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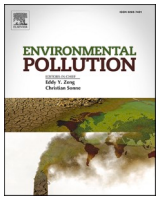

Evaluation of microplastic pollution using bee colonies: An exploration of various sampling methodologies☆

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ABSTRACT

Recent research has highlighted the potential of honeybees and bee products as biological samplers for monitoring xenobiotic pollutants. However, the effectiveness of these biological samplers in tracking microplastics (MPs) has not yet been explored. This study evaluates several methods of sampling MPs, using honeybees, pollen, and a novel in-hive passive sampler named the APITrap. The collected samples were characterized using a stereomicroscopy to count and categorise MPs by morphology, colour, and type. To chemical identification, a micro-Fourier transform-infrared (FTIR) spectroscopy was employed to determine the polymer types. The study was conducted across four consecutive surveillance programmes, in five different apiaries in Denmark. Our findings indicated that APITrap demonstrated better reproducibility, with a lower variation in results of 39%, compared to 111% for honeybee samples and 97% for pollen samples. Furthermore, the use of APITrap has no negative impact on bees and can be easily applied in successive samplings. The average number of MPs detected in the four monitoring studies ranged from 39 to 67 in the APITrap, 6 to 9 in honeybee samples, and 6 to 11 in pollen samples. Fibres were the most frequently found, accounting for an average of 91% of the total MPs detected in the APITrap, and similar values for fragments (5%) and films (4%). The MPs were predominantly coloured black, blue, green and red. Spectroscopy analysis confirmed the presence of up to five different synthetic polymers. Polyethylene terephthalate (PET) was the most common in case of fibres and similarly to polypropylene (PP), polyethylene (PE), polyacrylonitrile (PAN) and polyamide (PA) in non fibrous MPs. This study, based on citizen science and supported by beekeepers, highlights the potential of MPs to accumulate in beehives. It also shows that the APITrap provides a highly reliable and comprehensive approach for sampling in large-scale monitoring studies.

1. Introduction

The presence of microplastics (MPs) in the atmosphere has been of emerging concern in recent years. Significant quantities of these contaminants are discharged into the air throughout the manufacturing and regular use of plastic items (Liu et al., 2019a). But other factors, physical and chemical, such as abrasion or erosion, also contribute to the rise of MPs in the atmosphere (Chen et al., 2020a; Li et al., 2020). As particle size decreases, the amount of plastic waste and its mobility in the environment increases dramatically. Some recent works have shown that MPs have the capacity to travel over long distances and affect remote zones through atmospheric transport (Allen et al., 2019; Wright et al., 2020). Their presence suspended in air may pose a potential threat to human health, as they can be directly inhaled (Gasperi et al., 2018; Prata, 2018). Despite the publication of articles on this topic, there remains a notable deficiency in consistent and cost-effective sampling devices. This limitation hinders the implementation of extensive sampling programs and the establishment of standardized methodologies for

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evaluating microplastics in the atmosphere.

The primary challenge in detecting airborne microplastics lies in the effective collection of microplastic samples. To date, the predominant methods for gathering airborne MPs include passive atmospheric deposition samplers and active pump samplers, as noted in studies by Chen et al. (2020b) and Dong et al. (2023). Atmospheric deposition is a key technique for understanding how microplastic particles settle from the atmosphere onto sampling surfaces. This method is particularly suited for continuous, long-term sampling (e.g., weekly, or monthly) and is especially useful in remote areas where electricity to power active pump samplers might not be available. However, environmental factors like rain, snow, and wind can significantly impact the concentration and deposition of airborne MPs at collection sites, as discussed by Liu et al. (2019a). On the other hand, the pumped sampling system, which consists of a pump unit and replaceable filters, is widely used for quick collection of atmospheric samples. The air filtered through these units is quantitatively analyzed, providing data in terms of items per cubic meter.

There are certain limitations in data collection for both active and passive sampling methods. Active methods usually are expensive and require for electricity to function. This can prove a challenge for long-term sampling campaigns in remote areas. In contrast, outdoor passive samplers are usually very vulnerable to weather conditions, which can greatly affect the accuracy of the measurements. Additionally, both active and passive methods struggle to efficiently collect smaller microplastics, a problem that stems from the limited pore size of the filters in the samplers and the inherent difficulties in trapping minuscule airborne particles. As a result, the currently available data on the presence of airborne microplastics (MPs) are somewhat restricted and challenging to interpret.

