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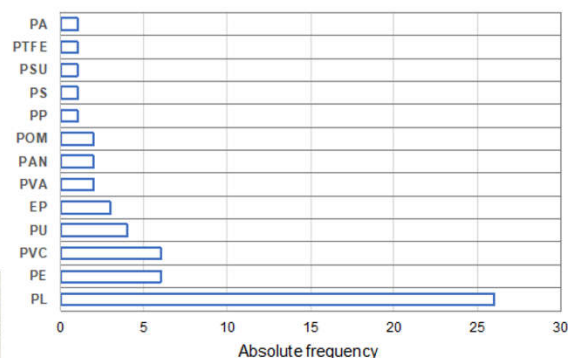
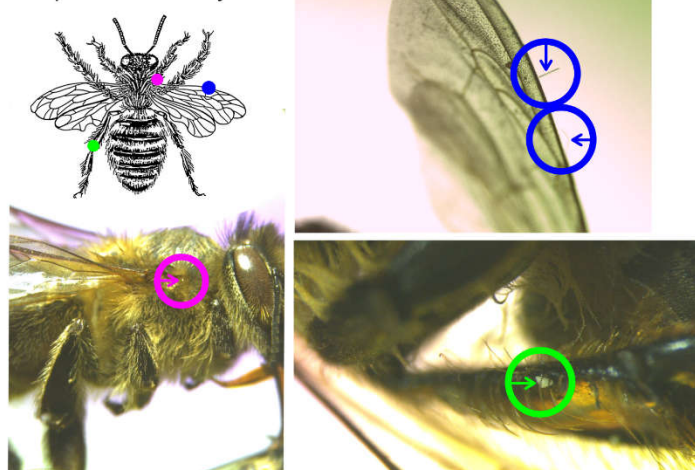
Honeybees as active samplers for microplastics

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Microplastics in honeybees



Polyester (PL), Polyethylene (PE), Polyvinyl chloride (PVC), Polyurethane (PU), Epoxy resin (EP), Polyvinyl acetate (PVA), Polyacrylonitrile (PAN), Polyoxymethylene (POM), Polypropylene (PP), Polystyrene (PS), Polysulfone (PSU), Polytetrafluoroethylene (PTFE) & Polyamide (PA)

Honeybees as active samplers for microplastics

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Abstract

Microplastics are ubiquitous and their sampling is a difficult task. Honeybees interact with the environment inside their foraging range and take pollutants with them. In this work, we demonstrated for the first time that worker bees can act as active samplers of microplastics. We collected honeybees from apiaries located in the centre of Copenhagen and from nearby semiurban and rural areas. We showed the presence of microplastics in all sampled locations mostly in the form of fragments (52 %) and fibres (38 %) with average equivalent diameter of $64 \pm 39 \mu\text{m}$ for fibres and $234 \pm 156 \mu\text{m}$ for fragments. The highest load corresponded to urban apiaries, but comparable number of microplastics was found in hives from suburban and rural areas, which can be explained by the presence of urban settlements inside the foraging range of worker bees and to the easy dispersion of small microplastics by wind. Micro-FTIR analysis confirmed the presence of thirteen synthetic polymers, the most frequently of which was polyester followed by polyethylene and polyvinyl chloride. Our results demonstrated the presence of microplastics attached to the body of the honeybees and opens a new research pathway to their use as active biosamplers for anthropogenic pollution.

Keywords: Microplastics; Honeybees; Biosampling; Environmental monitoring

1. Introduction

Microplastics (MPs) are defined as plastic particles with sizes ranging from $1 \mu\text{m}$ to 5 mm in their larger dimension (GESAMP, 2016). The definition is somewhat arbitrary and despite the prefix “micro”, the size of MPs expands into the millimetre range due to practical and historical reasons (GESAMP, 2019). Below the lower boundary of $1 \mu\text{m}$, plastic particles are referred to as nanoplastics, even though the definition is not coincident the usual size range of nanosized particles (Gigault et al., 2018). According to their origin, MPs can be primary or secondary. Primary MPs have been manufactured with their specific size for cosmetic or industrial purposes, while secondary microplastics come from the degradation of larger particles upon the effect of photochemical oxidation, hydrolysis, and mechanical forces (GESAMP, 2019). Fibres produced from synthetic polymers are considered MPs, but artificial fibres include extruded cellulose or industrially processed natural fibres like cotton

or wool, which can also be considered anthropogenic pollutants. These materials may contain additives and other chemicals and have received much less attention (Henry et al., 2019). MPs have been reported in all compartments, including apparently pristine environments in remote areas and are a global cause for concern due to their mobility and ubiquity and to the lack of knowledge about important aspects related to their fate and risk (Enyoh et al., 2019; Evangeliou et al., 2020; González-Pleiter et al., 2020b; Horton and Barnes, 2020; Li et al., 2018).

The atmospheric transport of MPs is still poorly known. The sources of airborne MPs are the disintegration of larger plastic products like building or packaging materials or point sources like industrial emissions (Wright et al., 2020). Urban sources are generally dominant with an important contribution of fibres produced during the wearing of synthetic textiles (Liu et al., 2019a). The presence of MPs in the atmosphere is a new field of research and the available data are still

very limited. The sources and fate of atmospheric MPs are poorly known because of their many potential origins, their low concentrations, and the difficulty of sampling. The dispersion and transport of MPs and the factors influencing their chemical and mechanical transformation are complex and still not fully understood (Zhang et al., 2020). The occurrence of airborne MPs has been studied at ground level using active or passive collectors or by measuring their ground deposition rate, but the available data are limited and difficult to interpret due to methodological issues, the rapid atmospheric mixing and the occurrence of unpredictable deposition events. The only broad study available showed deposition rates $> 100 \text{ MP m}^{-2} \text{ day}^{-1}$ in remote areas of North America. Air mass trajectory calculations and population metrics suggested an urban origin for wet-deposited MPs, while in the absence of precipitation, MPs might travel very large distances (Brahney et al., 2020). Overall, the data available in the literature showed deposition rates reaching values in the order of hundreds of MPs per square meter and day (Cai et al., 2017; Klein and Fischer, 2019).

