Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment

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Supporting Information

ABSTRACT: Municipal effluent discharged from wastewater treatment works (WwTW) is suspected to be a significant contributor of microplastics (MP) to the environment as many personal care products contain plastic microbeads. A secondary WwTW (population equivalent 650 000) was sampled for microplastics at different stages of the treatment process to ascertain at what stage in the treatment process the MP are being removed. The influent contained on average 15.70 (±5.23) MP·L⁻¹. This was reduced to 0.25 (±0.04) MP·L⁻¹ in the final effluent, a decrease of 98.41%. Despite this large reduction we calculate that this WwTW is releasing 65 million microplastics into the receiving water every day. A significant proportion of the microplastic accumulated in and was removed during the grease removal stage (19.67 (±4.51) MP/2.5 g), it was only in the grease that the much publicised microbeads were found. This study shows that despite the efficient removal rates of MP achieved by this modern treatment plant when dealing with such a large volume of effluent even a modest amount of microplastics being released per liter of effluent could result in significant amounts of microplastics entering the environment. This is the first study to describe in detail the fate of microplastics during the wastewater treatment process.

1. INTRODUCTION

Plastic pollution in the aquatic environment is well studied and has been given considerable attention for a number of decades.1−3 Because of the lightweight nature of plastic it is easily dispersed by wind and ocean currents across vast distances.4 In recent years, the issue of small plastic particles known as microplastics has been gathering increasing attention.5 Microplastic are plastics that are <5 mm in size6 and can be separated into two different types, primary microplastics and secondary microplastics. Primary microplastics are plastics that are manufactured to be of microscopic size; these can be found in many cosmetic products, where they are used as facial scrubs, or as an air blasting media.7 Secondary microplastics are formed from the breakdown of larger plastics debris,8 via exposure to sunlight, wind, water, and other environmental stressors.9

Microplastics are ubiquitously found in aquatic water bodies10,11 and sediments12,13 and have been ingested by various aquatic organisms.14−16 Trophic level transfer of microplastics has also been shown to occur.17 Because of their small size, microplastics may be more bioavailable to lower trophic organisms,17 who tend to display limited selectivity and will often ingest anything of appropriate size.18 While organisms of higher trophic levels may ingest microplastics indirectly through trophic level transfer via their prey or by mistaking microplastics for a prey item.19

However, despite this ubiquitous nature, the sources of microplastics in the environment are not fully understood. Wastewater Treatment Works (WwTW) could potentially be a major source of microplastics in the aquatic environment.20 Microbeads used in facial scrubs, toothpaste, and other personal care products are transported in the raw effluent to WwTW,21,22 where because of their small size they may bypass the waste treatment process. In recent years increased public pressure has led companies and governments to regulate and ban the use of microbeads.23 Synthetic clothing, such as polyester and nylon, is also a concern as these fabrics can shed thousands of fibers into the wastewater.24 The growing issue of microplastics released from WwTW was recently reported by the Norwegian Environmental Agency.25 This report highlighted the knowledge gap regarding the analysis of microplastics discharged from WwTW, particularly entering river systems and the need for analysis of the fate and removal of microplastics during the treatment

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Figure 1. (A) Diagram of WwTW showing the location of the liquid fraction sampling sites (S1−4), where S1 = influent, S2 = grit and grease effluent, S3 = primary effluent, and S4 = final effluent. Sludge cake samples were taken from the same area for both the 24 h SC duplicate comparison and the comparison between grit and grease. (B) Barplot of the number of microplastic (MP)·L−1 at each liquid fraction site sampled (S1−4) (error bars = standard deviation, * = significance <0.05). (C) Barplot of the number of MP/2.5 g from solid fraction comparison (error bars = standard deviation, * = significance <0.05). (D) Barplot of the number of MP/2.5 g sample of 24 h SC duplicate (error bars = standard deviation). (E) Barplot of mean length of microplastic (mm) from each study (liquid fraction, solid fraction, and 24 h SC duplicate) conducted (error bars = standard deviation, * = significance <0.05).
process. The report also highlighted the need for more detailed analysis of microplastic particles to classify them based on the polymer, size and type. Some research has been undertaken on microplastics in WwTW final effluent, but little work has been undertaken to determine their removal efficiencies and at what stage in the process microplastics are extracted and determining the composition of the polymers entering and exiting these treatment facilities.