Honey bees (*Apis mellifera*) and some bee products, such as honey, pollen, wax or royal jelly, have previously been used as biomonitors of environmental contamination (Bargańska et al., 2016). Their advantages include their large flying capacity, including inaccessible places, and their high reproductive rate and the fact that all bee return to the hive, and by doing this, accumulate the contamination inside the hive (Murcia-Morales et al., 2020). Thus, the bees during their foraging flights are exposed to contaminants, including airborne MPs. In a recent paper published in 2021, the authors demonstrated for the first time the possibility of using honeybees as an active biosampler to detect the presence of MPs in the environment (Edo et al., 2021). To date, a few publications have reported the presence of microplastic particles in honey (Liebezeit and Liebezeit, 2013). Nevertheless, to the best of the authors' knowledge, no data about the detection of MPs in pollen samples has been published to date.

Honeybees interact with their environment and transport pollutants back to their hives. Consequently, honeybee colonies have been studied as dynamic samplers for heavy metals (Gajger et al., 2019; Zarić et al., 2017), xenobiotic contaminants such as pesticide residues (Murcia--Morales et al., 2020), and polycyclic aromatic hydrocarbons (PAHs) (Murcia-Morales et al., 2024). The atmospheric stability and airflow within the beehive can provide an advantageous sampling point for environmental monitoring, which can be integrated with citizen science platforms to facilitate cost-effective evaluation of MPs. MPs can enter the hive through two primary pathways: during the bees' foraging flights or via air currents. The ventilation of a beehive involves two simultaneous processes: the first is the intra-hive air exchange between the honeybee and the air inside the beehive, caused by the temperature difference between the inside and outside the brood nest and the second is the active exchange of air between the beehive and the external environment (Peters et al., 2019; Sudarsan et al., 2012). Therefore, the development of in-hive passive samplers can be a valuable tool for assessing environmental contamination by MPs. These samplers can capture MPs through indirect adsorption from the air circulating within the hive.

The primary aim of this study centred on creating an innovative device designed for the extensive collection of atmospheric MPs over prolonged durations, functioning independently of electricity and resistant to adverse weather conditions. This newly developed device was deployed within beehives to facilitate long-term environmental surveillance of airborne MPs in Denmark's relatively secluded regions. We compared the data gathered using this in-hive passive sampler to the findings acquired from other biological samplers, specifically honeybees and one of their by-products, pollen.

2. Materials and methods

2.1. Field study and sample collection

In this study a new non-biological passive sampler "APITrap" was developed (Apiarian-Trap). The sampler device was a rigid polyethylene plastic with a polyvinyl acetate adhesive (called sticky sheets, 40 × 25 cm), which is part of the APITrap. The APITrap is basically a wooden frame covered with a stainless-steel mesh with a 2 mm gap into which acetate adhesive sheets are inserted. One APITrap was placed per hive brood chamber where there is the highest bee-activity. Two hives per apiary were selected for sampling. Five different apiaries were used for the field studies in Denmark (see Fig. 1). The sampling sites were divided into: (1) intensively agricultural area, (2) intensively agricultural area influenced by motorways, (3) and (5) urban near the coast, (4) wilderness area (recreation area, organic beekeeping). All the apiaries were situated within a 100-km radius, covered various pollution scenarios, from heavily agricultural areas to regions of organic or nature forest. Two APITraps were placed inside two neighboring hives for two weeks. Four samplings during two consecutive months from June to July 2022 were performed. Honeybees and pollen samples were collected in the same period from the same apiary but belonging to different hives. Honeybees (approx. 150/apiary) and pollen (approx. 25 g/apiary) were directly caught into 50 mL pre-cleaned glass jars. Jars were labelled and stored in zip lock bags. The samples were transported directly to the Danish national coordinator at the end of each sampling. APITrap and pollen samples were stored at room temperature until they were sent to the laboratory in Almeria, Spain, while honeybees' samples were frozen to avoid organic decomposition and microbial growth.