Concerning their risk, MPs have been associated to chemical toxicity due to the release of additives, non-intentionally added substances, and pollutants retained from the environment (Hahladakis et al., 2018; Wang et al., 2018). While relatively large MPs can produce physical harm, small debris may cause the blockage of the intestines of small animals and, for sufficiently small particles, translocation and transfer through the food webs, thereby originating true toxic effects. The accumulation in tissues and translocation are phenomena described for plastics in the range of hundreds and tens of nanometres respectively (Sendra et al., 2020a; Shen et al., 2019). It has been reported that small particles could cause damage at cellular and molecular levels, including immunotoxicity and genotoxicity (Ballesteros et al., 2020; Sendra et al., 2020b). An additional cause for concern is the possible migration of MPs to packaged food (Kedzierski et al., 2020). In fact, the presence of MPs in food is well documented with estimations of annual MP intake in order of tens of thousands of particles (Cox et al., 2019).

Honeybees (*Apis mellifera*) or honeybee products have been used as bioindicators for different pollutants (Devillers and Pham-Delègue, 2002). Their advantages include sensitivity to toxic substances, large flying capacity, including inaccessible places and high reproduction rate. The

wide-range activity of honeybees, whose foraging range reaches several kilometres and the existence of tens of thousands female worker bees per colony make them useful as active samplers (Bargańska et al., 2016; Murcia-Morales et al., 2020). Monitored pollutants include products specifically used for beekeepers to control pests, as well as different kinds of environmental pollutants gathered by honeybees during their foraging flights. Honeybees interact with essentially all elements in their environment and bring back pollutants to their hive, where they eventually accumulate and are transferred to honey, bee bread, and beeswax. The occurrence of distribution of pesticide residues in several beekeeping matrices including live honeybees, beeswax and pollen has been reported elsewhere (Calatayud-Vernich et al., 2018; Murcia-Morales et al., 2020). Honeybee colonies have also been explored as active samplers for heavy metals (Džugan et al., 2018; Gajger et al., 2019; Zarić et al., 2017). It has also been showed that honeybees can gather airborne particulate matter that concentrates in specific parts of their bodies like the edge of wings and the head (Negri et al., 2015).

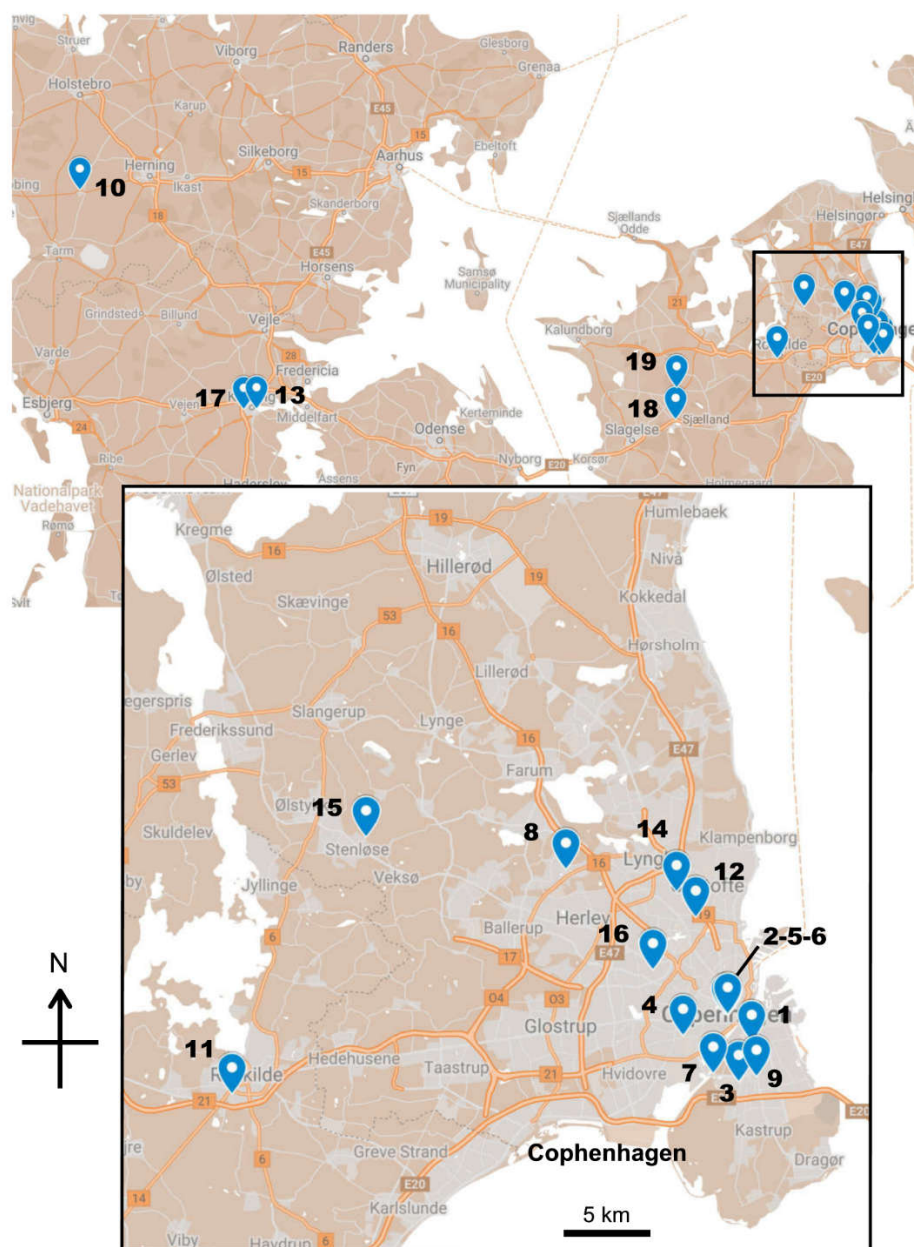
In this work, we tested the hypothesis that worker bees can take MPs from their foraging area, potentially acting as biosamplers of MP pollution. The research responds to the need for implementing wide geographical monitoring of airborne MPs to establish their distribution as new group of persistent anthropogenic pollutants (Bujnicki et al., 2019).