There has been some research carried out on microplastics in WwTW. These studies have mainly focused on the final effluent, with little work done on determining removal efficiencies and where in the process microplastics are extracted. A Swedish study investigated the ability of a WwTW to retain microplastics and found that 99% of the microplastic was removed from the final effluent. However, this was a relatively small WwTW serving 12 000 people and limited identification was conducted on the specific polymers found. While Carr et al., looked at microplastics from secondary as well as tertiary WwTW by filtering large volumes of effluent as well as attempting to analyze different stages of the treatment process. Identification of MP was primarily visual using MP extracted from personal care products as a visual reference with limited FT-IR conducted. Browne et al., also examined effluent from a tertiary WwTW finding 1 MP·L⁻¹, however only small 750 mL samples were filtered. Concentrations of MP measured previously in municipal effluents range from 0.00097 to 0.009 MP·L⁻¹ for secondary treatment and 0.0000027 to 1 MP·L⁻¹ for tertiary. However, comparison between these concentrations is made difficult because of the variable sampling techniques and identification methods employed.

In this study, we investigate the effectiveness of the WwTW process in the removal of microplastic from municipal effluent at different stages during the treatment process of a large secondary WwTW with a population equivalent of 650 000. We identify where in the treatment process microplastics are being removed, identify the physical and chemical composition of the microplastics found at each treatment stage. We provide the first systematic overview of the fate of MP in municipal treatment plant, identifying and quantifying where MPs are removed at various stage of the treatment process. There are three separate aspects to this study: the examination of (i) the liquid fraction; the (ii) solid fraction, comprising grit, grease, and sludge cake (SC); and the (iii) 24 h SC duplicate, SC sampled at two different time points on two consecutive days.

2. MATERIALS AND METHODS

2.1. Sampling. A large secondary WwTW located on the River Clyde, Glasgow was sampled for microplastics at different stages of the treatment process. This site has the population equivalent of approximately 650 000 and produces on average 260 954 m³ of treated wastewater every day that is discharged into Glasgow’s major waterway, the river Clyde. Samples were taken after coarse screening to avoid larger debris clogging or damaging the equipment used to filter the samples. Four stages of the treatment process were sampled (Figure 1A): influent after 19 mm coarse screening (S1), grit and grease effluent (S2), primary effluent (S3), and the final effluent (S4) before it is released to the river Clyde (see Figure S1 for detailed description of the treatment process).

Steel buckets (10L) attached to steel wire were lowered into the turbulent effluent stream (<50 cm) for sample collection by an on-site technician. The sample was then passed through steel sieves (65 μm) to collect any debris present. Due to the large amount of debris it was only possible to filter 30L (3 × 10L pooled sample) from sites 1–3 and 50L (5 × 10L pooled sample) from site 4 before the sieves became clogged. This debris was then washed into clean glass bottles using distilled H₂O, and all equipment was cleaned using on-site hoses between samples. All samples were taken in duplicate. The bottles were then sealed and brought to the laboratory, where the samples were vacuum filtered through Whatman No. 1 qualitative circles, 90 mm filter paper, with a pore size of 11 μm.

Samples of grit and grease were taken from the grit and grease removal stage (Figure 1), and SC from the sludge centrifuge treatment for comparison of microplastics present in the solid effluent fraction from the WwTW. On a separate day, SC samples were also collected at the centrifuge treatment stage (Figure 1) at 09:30AM and again at 14:30PM on two consecutive days in order to determine any variation in the amount of microplastic present.

2.2. Contamination Mitigation. A number of steps were taken to reduce the incidence of microplastic contamination. During these steps clean white cotton lab coats were worn at all times, only natural fabric and no clothing made from synthetic fibers was worn underneath the lab coats.

2.2.1. Cleaning. All equipment used was cleaned three times with distilled H₂O. All Petri dishes, filter papers, and forceps were examined underneath a dissection microscope before use to ensure no contamination was present. All work surfaces were wiped down with 70% ethanol three times prior to work commencing.