2.2. Sample extraction

The desorption procedure of the MPs from the plastic polyethylene surface was carried out as follows: first, each APITraps were placed inside 500 mL pre-cleaned glass jars. APITrap sheets were folded, no cuts were made during the extraction stage to avoid the possible generation of films or fragments. Then, 75 mL of filtered n-hexane was added. The bottles were covered to avoid contamination, and placed in an ultrasonic bath for 10 min, applying three extraction cycles. The supernatant was then filtered under vacuum on a cellulose esters filter (S-Pak Filters, pore size 1.2 μm, diameter 47 mm, Merck Millipore, Milford, MA, USA) which were kept in Petri dishes to avoid contamination. In the filtration process, each beaker was flushed with n-hexane to rinse adhering materials.

The extraction of the honeybee samples was carried out following a similar approach. Twenty-five honeybees (5 g) were transferred to a pre-cleaned glass jar of 250 mL, using ethanol and ultrapure water filtered. To extract MPs from the bees, 30 mL of a saturated sodium chloride solution (1.2 g cm^{-3}) was added. This solution was chosen because of its capacity to separate particles and low-density polymers such as PE, PP, and PS from the bee's body, while being cheap, easily available, and environmentally friendly. To prepare the saturated NaCl solution, 360 g of NaCl were added to 1 L of ultrapure water. After stirring and heating the solution to 50 °C, it was allowed to cool to room temperature and finally filtered using filter paper to remove any excess undissolved salt. The bottles were then covered to avoid contamination and shaken using an ultrasonic bath. After 3 cycles for 10 min each cycle, the supernatant was vacuum filtered through a cellulose esters filter (S-Pak Filters, pore

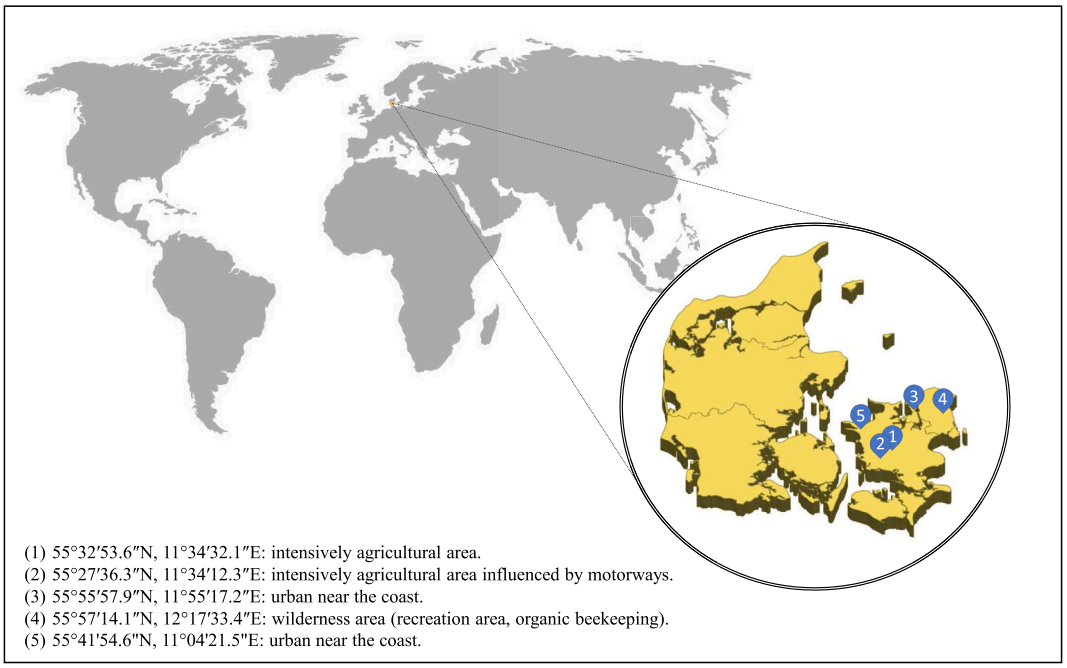

Fig. 1. Location of sampling points.

size 1.2 μm, diameter 47 mm, Merck Millipore, Milford, MA, USA) to collect the MPs. Finally, all filters were placed in Petri dishes and left in the dark at room temperature in a silica gel drying chamber.