2. Materials and methods

2.1. Sampling

The sampling was conducted with the collaboration of the Danish association of beekeepers (Danmarks Biavlerforening). Nineteen different urban apiaries from Copenhagen (9) and other areas (10) in Denmark, were selected to test the interaction between environmental MPs and honeybees. The complete list of apiaries and their location is shown in Fig. 1.

The protocol for sampling honeybees was developed and delivered to the different beekeepers based on the recommendations for the analysis of MPs in biota samples stated elsewhere (Hermesen et al., 2018). The same sampling method was used in all locations and the honeybees were taken from the same place of the hive in all cases. Samples were taken in the early spring season in



Apiary	Colonies	Location	Site description
1	3	Sundholm, Copenhagen	High density urban, with few green areas
2	2	Dansk Industri, Copenhagen	High density urban, with few green areas
3	3	Bella Centret, Copenhagen	High density urban, with some green areas in the neighbourhood
4	3	Valby, Copenhagen	High density urban, with green areas in the surroundings, including railroads with flowers
5	3	Tivoli, Copenhagen	High density urban, with green areas that include Tivoli Garden
6	2	Majors House, Copenhagen	High density urban, with few green areas
7	3	Kalvebod, Copenhagen	High density urban, with green areas in the surroundings, including railroads with flowers
8	2	Værløse, Copenhagen	Suburban, with many gardens in the neighbourhood
9	2	Westi, Copenhagen	High density urban, with green areas in the surroundings, including railroads with flowers
10	2	Flydtkjær, Videbæk	Countryside, small village
11	2	Germuth, Roskilde	Suburban, green areas and gardens
12	2	Branner, Hellerup	Suburban, with many gardens in the neighbourhood
13	2	Korsholm, Kolding	Suburban with green areas and gardens
14	2	Andersen, Gentofte	Suburban, with gardens in the neighbourhood
15	2	Ask Laurberg, Stenløse	Countryside, small village
16	1	Holm-Petersen, Brønshøj	Urban with gardens
17	2	Wichmann-Hansen, Kolding	Town with green areas and gardens
18	3	Sorb	Countryside, small village
19	2	Stenlille	Countryside, small village

Figure 1. Location of sampling points and site description.

Danish beekeeping. This is a period in which colonies are building up and nectar flow has just started. Samples were taken close to the brood area of the colony, where bees feed larvae and start to store nectar. Honeybees were directly caught in the interior of 50 mL glass jars. Jars were labelled and stored in zip lock bags before being frozen. They were frozen directly into the jars without solution to avoid plastic degradation, organic decomposition and microbial growth, and were transported as soon as possible to the laboratory. The direction and intensity of wind during sampling days was recorded and shown in Fig. S1 (SM, Supplementary Material, SM). A minimum of two samples was recommended per apiary to have at least one replicate. For technical reasons, replicates, although from the same apiary, belonged to different hives. For each replicate, at least 50 honeybees were taken per sample with a minimum of 120 honeybees per apiary. A total number of 4187 honeybees was analysed.

2.2. Laboratory procedures

At the laboratory, the samples were defrosted and put inside beakers filled with 150 mL of ultrapure water and 50 mL ethanol. All honeybees in the same sample were washed together, with a minimum of 120 bees per sample. The mixture water-ethanol was chosen due to its capacity to detach particles from the body of the bees. After 15 min of gentle stirring, the liquid was filtered using 47 mm, 25 µm stainless steel filters in a Millipore stainless steel pressure holder system. Afterwards, their bodies were placed on the same filters and thoroughly washed to remove all possible particles. This procedure allowed recovering the material attached to the body of the honeybees without affecting their integrity. After this procedure, the filters were treated with 33 % H₂O₂ at 60 °C for 24 h to digest the remains of organic matter. Most materials coming from insect bodies were destroyed using this procedure. Finally, filters were dried at 60 °C, placed into glass Petri dishes and sealed to avoid contamination during visual inspection and particle count.

2.3. Quantification and identification of microplastics

MPs samples, kept inside closed glass Petri dishes to avoid contamination, were observed using a stereomicroscope Euromex-Edublu equipped with camera and ImageFocus 4 software. All particles measuring < 5 mm along their larger dimension were photographed and classified by

morphological characteristics: size, shape, and colour. When reporting shape, researchers in the field of plastic litter use categories like fragments or films for irregular shaped particles, and fibres and filaments for high aspect ratio debris as well as other specific categories that vary somehow among research groups. In our case, we classified microplastics into fragments, films, fibers, and filaments with the characteristics detailed below (Frias and Nash, 2019; Lusher et al., 2017). Fragments were defined as particles with irregular shape and edges, with possible origin in the fragmentation of larger particles. Films are also irregular, but thinner than fragments and with flexible aspect. Fibers and filaments are characterized because their larger dimension (length) is considerably higher than the second projected area dimension (width or diameter). For the purpose of this work, we considered fibres or filaments those microparticles with aspect ratio (length/width) > 4; otherwise they were classified as fragments or films. Filaments differentiate from fibres because they have the same thickness along their length and show sharp ends (Magni et al., 2019). We computed the projected dimensions of all microparticles using micrographs and the image analysis program ImageJ. The equivalent diameter was calculated for particles and films as projected area diameter, and for fibres and filaments as aerodynamic diameter calculated as follows (Prodi et al., 1982):

$$D = \frac{3W}{2} \sqrt{\frac{\rho}{\frac{0.385}{\ln(2\beta) - 0.5} + \frac{1.23}{\ln(2\beta) + 0.5}}} \quad [1]$$

where b is the aspect ratio based on projected dimensions (length/width or L/W), W the width of diameter of the fibre or filament and ρ its relative density (taken as 1.000).