2.2.2. Taping. The tape lifting techniques use in forensic science laboratories to check laboratory benches for fiber and particle contamination was used in this study. The tape consists of a plastic film with one side covered with a layer of glue and is placed so that the glue makes contact with the area being examined. The tape is then lifted and any trace particles present should adhere to the tape, which is then placed on a clean sheet of acetate. To take a taping, a piece of tape measuring 5 cm × 5 cm was randomly placed three times on the work surface after it was cleaned. After all lab work was completed, another taping was taken and was also placed on a sheet of acetate. The tapings were then examined under a microscope for identification. Tapings were carried out before and after all procedures. On average 3.3 fibers per taping taken were collected ranging from 0 to 14 fibers per taping.

2.2.3. Atmospheric Microplastic (MP). Clean filters in Petri dishes were left out for the duration of the filtration to collect any atmospheric MP that may be present. Before the liquid fraction filtering began 750 mL of distilled H₂O was vacuum filtered, and the filter examined for contamination. Clean filters in Petri dishes were also left out for the duration of lab work and were then checked for any contamination.

2.3. Analysis. Samples were vacuum filtered and all bottles containing the liquid fraction were rinsed three times with distilled H₂O and filtered after each rinse. The filter was then observed under a dissection microscope. Initially, all debris present was considered to be microplastic until proven otherwise by FT-IR, as relying solely on visual identification is open to bias. Following the FT-IR identification large amounts of material could be discounted (e.g., plant material) and the microplastics were removed and characterized based on their color, length and type (fiber, bead, flake, etc.). Because of the large amount of debris on the filters from sites 1–3, it was neither practical nor viable to identify all material present. It was therefore necessary to take sub samples from these sites. It should also be noted that because of the complexity of the samples, items of similar color to the background filter paper may have been overlooked.

To subsection the samples from sites 1–3 the filters were divided into 24 pie sections, and numbered 1–24. Using a random number generator, four sections were selected for each filter. These sections were then excised using scissors and thoroughly analyzed for microplastics. An average of the four sections was used to get an estimate of the amount of microplastics present for the whole filter. Using the equation below an estimated amount of microplastics released at each site was made.
One FT-IR Microscope was used in the reactivation and collect material for identification. A dissection microscope was used to separate out the presence of characteristic functional group signals at the correct wavenumber values were checked to confirm the likely chemical structure of materials being examined.

2.5. Statistical Analysis. Statistical analysis was conducted using R statistical computing software. Differences in the number of microplastics and their sizes between sites were determined using one-way ANOVA. Log10 transformation was used to transform data relating to the number of microplastics present in order to meet the assumptions of normality and equal variance. Size data did not need to be transformed as it already met the assumptions needed to carry out an ANOVA. A Pearson correlation analysis was conducted on the stage of treatment and the amount of MP per liter present.

3. RESULTS

In total, 430 plastic items were identified across all the samples examined, the majority of which came from the liquid fraction (n = 303), followed by the solid fraction (n = 79), and 24 h SC duplicate (n = 48) samples. Of the 430 items identified as plastic, 8 were >5 mm.

3.1. Liquid Fraction. There was a significant difference in the amount of microplastic (MP) found between the four sampling sites (p = 0.0002) (Figure 1B). The influent sampled at S1 contained on average 15.70 (±5.20) MP L−1, which was reduced by 98.4% in the final treated effluent sampled at S4 to 0.25 (±0.04) MP L−1 (Table 1). Despite the highly efficient removal rate, using three years of flow rate data from the WwTW an estimated 65 238 500 MP could be released from the WwTW every day in the final effluent or 23 billion microplastics annually from this WwTW alone (Table 1).
(grit and grease removal) showed the biggest reduction in the amount of microplastic at 44.59%; this was further reduced by the primary settlement tanks by an additional 33.75%. Aeration and clarification reduced the amount by 20.07% before the effluent was released into the receiving water.

There was a significant negative correlation between the treatment stage and the number of MP/L⁻¹ (p = 0.014). The most common polymers found in S1 were alkyls (28.7%), polystyrene-acrylic (19.1%), polyester (10.8%), polyurethane (8.9%), and acrylic (8.3%) (Table 2). The most common polymer found in the final effluent (S4) was polyester (28%), polyamide (20%), polypropylene (12%), acrylic (12%), alkyd (8%), polyethylene (4%), polystyrene (4%), and PET (4%) (Table 2).