The following procedure was used to extract the pollen samples. Two grams of homogenized pollen were weighed and put inside jars filled with 30 mL of a sodium chloride solution. After 10 min of sonication in an ultrasonic bath, the supernatant was centrifuged (3 min x 4000 r.p.m) and filtered using 47 mm, 25 μm stainless steel mesh in a vacuum support system. The steel filter was then washed with 10 mL of ultrapure filtered water to remove the organic matter retained on the mesh. After that, the steel filter was transferred to a pre-cleaned glass jar filled with 20 mL of n-hexane and extracted during 10 min using an ultrasound bath (3 cycles). Finally, the solution was filtered a cellulose esters filter and stored in Petri dishes at room temperature.

2.3. Sample analysis, characterization and chemical evaluation

After MPs extraction, Petri dishes were examined using a stereomicroscope S9i (Leica S9i) equipped with an integrated digital camera with 10 Mpixels and LAS-X 3.7 software. Any particles with a dimension between 25 μm and 2 mm along their larger axis were photographed and classified based on their morphological characteristics (size, shape, and colour). The MPs were classified into three main groups (fragments, films, and fibres) following the same criteria used by other researchers (Edo et al., 2021). Fragments were defined as particles with irregular shapes and edges, likely resulting from the breakdown of larger particles. Films also exhibited irregular shapes but were thinner than fragments and with a more flexible appearance. Fibres were distinguished by having a significantly greater length compared to their width or diameter. Every suspected particle was counted and classified under two lenses with a 1 × magnification objective, which providing a 9:1 zoom at 6x-55x magnification. Furthermore, high-resolution photographs of MPs were taken using a visual objective set at 2 × magnification providing a magnification range of 12–110. The images were processed with LAS-X 3.7 to obtain the projected length, width, area, and roundness of the particles. Roundness values close to 1 indicate that the particle is spherical.

All particles suspected of being MPs were deposited on macroporous silicon membrane filters (pitch 12 μm, pore length 500 μm, pore diameter 4–6 μm (MakroPorP12M5-500, SmartMembranes GmbH, Germany). The semi-automated analyses of MPs were collected using the mapping mode, and a micro–Fourier Transform Infrared Spectroscopy (μ-FTIR) microscope (Nicolet iN10; Thermo Fisher Scientific), in transmission mode and spectral ranging from 4000 to 650 cm^{-1}, at 8 cm^{-1} spectral resolution and after 16 scans. Liquid nitrogen was used to cool the internal mercury cadmium telluride (MCT) detector. Prior to analysis, calibration was performed to account for background interference caused by ambient carbon dioxide and water vapor levels. The spectra obtained were manually confirmed with OMNIC polymer spectra libraries (Thermo Fisher Scientific, USA) and with our own spectra to distinguish the polymer type. Matching >65% was considered enough for positive identification of plastic materials (Liu et al., 2019b; Mecozzi et al., 2016). Spectrum matches lower than 65% were discarded.

2.4. Prevention of procedural contamination

Quality control measures were used to present the external contaminations. The person involved to the collection of field samples (honeybees, pollen, and APITrap) was wearing clothes from non-synthetic materials and nitrile gloves. No plastic material was used in the hives. An APITrap sticky sheets (control) was activated at each sampling. The sticky sheets were opened for the approximately 30 min it took to perform the sampling. These sticky sheets did act as a control outside the hives.

In the laboratory, all glassware and stainless-steel tweezers were thoroughly cleaned using ethanol and ultrapure water. Prior to use, they were carefully wrapped with aluminium foil and placed in an oven at 250 °C for 6 h. All solvents used in this study were filtered using 0.45 μm filters, at least twice. The sample treatment was performed inside an ultra-clean, closed laminar flow hood, with a vertical wind of 0.6 m/s. Lab coats (100% white cotton) and nitrile gloves were worn throughout the whole procedure. In addition, each day, before starting, a blank control was performed during the sample treatment (without APITrap, honeybees or pollen), to evaluate the background pollution of the laboratory due to solvents or laboratory material. Furthermore, an APITrap blank was evaluated to ensure that the sampler material did not affect to the measurements. All the results were corrected by subtracting the MPs

found in each control batch. In all cases, we only found one or two blue fibres.

3. Results and discussion

3.1. Morphological characteristics of observed MPs

Particles clearly of natural origin, such as plant debris, (parts of) bee wings, and fine hairs, were excluded from our analysis. These particles are likely released by bees during their activities inside the hive. Similarly, white fibres, particles, and films were not considered in our results, as they could originate from textiles from the beekeepers or the exoskeletons of insects (Mühlschlegel et al., 2017). Our study detected microplastics (MPs), including fibres, fragments, and films, at all locations and throughout all monitoring campaigns using APITrap devices. Interestingly, results varied when using honeybees or pollen for microplastic monitoring.

