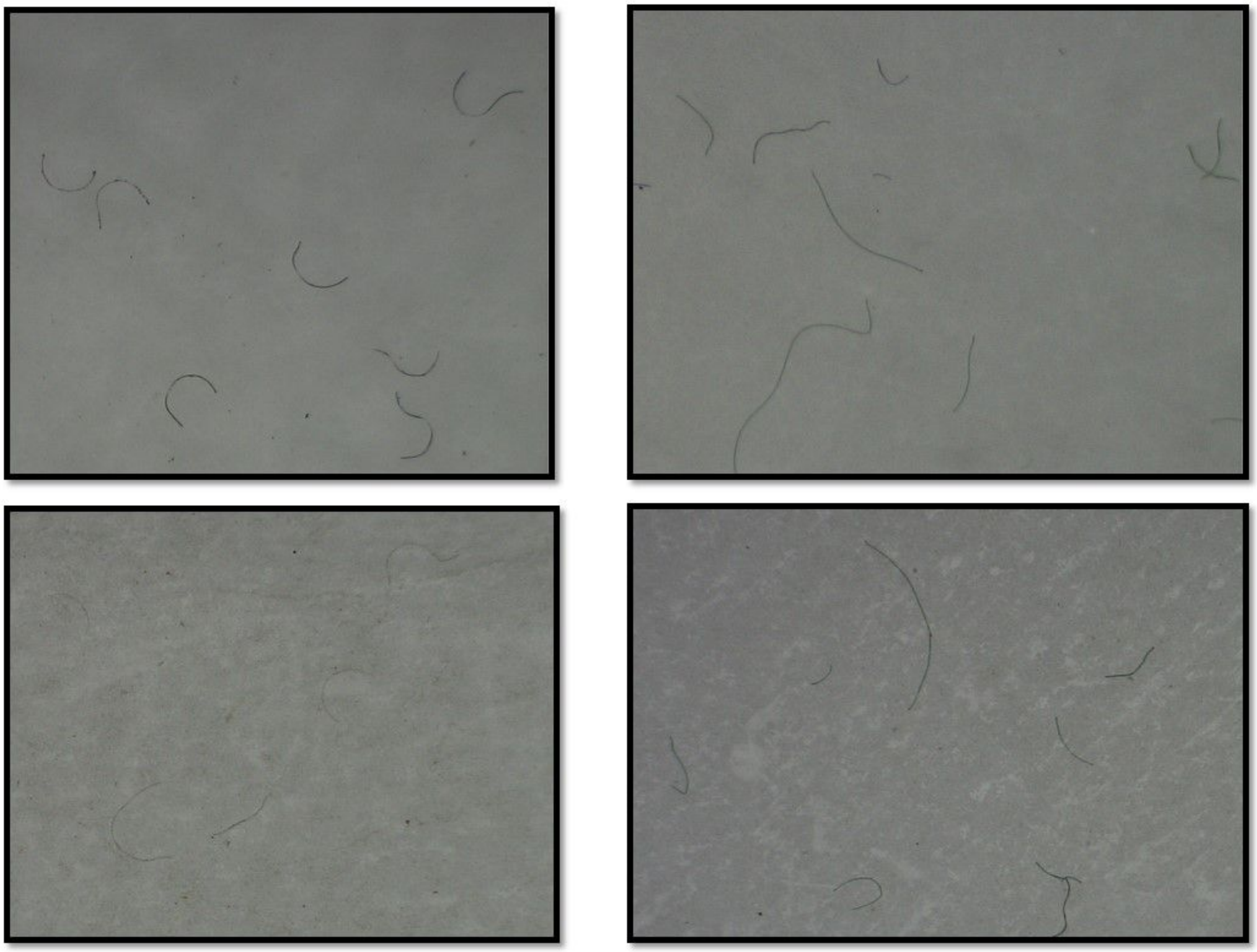
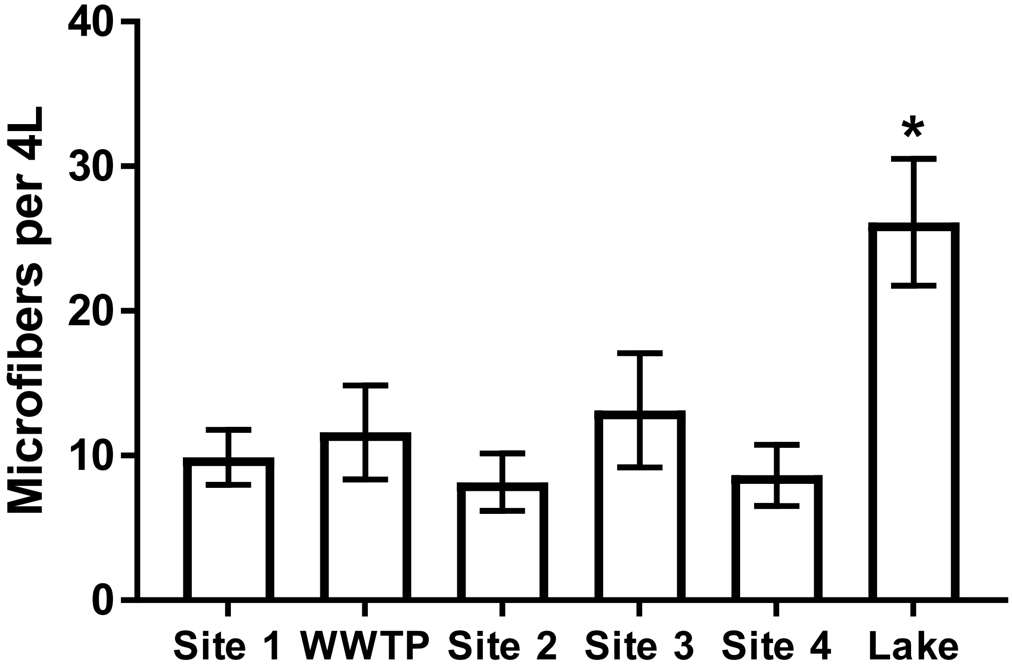
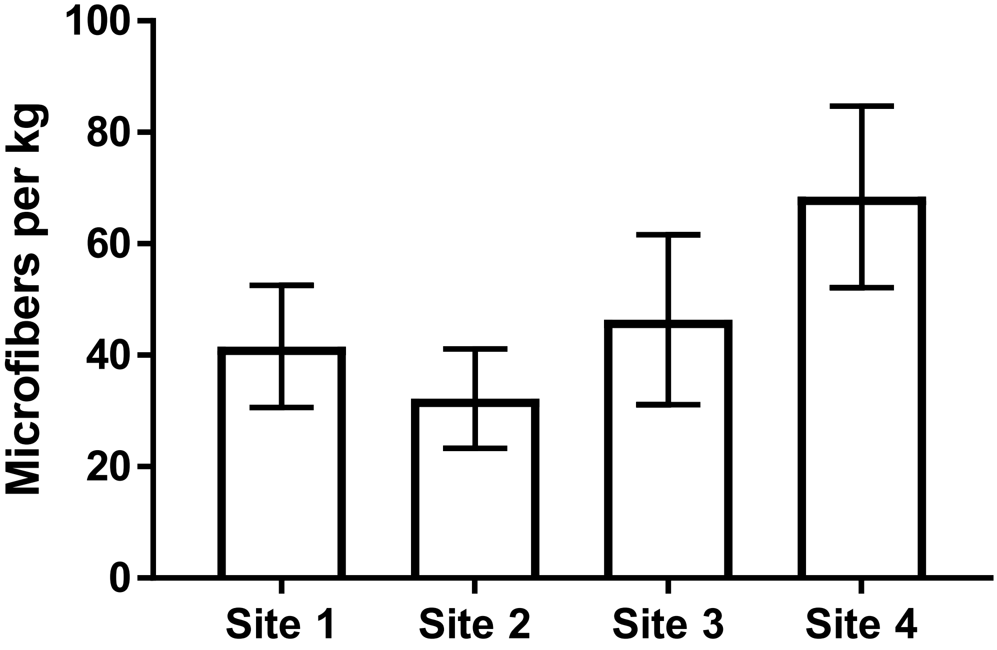
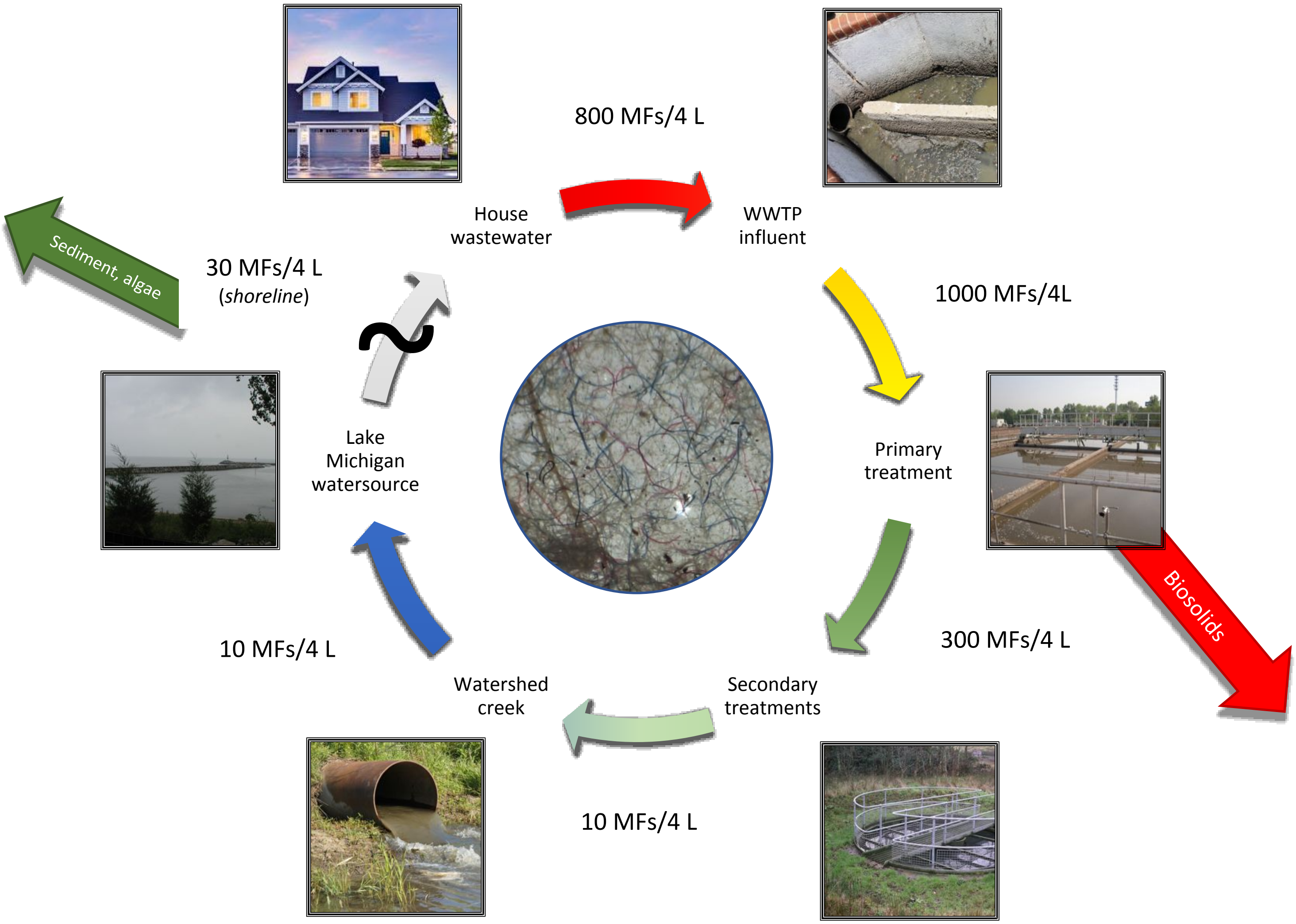
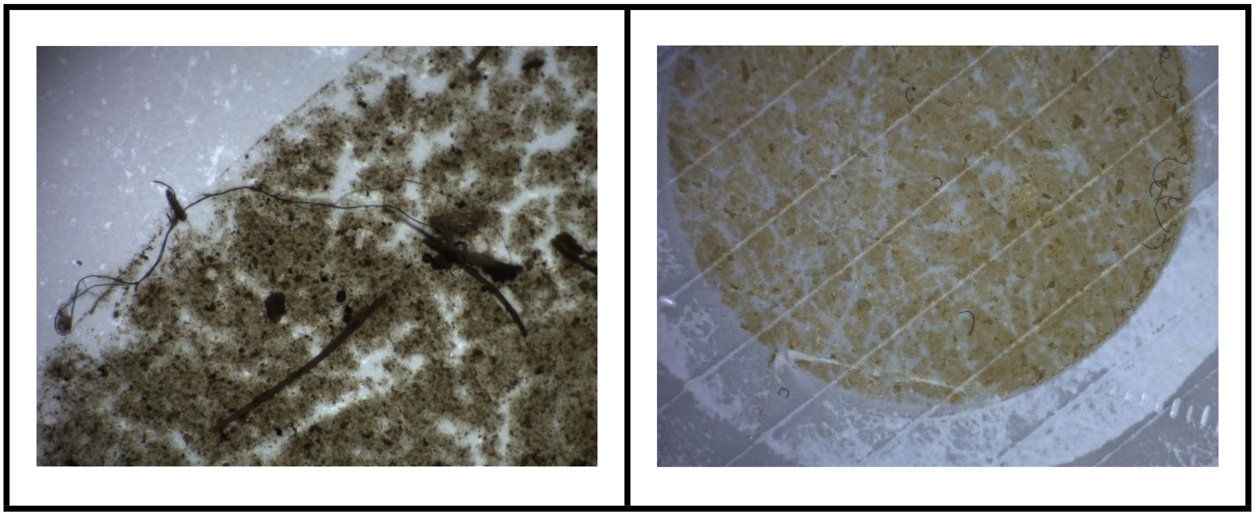


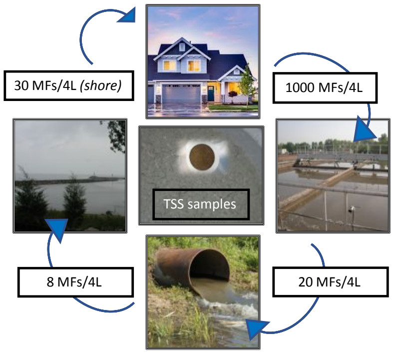

40
30
20
10
0
Microfibers per 4L
*
Site 1
WWTP
Site 2
Site 3
Site 4