The liquid fraction contained mainly flakes (67.3%), fibers (18.5%), film (9.9%), beads (3.0%), and foam (1.3%) (Figure 2A). The sampling process may have resulted in the number of flake items being overrepresented as these flakes were very brittle and fragmented easily during identification. There was no significant difference between the sizes of plastics found at each site (p = 0.913). The MP found were predominantly red (26.7%), blue (25.4%), and green (19.1%) but other colors were also present (Figure 2B).

3.2. Solid Fraction and 24 h SC Duplicate. There was a significant difference between the number of MP/2.5 g in the three different solid fractions investigated (p = 0.002) (Figure 1C). The grease sample contained an average of 19.67 (±4.51) MP/2.5 g sample, which was significantly higher than both the grit sample (p = 0.009) and the SC sample (p = 0.002). From the 24 h SC duplicate study, there was no significant difference in the number of MP/2.5 g SC found between the day 1 and day 2 or 09:30 and 14:30 (p = 0.383), or between the time of day the samples were taken (Figure 1D). Polyester, acrylic, polypropylene, alkyd, and polystyrene were the most commonly found polymers in the 24 h SC duplicate study (Table 2).

3.3. Size Comparison. There was a significant difference in the size (mm) of MP found between the liquid fraction, solid fraction, and 24 h SC duplicate study (p = 0.002) (Figure 1E). MP taken from the liquid fraction were on average 0.598 mm (±0.089) in size and were significantly smaller than both the solid fraction 1.342 mm (±0.519) and the 24 h SC duplicate study 1.618 mm (±0.394) (p = 0.002). There was no significant difference between the solid fraction and the 24 h SC duplicate study (p = 0.4). There was no significant difference in the sizes of the microplastics between the times sampled in the 24 h SC duplicate study (p = 0.782).

3.4. Contamination. Throughout the course of the study 25 items were found to have accumulated on the filters that were left out to test for atmospheric microplastic contamination. Following FT-IR examination just one item was identified as a microplastic (polyester), with the rest identified as blue/black cellulose/cotton fibers with a very distinctive ribbon like morphology when examined under light microscope (Figure S3). Similar fibers were also found on the tapings taken and following observation under light microscope these were also identified as cellulose/cotton.

4. DISCUSSION

4.1. Liquid Fraction. Preliminary and primary treatment effectively removed 78.34% of the microplastics from the liquid fraction. Preliminary treatment involves the removal of large items such as rags and sticks as well as the removal of floatables, grit and grease that may damage or interfere with the equipment used in the treatment process. Primary treatment involves the removal of a portion of the suspended solids and organic matter, achieved through the use of chemical additives (floculation agents) and sedimentation. The secondary treatment stage managed to remove a further 20.1%. Secondary treatment involves the removal of biodegradable organic matter as well as suspended solids during the aeration and clarification treatment (Figure 1A).

Chemicals such as ferric sulfate are used in the treatment process in order to cause suspended particulate matter to aggregate together forming a “floc”. It is likely that the amount of ferric sulfate or other floculating agents will have an effect on the particulate matter present in the wastewater. For the WWTW in the current study the amount added is flow dependent but on average a total of 7−9 g/m³ are used, with primary treatment receiving 2 g/m³ and secondary treatment getting 5−7 g/m³ during aeration (Figure 1A). Polyacrylamide is also used as a floculation agent (Figure 1A), this is a white water-soluble powder. Samples of this were taken and examined to exclude it from the final results. Bacterial jelly like balls are also formed at the aeration stage, likely aiding in the accumulation of particulate debris.