Fibres were the most commonly found, the occurrence of fragments and films showed significant variation based on location and time period, both in honeybees and in pollen samples. Fig. 2 presents a summary of the distribution and average number of MPs detected across four sampling rounds, categorized by shape in APITrap (A), honeybees (B), and pollen (C). Fibres were the predominant shape detected in all samples, with fragments and films following. Specifically, in the APITrap, fibres, fragments, and films had detection frequencies of 91%, 5%, and 4% of the total MPs detected, respectively. In contrast, the proportion of fibres in honeybees and pollen was lower than in APITrap, at 55% and 76% respectively, while fragmentraised detected values of 30% in honeybees and 22% in pollen. The data found in APITrap were in line with previous scientific works. Thus, approximately 90% of the airborne MPs detected were in form of fibres, similarly to results reported by Dris et al. (2017); Li et al. (2020). However, our results were different from those reported in a recent study in Denmark. In our study, we found that fibres were the most frequently detected form of MPs (55%) in honeybees, followed by fragments (30%), whereas in the work published by Edo et al. (2021), the dominant forms of MPs in honeybees were fragments (52%) followed by fibres (38%). While it is true that the data differ somewhat, it must be taken into account that the sampling points were different and in the case of the work previously published in 2021 part or most of the points were urban.

Important to note was different the relative standard deviation (R.S.D, %) obtained over the four sampling periods. In the case of fibres, the R.S.D ranged from 10% to 38% in APITrap, with an average of 27%. For honeybees, this range was between 35% and 124%, averaging at 76%, and for pollen, it was between 8% and 60%, with an average of 35%. The variability of values for fragments and films were higher than those for fibres. This increase is attributable at least in part to the lower count of these items, where even minor variations between data sets significantly amplify the percentage change. The average R.S.D values observed for fragments and films were 52% and 40% in APITrap, 113% and 145% in honeybees, and 113% and 143% in pollen, respectively. These findings indicate a much better reproducibility of the APITrap sampler results compared to results of honeybee and pollen samples that only are snapshoot procedures. These results are in agreement with the consideration of the stability of flow at the interior of bee hives and not consider high variation in pollution effects on the selected sampling points. For more detailed information on the presence and distribution of the detected MPs across various matrices, apiaries, and periods, please refer to the supplementary material (Fig. S1).

Microplastics were classified into five colours. Overall, the proportion of them was different according to the shape, the sampling method used or the sampling sites, except to fibres, which presented no significant variation between apiaries or sample type (see Fig. 2). Black and blue were the dominant colours of the microfibres detected in all cases, with average percentages of 67% and 23% in APITrap, 40% and 35% in honeybees, and 51% and 32% in pollen, respectively. In addition to these colours, green, red, and translucent fibres were also observed in all samples. Fragments were mainly blue and green in APITrap and honeybees (between 32% and 51% blue and 21%–15% green), followed by red and black fragments (approx. 15%). Red and green were the dominant colours of fragments observed in pollen, accounting for 43% and 31% of all items observed, respectively. Blue and translucent were the main colours of the films found. The APITrap sampler was the only one in which translucent fragments and red films were detected.

Table 1 summarized the number, size, roundness, and area of MPs detected over the four sampling rounds according to their shape in (A) APITrap, (B) honeybees, and (C) pollen. Fibres found in the APItrap ranged in length from 59 to 887 μm, with a mean of 384 μm, while the