Photographs were processed with ImageJ software for obtaining projected particle length and width. All microparticles suspected of being MPs were analysed using micro Fourier Transform Infrared Spectroscopy (micro-FTIR). For it, particles were deposited on KBr disks and measured in transmission mode using a Perkin-Elmer Spotlight 200 Spectrum two apparatus with mercury cadmium telluride detector that allows obtaining optimum results in the mid-infrared region. The conditions for the analyses were: 50 µm spot size, a minimum of 20 scans, resolution of 8 cm⁻¹ and a spectral range 550-4000 cm⁻¹. Resultant spectra were processed through OMNIC 9 software and

compared with existent databases and with our own spectra. Matching > 70 % was considered enough for positive identification of plastic materials (Liu et al., 2019). In some cases, spectra with noisy signals and matching > 65 % were judged satisfactory based on the identification of representative bands as explained below and in Supplementary Material.

2.4. Prevention of procedural contamination

The measures taken to avoid sample contamination included field and laboratory procedures. For the collection of honeybees, only one person was involved wearing controlled clothes from non-synthetic materials and placed against the wind. Plastic materials such as synthetic polyester or acrylic goods were avoided, and no plastic material was used in hives. Nitrile gloves and metal tweezers were used if needed. Glass material was used to store the samples, previously cleaned carefully with pure water. During laboratory manipulation, only glass and steel material was used previously cleaned with ultrapure water (filtered through a 0.22 µm filter, particle and bacteria free) a minimum of three times. Glass beakers were always covered with aluminium foil using specifically designed metallic cages. Prior to use, all laboratory materials were wrapped with aluminium foil and heated to 450 °C for 4 h to remove all possible contamination from fibers or other potentially interfering materials. The clothes worn by laboratory personnel were 100% cotton with non-typical colours. During all field and laboratory sample manipulation steps, Petri dishes were kept open with glass fibre filters in order to identify possible contamination from the environment. Plastics like those found in procedural controls were not considered.

2.5. Statistics

Pearson correlation was used to assess matching between samples and database or standards. A one-way ANOVA coupled with Tukey's HSD (honestly significant difference) post-hoc test was performed for comparison of means. The p-value for statistically significant difference was 0.05.

3. Results and discussion

By inspecting the whole set of sealed filters, we selected 125 microparticles < 5 mm along their larger dimension of putative anthropogenic origin, which were individually studied using micro-FTIR. We only excluded particles of clearly natural origin. Fig. 2 shows size distribution and the

relative abundance of fragments, films, fibres and filaments among MPs. The dominant shapes of MPs were fragments (52 %) followed by fibres (38 %) with lower amounts of filaments and films. Clearly, fibres and filaments displayed lower equivalent size because of their small diameter or lower projected dimension, which was in the 10.5-69.9 µm range. The average equivalent diameter was 64 ± 39 µm for fibres and 234 ± 156 µm for fragments, the intervals corresponding to standard deviations. The data on size distribution and shape are difficult to compare with literature data because atmospheric transport of MPs is new research area with still very limited data. Specifically, concerning shape, there is no agreement in the available literature and fibre and fragments are found dominant depending on the source (Cai et al., 2017; Klein and Fischer, 2019).

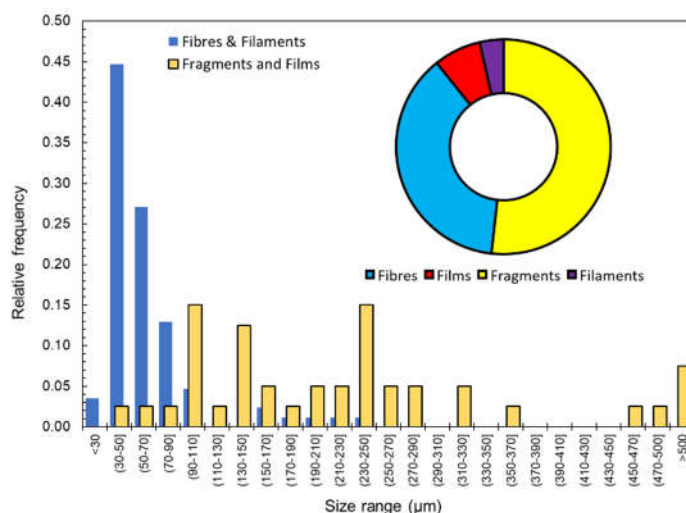


Figure 2. Size distribution of equivalent diameter. Blue bars for the sum of fibres and filaments, and orange for the sum of fragments and films. The inset shows the distribution of the different classes of microparticles: fibres, filaments, fragments, and films.

All microparticles were analysed by micro-FTIR as described above resulting in 56 microparticles positively identified as MPs (21 fibres, 2 filaments, 29 fragments and 4 films). The MPs identified were polyester (PL), polyethylene (PE), polyvinyl chloride (PVC), polyurethane (PU), epoxy resin (EP), polyvinyl acetate (PVA), polyacrylonitrile (PAN), polyoxymethylene (POM), polypropylene (PP), polystyrene (PS), polysulfone (PSU), polytetrafluoroethylene (PTFE) and polyamide (PA). The absolute abundance of MPs is shown in Fig. 3. Clearly PL, > 80 % of which were fibres and filaments, was dominant, followed by PE and PVC, which were mostly fragments and films.