A study conducted by the Swedish Environmental Research Institute found high removal rates of 99% in a smaller WWTW serving a population equivalent load of 12 000. Although microplastic polymer composition was not fully described as the identification of items was mainly visual, several items were identified using FT-IR and included polyester, polyethylene,
and polypropylene. This study estimated that 2000 microplastics were released in the effluent on one particular day, equivalent to 0.16 MP/person/day or 0.009 MP·L⁻¹. This microplastic concentration is considerably lower than the 100 MP/person/day or 0.25 MP·L⁻¹ of final effluent found in the current study. While a concentration of 1 MP·L⁻¹ was found in the effluent from two tertiary treatment plants in New South Wales, Australia.²⁴ This comparatively high concentration was surprising considering the additional treatment process involved in tertiary treatment. However, direct comparison is difficult as the specific treatment processes, the volumes of effluent treated and the population equivalent was not described in this study. A recently published study carried out in Southern California also examined effluent from several tertiary and a secondary WwTW.²⁷ 423 000 L of effluent were filtered at the secondary WwTW and 373 MP were counted or 0.0009 MP·L⁻¹. However, this study conducted limited FT-IR, identification was mainly visual and relied upon comparison of MP derived from personal care products. This may have resulted in MP being underestimated, while the number of MP found in the tertiary effluent was even lower with the highest count being 0.000002 MP·L⁻¹ at one site. This study also attempted to investigate the transport of microplastics at each stage of the tertiary treatment process. But owing to practical issues with clogging of filtration equipment (sieves) and the use of density separation, only 5 L of the raw influent was examined with no MP identified making determining reduction rates difficult. Therefore, the current study is the first to provide detailed data on the removal rates of MP at various stages of municipal effluent treatment as well as the detailed characterization of MP found. The concentrations reported in these four studies are all considerably lower than that was reported in a Dutch survey of microplastics in the environment.³³ The concentrations reported ranged from 9 to 91 MP·L⁻¹, averaging 52 MP·L⁻¹ however identification of microplastic was visual so the concentrations are likely to have been overestimated.

The three most common polymers found in the final effluent in the Australian study were polyester (30.4%), polyamide (21%), and acrylic (13%) the same as in the current study. Browne et al.²⁴ reported the presence of only fibers in municipal effluent which is in contrast to the various different types of microplastics found in the current study. This is most probably due to the large difference in the sizes of the two facilities but may also be influenced by the difference in influent composition from the surrounding catchment, the degree of urbanisation and the time of day that sampling was conducted as well as the specific treatment process used at that facility.

4.2. Solid Fraction. Analysis of the solid fraction (grit, grease, and SC) samples showed high amounts of microplastic accumulating in these three stages. This was most evident in the grease stage, which showed a significantly higher amount of microplastic present. It was only from the grease samples that the much publicised microbeads from face washes were found²² (Figure 3). It has been suggested that due to their small size, microbeads are capable of passing through the coarse and fine screens (designed to remove large debris to prevent damage to the equipment) and through the wastewater treatment process without removal. The microbeads found in the majority of face washes consist of polyethylene,³⁴ which is positively buoyant in water and is likely to sit on the surface of the wastewater where it can be easily skimmed off the surface layer during grease removal. In this study no microbeads were found in the final.
effluent, indicating that microbeads from face washes may not be a major issue for the receiving environment if appropriate treatment processes are implemented. However, owing to the small sample size, this study may not be entirely representative. Microbeads from face wash products have been previously found in the Great Lakes in North America, but because of the size of the area studied, it is difficult to determine their source.

Certain aspects of the grit and grease removal stage implemented in this WwTW are site specific and may not be normal practice in other locations. Typically, at this stage of the treatment process skimmers are placed on the surface of the effluent to skim off any floating grease, while the grit portion settles at the bottom. The WwTW sampled in the current study uses aeration to cause frothing of the grease, making it more likely to be collected and removed from the effluent. On average 12–15 m³ of grease is removed from the effluent each day. This is then incorporated along with the grit to the sludge (Figure 1A), where it is then sent for incineration in a waste to energy scheme.

Synthetic material has previously been found in sludge samples as well as effluent samples. A study examining the presence of synthetic fibers in WwTW sludge found 4 fibers per gram of sludge sampled. However, this study only examined synthetic fibers as an indicator of soil pollution and may have resulted in other nonfibrous polymers being overlooked.

4.3. 24 h SC Duplicate. The 24 h duplicate study showed no difference in the amount of microplastic present in the sludge over this time period investigated. However, this study may have used too short a time between sampling periods to provide a solid conclusion and it would be more appropriate to look at longer time frames such as a monthly comparison and more frequent sampling in future studies.