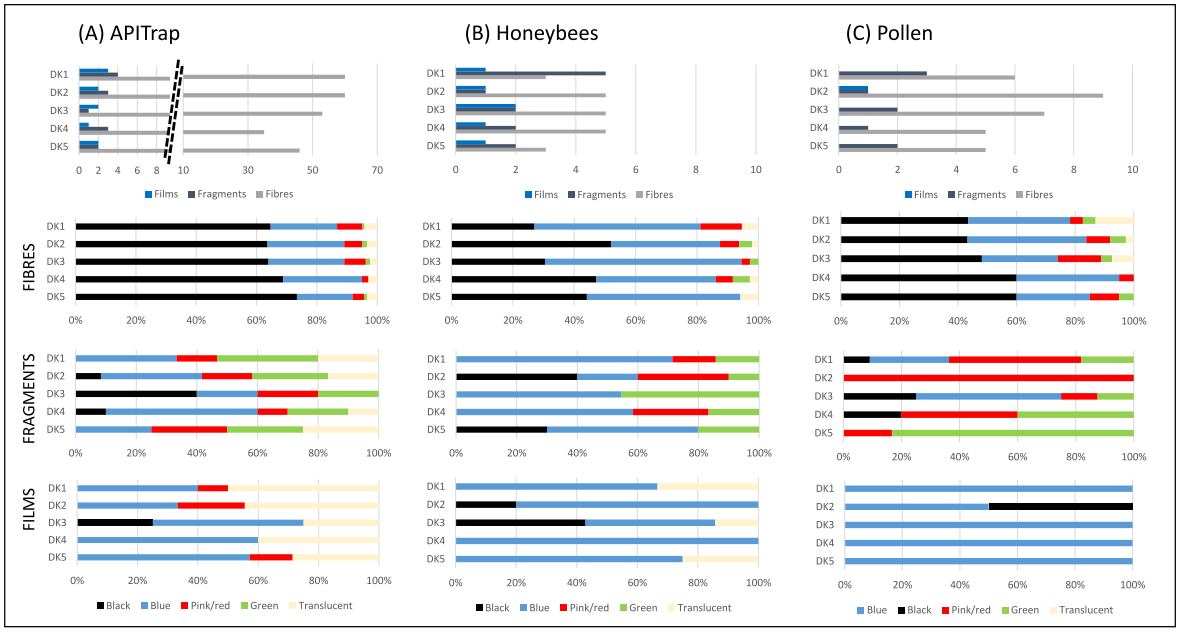

Fig. 2. Distribution, colour and average number of MPs detected over four monitoring studies according to their shape in (A) APITrap, (B) honeybees, and (C) pollen. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Number, size, roundness, and area of MPs detected over four monitoring studies according to their shape in (A) APITrap, (B) honeybees, and (C) pollen.

	DK1			DK2			DK3			DK4			DK5		
	Fibres	Fragments	Films	Fibres	Fragments	Films	Fibres	Fragments	Films	Fibres	Fragments	Films	Fibres	Fragments	Films
APITRAP															
Items[a]	47-72 **(60)**	1-5 **(4)**	2-3 **(3)**	53-66 **(60)**	2-4 **(3)**	1-3 **(2)**	41-83 **(53)**	1-2 **(1)**	1-3 **(2)**	28-51 **(35)**	1-5 **(3)**	1-2 **(1)**	28-65 **(46)**	1-4 **(2)**	1-3 **(2)**
Length (μm)[a]	59-699 **(358)**	51-209 **(118)**	148-1237 **(502)**	58-788 **(346)**	40-251 **(113)**	108-743 **(328)**	84-887 **(442)**	48-250 **(106)**	168-751 **(350)**	78-634 **(366)**	37-90 **(65)**	176-314 **(218)**	64-711 **(409)**	79-185 **(117)**	162-1073 **(445)**
Average Roundness	0.04	0.21	0.06	0.03	0.22	0.04	0.04	0.20	0.05	0.05	0.21	0.05	0.05	0.18	0.07
Average Area (μm^2)	7917	5822	7995	7757	4102	8972	9249	4052	7549	7979	4032	8785	8933	6033	8149
Total Area (μm^2)	475031	23289	23984	465394	12305	17943	490187	4052	15097	279258	12095	8785	410925	12067	16298
Total (mm^2)	0.52			0.49			0.51			0.30			0.44		
HONEYBEES – 5 g															
Items[a]	0-7 **(3)**	2-8 **(5)**	0-2 **(1)**	1-9 **(5)**	0-2 **(1)**	0-3 **(1)**	0-9 **(5)**	0-3 **(2)**	0-5 **(2)**	0-7 **(5)**	0-5 **(2)**	0-3 **(1)**	2-4 **(3)**	0-6 **(2)**	0-3 **(1)**
Length (μm)[a]	79-911 **(415)**	31-57 **(44)**	270-801 **(457)**	73-612 **(367)**	46-76 **(63)**	185-366 **(268)**	88-778 **(389)**	27-73 **(50)**	144-767 **(390)**	72-542 **(356)**	53-314 **(115)**	223-273 **(256)**	81-588 **(406)**	37-165 **(79)**	300-784 **(445)**
Average Roundness	0.10	0.20	0.01	0.01	0.31	0.10	0.10	0.22	0.01	0.01	0.21	0.10	0.10	0.22	0.10
Average Area (μm^2)	6123	4336	5688	5998	5011	5788	6812	4988	5815	6542	4124	5477	5989	3812	5476
Total Area (μm^2)	18369	21680	5688	29990	5011	5788	34060	9976	11630	32710	8248	5477	17967	7624	5476
Total (mm^2)	0.05			0.04			0.05			0.05			0.03		
POLLEN – 5 g															
Items[a]	1-8 **(6)**	0-4 **(3)**	0-1 **(0)**	8-10 **(9)**	0-1 **(1)**	0-1 **(1)**	6-8 **(7)**	0-5 **(2)**	0-1 **(0)**	2-6 **(5)**	0-3 **(1)**	0-1 **(0)**	2-9 **(5)**	0-5 **(2)**	–
Length (μm)[a]	110-875 **(486)**	20-106 **(50)**	322 **(322)**	124-678 **(419)**	82 **(82)**	276 **(276)**	197-766 **(453)**	54-55 **(55)**	316 **(316)**	206-601 **(426)**	63 **(63)**	412 **(412)**	102-537 **(392)**	61-75 **(68)**	–
Average Roundness	0.06	0.31	0.07	0.04	0.24	0.05	0.06	0.22	0.02	0.06	0.31	0.08	0.06	0.28	–
Average Area (μm^2)	9433	4026	8876	8523	4762	6706	9016	3931	8789	8730	4364	8059	7543	4499	–
Total Area (μm^2)	56595	12078	8876	76707	4762	6706	63112	7862	8789	43650	4364	8059	37714	8998	–
Total (mm^2)	0.08			0.09			0.08			0.06			0.05		