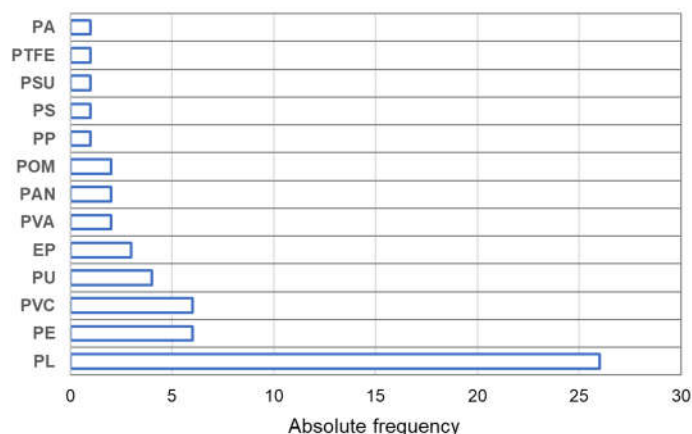


Figure 3. Chemical composition of MPs sampled in honeybees. PL: Polyester; PE: Polyethylene; PVC: Polyvinyl chloride; PU: Polyurethane; EP: Epoxy resin; PVA: Polyvinyl acetate; PAN: Polyacrylonitrile; POM: Polyoxymethylene; PP: Polypropylene; PS: Polystyrene; PSU: Polysulfone; PTFE: Polytetrafluoroethylene; PA: Polyamide.

The identification of sampled microparticles showed, besides MPs, the presence of cotton, wax, vegetal debris, and different parts of honeybee bodies together with some particles identified with insufficient evidence. Besides, we found 30 cotton fibres with non-natural colours, which included 19 blue, 7 black, and 1 red, apart from several more white, transparent and grey. In controls, we identified only one blue cotton fibre, possible from textile origin. Overall, the results indicated the presence of natural fibres of non-natural colours that can also be considered a tracer of anthropogenic pollution (González-Pleiter et al., 2020a). Natural fibres with evidence of industrial origin result in environmental concerns due to their content of dyes and other chemicals and are a class of ubiquitous airborne anthropogenic pollutants that received limited attention so far (Stanton et al., 2019).

Fig. 4 shows the FTIR spectra of six representative MPs; two fragments, 1 film, 2 fibres and 1 filament. The FTIR spectra of fibres and fragments showed the typical bands of the materials identified. The main features are as follows. The broad band centred at 3350 cm^{-1} and the absorption at 1720 cm^{-1} corresponded to the N-H stretching vibration and the stretching band of -C=O in the urethane bond (Fig. 4A). The characteristics bands at 2914 cm^{-1} , 2847 cm^{-1} , 1460 cm^{-1} , and 715 cm^{-1} of PE (Fig. 4B). The bands of C=O vibration at 1715 cm^{-1} , the stretching of the aromatic ring at 1410 cm^{-1} and the carboxylic anhydride from PL at 1021 cm^{-1} (Fig. 4C). The features of PVC (Fig. 4D) include the C-H stretching bands at $2850\text{-}2920$

cm^{-1} , and the typical small shoulder at from C-Cl stretching at 840 cm^{-1} . The blue fibre of Fig. 4E could be identified as PAN based on the $\text{C}\equiv\text{N}$ stretching band at 2240 cm^{-1} , and the aliphatic bands from methylene C-H stretching. The bands of EP that appear in Fig. 3F are the C-O-C epoxy vibration at 920 cm^{-1} , and the characteristic O-H stretching in the $3500\text{-}3200\text{ cm}^{-1}$ range. Other details about the identification are provided as Supplementary Material. FTIR standards are provided in Fig. S2 (SM). Fig. 4 also shows the micrographs of the same fibres and fragments. For comparison, the typical hair length in honeybees is about 1 mm (Roquer-Beni et al., 2020).

The results reporting the abundance of MPs on honeybees are shown in Fig. 5 for all the locations studied in this work. Fig. 5A shows the number of MP per 100 honeybees for the different apiaries. The average for all apiaries is also indicated as a dashed line. Fig. 5B shows the boxplot of MPs relative to the number of sampled honeybees for locations grouped into urban (a), suburban (b) and rural areas (c). Our work showed the maximum concentration of MPs in the centre of Copenhagen (Location 1). The limited overall variability, could be in part attributed to the fact that all points marked a (urban) were separated by less than 4.3 km, which is inside the foraging radius of *Apis mellifera*, reported as 5-6 km, with 50 % of the workers foraging 6 km and 10% more than 9.5 km from their hive (Beekman and Ratnieks, 2000). Locations 18 & 19 were separated 6.5 km from the city and, noteworthy, locations 13 & 17 and 10 were separated from the centre of Copenhagen by 20 and 25 km respectively. However, in all cases there were population nucleus nearby. For example, location 10 is a rural place, but surrounded by several towns including Herning, with 47000 inhabitants. In all cases except location 16, a minimum of two neighbouring hives were sampled. The results showed that deviations from hives in the same apiary did not differ more than 60 % (minimum 2 %, 35 % in average) expressed as MPs/bee, which supports the strength of the sampling procedure. Another reason for the relatively homogeneous concentrations obtained may be the homogenization produced by the wind dispersion of microplastics over large areas as explained below.

Our results can be interpreted in the light of other studies on the atmospheric deposition of MPs. The few data available point to an abundance of MPs decreasing when moving away from urban areas