Lake


Microfibers per kg
100
80
60
40
20
0
Site 1
Site 2
Site 3
Site 4


800 MFs/4 L
House wastewater
WWTP influent
1000 MFs/4L
Primary treatment
Biosolids
300 MFs/4 L
Secondary treatments
10 MFs/4 L
Watershed creek
10 MFs/4 L
Lake Michigan watersource
~
30 MFs/4 L
(*shoreline*)
Sediment, algae



| **Fabric and color** | **Average % recovery post oxidation** | **Range** | **Color, post oxidation** |
|---|---|---|---|
| Acrylic, purple | 100 | - | Some colorless fibers |
| Modal, white | 100 | - | White |
| Polyester, blue | 85 | 70-95 | Blue |
| Rayon, black | 82 | 66-92 | Mostly colorless fibers |
| Polyester, green | 76 | 63-100 | Green |
| Polyester, maroon | 72 | 63-90 | Maroon |
| Polyester, red | 57 | 38-90 | Some colorless fibers |
| Polyester, gold | 36 | 12-71 | Lighter gold |
| Cotton, maroon | 0 | - | - |
| Linen, blue | 29 | 3-41 | Colorless fibers |


30 MFs/4L (shore)
1000 MFs/4L
TSS samples
8 MFs/4L
20 MFs/4L