[a] Minimum-Maximum (average).

length of fragments and films ranged from 37 to 251 μm (average 104 μm) and from 108 to 1237 μm (average 368 μm), respectively. The length of fibres identified in honeybees ranged from a minimum of 72 μm to a maximum of 911 μm, with a mean size of 387 μm, while the length of fragments and films ranged from 27 to 314 μm (mean 72 μm) and 144–801 μm (mean 363 μm), respectively. Pollen contained fibres ranging in size from 102 to 875 μm (average 435 μm), fragments from 20 to 106 μm (average 64 μm) and films from 276 to 412 μm (average 332 μm). Thus, no significant differences in the sizes of fibres, fragments and films were found comparing APITrap, honeybees and pollen. It should be noted, however, that the quantity of MPs detected varied based on the sampling tool utilized. On average, the number of MPs identified over four sampling rounds ranged from 39 to 67 items in APITrap, from 6 to 9 in 5 g of honeybees, and from 6 to 11 in 5 g of pollen samples. Although fibres were much more numerous than fragments or films, the latter have a substantial surface area, making their contribution noteworthy. This relevance is consequence of the plastic particles can initiate effects through different mechanisms. For instance, based on available data on the characteristics of microplastic particles such as particle surface area, specific surface area, or particle volume, a probability density function (PDF) can be defined from an ecological or toxicological perspective (Koelmans et al., 2022). Furthermore, the surface area of MPs plays a crucial role in their interaction with organic compounds. A larger surface area provides more potential binding sites for compound adsorption onto MPs, therefore greater adsorption is expected (Moura et al., 2023). Consequently, the quantification of the surface area of MPs can provide valuable insights into the impact of plastic pollution on ecosystems and human health as well as to assess the environmental impact of plastic pollution. Over the complete sampling period, the average surface areas of fibres, fragments, and films did not differ greatly, with average values in APITrap being 8367 (±604) μm^2 for fibres, 4808 (±917) μm^2 for fragments, and 8290 (±523) μm^2 for films. Honeybees and pollen samples showed similar values, with average areas of 6293 (±329) μm^2 and 8649 (±632) μm^2 for fibres, 4454 (±475) μm^2 and 4316 (±306) μm^2 for fragments, and 5649 (±147) μm^2 and 8108 (±869) μm^2 for films, respectively (see Table 1).