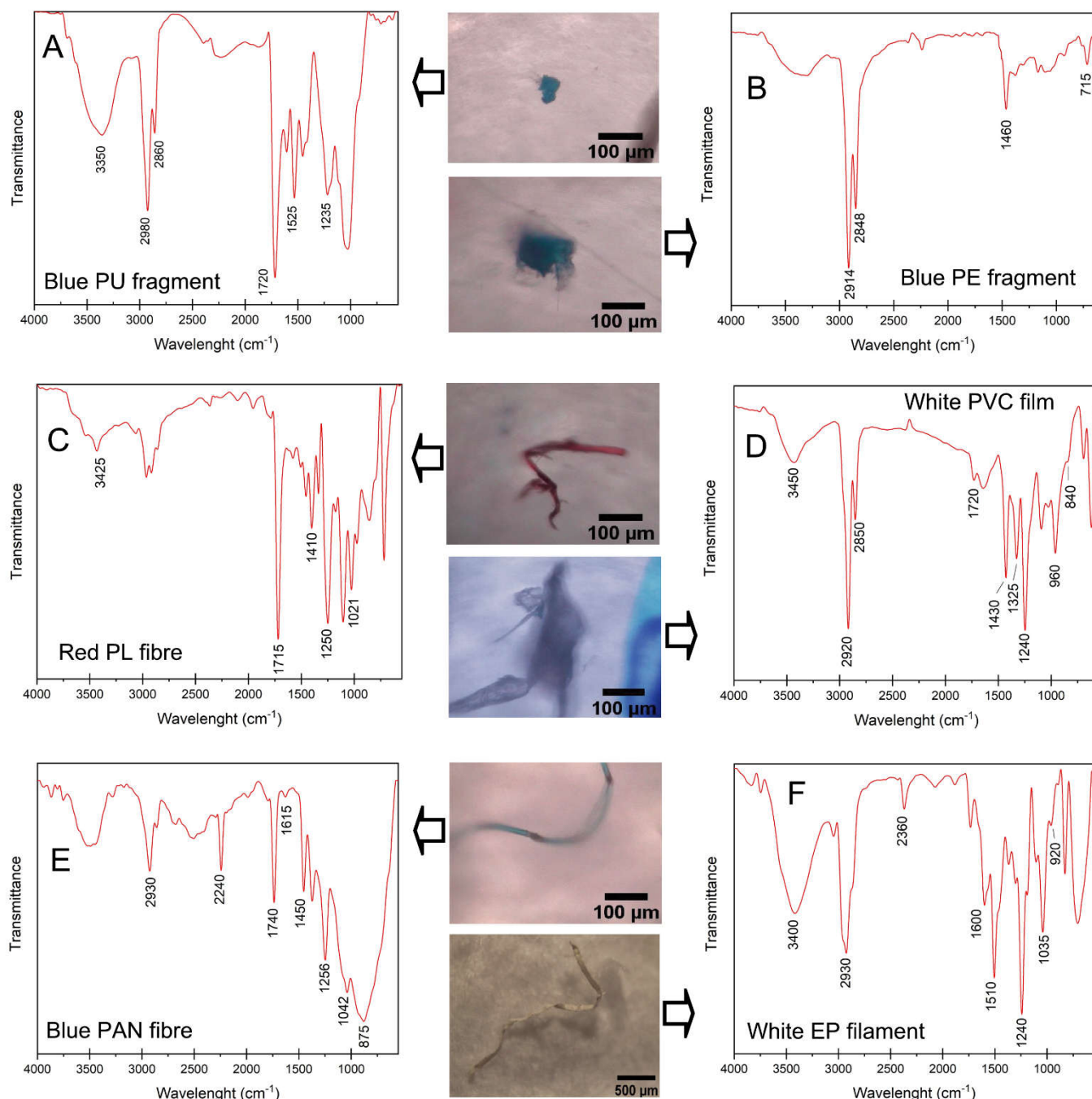


Figure 4. Micro-FTIR spectra and representative images of some of the MPs found in this work. (A) blue PU fragment, (B) blue PE fragment, (C) red PL fibre, (D) white PVC fragment, (E) blue PAN fibre and (F) white EP fragment.

(González-Pleiter et al., 2020a; Liu et al., 2019). Besides, it has been recently shown that plastic fragments and small fibres can be transported by wind across long distances before being deposited even in very distant places (Brahney et al., 2020). Brahney et al. (2020) studied wet and dry deposition of MPs in remote areas of the United States. Their analysis of air mass trajectories suggested that urban centres are one of the main sources for at least wet-deposited MPs and that small plastic debris can be transported long distances before being deposited. Recently, the first direct evidence of the presence of MPs at high

altitude showed a higher concentration in the air above cities in comparison with rural areas (González-Pleiter et al., 2020a). Overall, the available data show that MPs are emitted in densely populated areas and reach high altitude, which allows their transportation by wind to distant places.

The data available on the atmospheric precipitation of MPs in a Chinese city (Dongguan) showed an average deposition rate in the 31 ± 8 to 43 ± 4 MP $\text{m}^{-2} \text{day}^{-1}$ range for MPs between 200 and 4200 μm (Cai et al., 2017). Allen et al. (2019) reported, for a

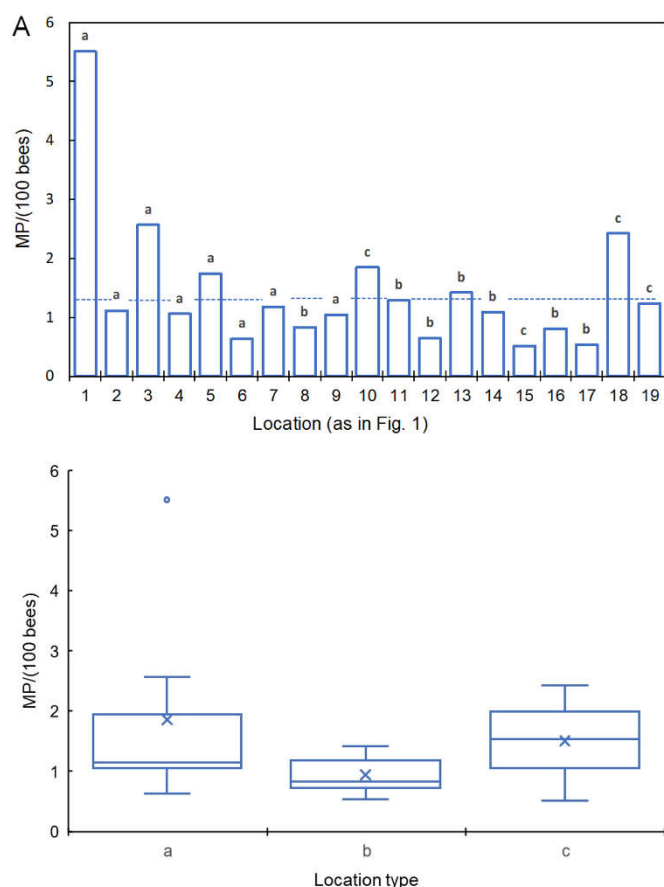


Figure 5. Relative abundance of MP per insect for all sampling points as indicated in Fig. 1. Urban zones with high population (a), suburban areas (b) and rural zones (c). In Panel B the abundance is represented for each type of zone as boxplot with crosses representing the average values. The dashed line in Fig. 5A represents the average for all apiaries.