4.4. Destination. Microplastic size was considerably smaller in the liquid fraction than in the solid fraction and 24 h SC duplicate study samples. This may be due to the smaller items remaining suspended within the liquid fraction, while the larger items are more likely to settle at the bottom of settlement tanks or be captured in the grit and grease stage. It could also be due to only the smallest items being capable of passing through the treatment process. The final destination of these microplastics released in the treated effluent remains unknown but evidence suggests they may be accumulating in the river banks of the Clyde or carried out into the estuary and eventually the Clyde Sea. A preliminary study conducted by Habib et al., examined sediments collected from a bay downstream of a sewage treatment plant. It was found that the sediment contained numerous synthetic fibers and as distance increased from the sewage treatment plant the size and number of fibers decreased. The difference in concentrations of microplastics up and downstream from a WwTW has also been examined finding a higher downstream concentration of MP (17.93 m³) compared to the upstream concentration (1.91 m³), with primarily fibers and fragments being found. The river Clyde receives the effluent from a number of WwTW which could all be contributing to the microplastic load. Microplastics have been previously identified in the Clyde Sea, with 83% of Nephrops examined found to contain microplastics, although it was thought that these were primarily sourced from discarded fishing line and rope.

4.5. Contamination. An important aspect of this study was the implementation of various contamination controls to ensure the validity of the findings. Contamination has been put forward as a topic of concern in microplastic research. Similar methods to reduce and determine the incidence of contamination such as avoiding wearing synthetic clothing, thorough cleaning, the use of filters to collect atmospheric microplastics, as well as forensic tape techniques were also developed for microplastic sediment analysis. Through the use of these contamination controls it was determined that the incidence of microplastic contamination from clothing or atmospheric particulate matter is very small provided appropriate controls are put in place. The methods used in the current study are simple, cheap and require little technical training to carry out but do require care to be taken to prevent contamination. Implementation of these contamination controls in future microplastic research should be included to provide additional validity to the results obtained.

4.6. Limitations of This Study. Sampling in wastewater treatment systems presents a number of challenges as reviewed by Ort et al. This review examined the study of pharmaceuticals and personal care products but should also be applicable to sampling for microplastics. The review highlights practical limitations in sampling such as environmental and the daily variability of flow rates as well as variability in pollutant concentration. In future studies the time of day, year and weather patterns should all be considered when sampling. Because of the the great variation of flow rates it may be more appropriate to take frequent samples throughout the day rather than taking a snapshot as was done in this study.

This current study did not take into account stormwater runoff, where untreated effluent is released directly into the river when the volume of incoming water exceeds the treatable volume. According to flow rate data taken from the WwTW, when averaged out over the year 39 000 m³ of effluent with limited treatment (settlement in storm tanks) is released every day or potentially an additionally 620 million microplastics/day using the figure of 15.70 MP·L taken from S1. However, this normally occurs in large volumes across short periods of time during spells of bad weather, for example on one particular day over 700 000m³ was recorded to have been released as stormwater. This untreated wastewater may potentially heavily increase the amount of microplastic entering the receiving environment. However, it is important to take into account the dilution factor that would occur, although the volume of wastewater increases it is unlikely to increase the amount of microplastics present. Although large storm tanks used to hold excess untreated wastewater, allow some settlement to occur reducing the amount of particulate matter and the amount of denser microplastic present before being released/treated, the issue of stormwater overflow has yet to be investigated in relation to microplastic contamination.

5. CONCLUSION
The results of this study show that WwTW can be effective in the removal of microplastic from the municipal effluent. However, even a small amount of microplastic being released per liter can result in significant amounts of microplastics entering the environment due to the large volumes being treated. These treatment processes are standard wastewater treatment practices and are implemented worldwide. The study goes someway to determine what the most important steps in the treatment process are in the removal of microplastics, that is, grit and grease removal and primary settlement and to address the knowledge gaps highlighted by the Norwegian
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Environmental Report. Treatment facilities where these particular processes are less efficient may be making a greater contribution to microplastic pollution in the environment. This will provide important information in the reduction of microplastic pollution in guiding waste management processes. It is also important that future research on microplastics in WWfW takes a site specific approach by detailing any unique practices carried out by the WWfW studied. More research is needed to determine the difference between the ability of primary, secondary and tertiary WWfW to remove microplastics, as well as the potential temporal differences in the release of microplastics from this source into the environment.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b05416.

Diagram of WWfW showing the location of the sampling sites, comparison of FT-IR spectra found from a polymer library with samples of the same polymer type found in the environment, and photographs of cotton fibers collected on the forensic tapings (PDF)

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Notes
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