remote area in the Pyrenees, a deposition rate of $365 \pm 69 \text{ MP m}^{-2} \text{ day}^{-1}$ for 25-3000 μm MPs. In an urban and peri-urban study in Hamburg, Germany, Klein and Fisher reported deposition rates in the $136.5\text{-}512.0 \text{ MP m}^{-2} \text{ day}^{-1}$ range for sizes $< 63 \mu\text{m}$ and up to 5000 μm (Klein and Fischer, 2019). Accordingly, and despite the methodological differences among studies that make comparisons difficult, the literature data point towards deposition rates from tens to hundreds of MPs per square meter and day without important differences between urban, rural and less populated areas. The fact that MPs can be dispersed long distances that may reach thousands of kilometres away from their initial point of release, explain that MPs reach remote areas and also that samples taken from rural areas, apparently safe from most of the emissions associated to human activity reported similar values to samples taken inside densely populated cities. In our case, the samples recovered from

honeybees in rural areas near Copenhagen contained relatively high number of MPs, not too different from those hiving in central Copenhagen. This finding is also consistent with the westward winds, dominant during the sampling period as shown in Fig. S1 (SM).

Using honeybees as active MP samplers opens a new research pathway to compare sampling in environments with different practices and urban/industrial pressure. They offer the possibility to compare with dry and wet deposition campaigns or other types of research on the occurrence of MPs. Honeybees are particularly adapted to transport particulate material because of their morphological structures and their grooming behaviour for pollen collection, which, together with their foraging range and worldwide distribution, make them ideal as living samplers for environmental monitoring. When flying, their bodies become positively charged with static electricity, so that when the bee lands on a flower, the pollen particles stick to their static-charged hair and the same happens with other microparticles in their environment (Negri et al., 2015). Honeybees may complement other sampling systems in common use for monitoring air quality. It is important to note that very limited research has been performed so far on the atmospheric transport of MPs. Another feature of our research refers to the translocation of MP pollution to honeybee products like honey or beeswax, which could raise concerns about human health. Once, we demonstrated that honeybees may act as collectors for MP pollution, further studies could involve the use of in-hive passive samplers capable to collect MPs from a large number of honeybees in a less intrusive way. Further research is also needed on the occurrence of MPs in honeybee products and to assess the possible use of honeybees or other active samplers to assess human exposure to microplastics.

4. Conclusions

Honeybees were collected and processed from nineteen different apiaries from the centre of Copenhagen and other locations including suburban and rural areas. Our results showed the presence of MPs in all locations. The highest load corresponded to apiaries located in Copenhagen. Nevertheless, honeybees from suburban and rural areas also bore a considerable number of MPs adhered to their bodies. The presence of urban

settlements inside the foraging range of honeybees, and the wind dispersion of MPs are the probable reasons explaining the presence of MPs in honeybees from rural hives.

Micro-FTIR analysis confirmed the presence of thirteen synthetic polymers, the most frequently of which was polyester. Fragments followed by fibres were the dominant shapes. Industrially processed cotton fibers were also frequently found. The results proved the presence of MPs and other anthropogenic materials adhered to the body of the honeybees, allowing their use as active samplers. This work demonstrates for the first time the possibility of using honeybees (*Apis mellifera*) as bioindicator for the presence of MPs in the environment.

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Supplementary Material

Honeybees as active samplers for microplastics

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Contents

Supplementary Information on the analytical determination of microplastics.

Figure S1. Compass rose for the average winds during daylight hours on sampling days (data from the meteorological observatory at Copenhagen Airport)

Figure S2. Reference spectra for the microplastics identified in Fig. 3 in the body of the article.

Supplementary Information on the analytical determination of microplastics. (Referred to Fig. 4 in the body of the article). The FTIR spectrum of PU blue fragment (Fig. 4A) displayed the typical broad band centred at 3350 cm^{-1} that corresponds to the N-H stretching vibration of the urethane bonds. The bands in the $2920\text{--}2860\text{ cm}^{-1}$ region are associated to vibrational modes of the -CH_2 groups and the absorption at 1720 cm^{-1} corresponds is the characteristic stretching band of the -C=O group of the urethane bond. The characteristic bands of the CN and C-O-C groups appeared at 1525 cm^{-1} and 1235 cm^{-1} , while the region just above 1000 cm^{-1} displayed bands attributed to the N-CO-O and other typical absorption of polyurethanes (Demétrio-da-Silva, et al. 2013). The blue fragment of Fig. 4B was clearly identified as polyethylene (PE) in view of the presence of its four characteristics bands at 2914 cm^{-1} , 2847 cm^{-1} , 1460 cm^{-1} , and 715 cm^{-1} (Gulmine et al., 2002). The red polyester (PL) fibre (Fig. 4C) showed the characteristic bands of C=O vibration at 1715 cm^{-1} , the stretching of aromatic ring at 1410 cm^{-1} and the carboxylic anhydride at 1021 cm^{-1} . The white film (of Fig. 4D) showed the characteristic features of polyvinyl chloride (PVC), which include the bands of C-H stretching at $2850\text{--}2920\text{ cm}^{-1}$, CH_2 deformation at 1325 cm^{-1} , CH rocking at 1240 cm^{-1} , trans-CH wagging at 960 cm^{-1} and the typical small shoulder at from C-Cl stretching at 840 cm^{-1} . The carbonyl stretching at 1720 cm^{-1} may indicate ageing through dehydrochlorination and oxidation processes or the presence of stabilizers like phthalates. The band at $3300\text{--}3500$ corresponds to a hydrated fragment. The blue fibre of Fig. 4E could be identified as PAN based on the bands at 2240 cm^{-1} , which corresponded to the $\text{C}\equiv\text{N}$ stretching, and the aliphatic bands at 2930 cm^{-1} and 1450 cm^{-1} from methylene C-H stretching. The weak absorption at 1615 cm^{-1} could be attributed to C=C probably from a butadiene copolymer (Ju et al., 2013). Finally, the white filament of Fig. 4F was attributed to epoxy resin (EP). The C-O-C from the epoxy group appeared at 920 cm^{-1} . The band at 2930 cm^{-1} corresponded to methylene C-H stretching and the characteristic O-H stretching band was clearly observed in the $3500\text{--}3200\text{ cm}^{-1}$ range. The stretching of benzene ring appeared at 1600 cm^{-1} , and 1510 cm^{-1} and the absorptions at 1240 cm^{-1} and 1035 cm^{-1} corresponded to the C-O stretching in the backbone of the polymer. Reference spectra are shown in Fig. S2.

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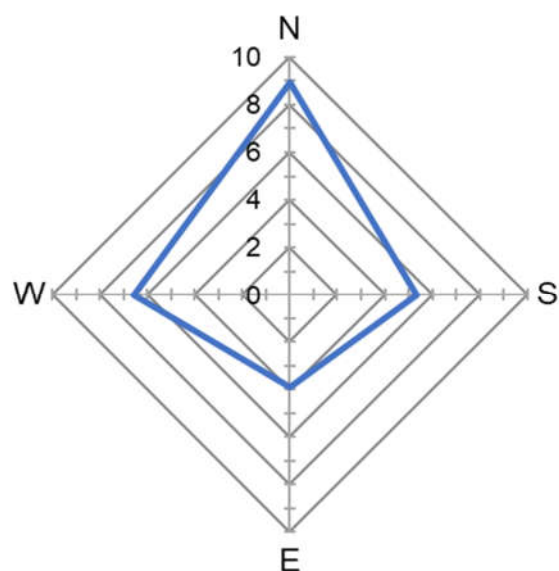


Figure S1. Compass rose for the average winds during daylight hours on sampling days. The scale is the average wind velocity in km/h (data from the meteorological observatory at Copenhagen Airport)

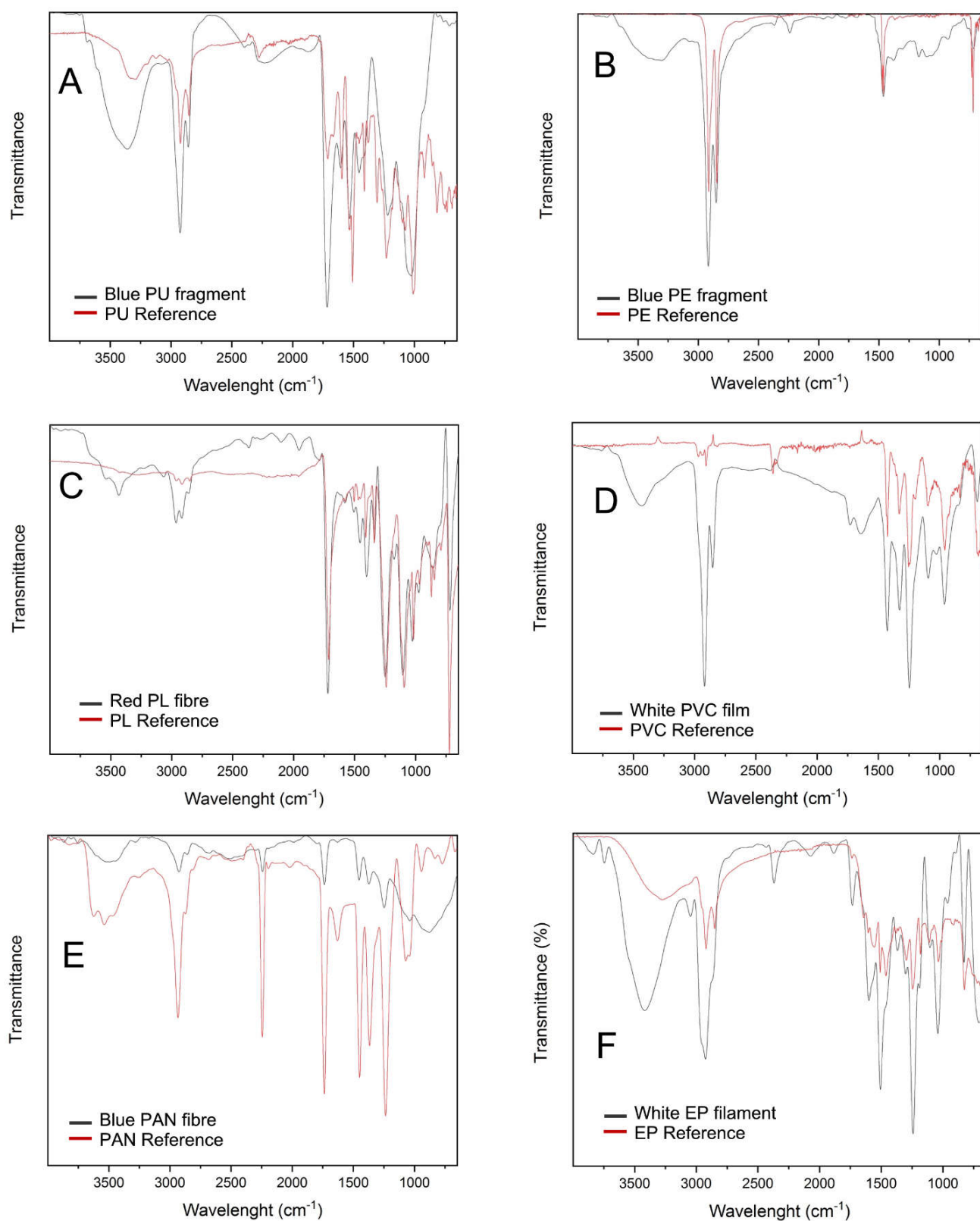


Figure S2. Reference spectra for the microplastics identified in Fig. 4 in the body of the article.