Current scientific research indicates that the presence of atmospheric microplastic pollution in a region is often closely linked to human activities, anthropogenic factors, population density, and the level of industrialization (González-Pleiter et al., 2021; Liu et al., 2019a). Recent studies have shown that microplastics can ascend to high altitudes and be carried over long distances by wind, eventually reaching remote areas (Brahney et al., 2020). Consequently, microplastic concentrations in rural or sparsely populated areas, which may appear to be less affected by anthropogenic emissions, may become like those in urban or industrial areas, as it was observed by Edo et al. (2021). The results of our study support this claim, where the variations in the quantity and total load of MPs found between rural and urban environments were less than 40%. Several factors, including transport, dispersion, and deposition mechanisms, influenced the movement of airborne MPs in the atmosphere. Previous studies have reported that MPs could come from synthetic textile, chopping, shredding or degradation of industrial synthetics macroplastics, tyre wear, waste incineration or agricultural activities (Ahmad et al., 2023; Li et al., 2020). Table 1 illustrates the number of MPs detected using the APITrap together with the geographical distribution. The lowest amount was found in location 4, a wilderness area, with 39 MPs having a total surface of 0.30 mm^2. Locations 1 and 2, characterized by large agricultural influence and situated 7 km from urban centres, exhibited total MPs surface of 0.49 mm^2 and 0.52 mm^2, respectively. In coastal residential areas, represented by locations 3 and 5, the total MPs surface extracted from the APITrap comprised 0.51 mm^2 and 0.44 mm^2, respectively. Consequently, samples from rural areas close to population centres collected using the APITrap showed a number and concentration of MPs like those in urban areas, with ranges from 50 to 67 MPs and total load of MPs between 0.44 mm^2 and 0.52 mm^2. Analysis of honeybees and pollen yielded comparable results, though the total surface areas of MPs found, were smaller: 0.03 mm^2–0.05 mm^2 using honeybees and 0.05 mm^2–0.09 mm^2 with the pollen sampler, respectively.

The sizes of the microplastics (MPs) were categorized into three ranges: less than 100 μm, 100–500 μm, and greater than 500 μm. Most fibre lengths were in the 100–500 μm range, comprising 48%, 60%, and 64% of all items in the APITrap, honeybees, and pollen samples, respectively. This was followed by fibres larger than 500 μm, which accounted for approximately 35% in all cases. For fragments, those smaller than 100 μm constituted 66%, 88%, and 93% of all items in APITrap, honeybees, and pollen, respectively. Films were predominantly in the 100–500 μm range, representing 74% of the total films detected in APITrap, 83% in honeybees, and 60% in pollen. Consequently, the size distribution of MPs based on length did not show clear variations across different hives and sample types. For additional details on the size distribution of microfibres and microparticles in each matrix, refer to the supplementary material (Fig. S2).

3.2. Chemical composition of observed MPs

Fibres, films with colour, and fragment-like particles were selected for analysis using micro-Fourier Transform Infrared Spectroscopy (μ-FTIR). Through comparison with an infrared database library, we identified up to five distinct synthetic polymers and three types of natural or regenerated materials. The primary synthetic polymers detected were polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polyacrylonitrile (PAN), and polyamide (PA). Among the natural or regenerated materials, cotton, cellulose, and rayon (RY) were found. Fig. 3 displays the average percentage abundance of the different types of microplastics (MPs), categorized by their shape and the sampling method used. Overall, the proportion of microplastics varied with the sampling method used. However, when comparing the same sampler across different sampling locations, no significant differences were observed in fibres. Previous studies, such as Dris et al. (2016), have documented the presence of fibres in natural environments, noting that approximately 30% of these fibres are synthetic. In our study, polyethylene terephthalate (PET) was the most commonly detected synthetic polymer in APITrap samples. Its average abundance varied, ranging from 15% to 31% in fibres, 33%–63% in fragments, and 38%–71% in films as well as the last percentages are affected by the low number of detections. Polypropylene (PP) followed, with consistent values around 15% across all shapes. Polyacrylonitrile (PAN) fibres, commonly used in the textile industry for their soft texture, easy dyeing, and resistance to light (Liu et al., 2019a), were infrequently observed, constituting only 5% of the total synthetic polymers in APITrap. Polyethylene (PE) fragments were detected exclusively in one apiary.

The APITrap also revealed the presence of natural or regenerated microplastics displaying non-natural colours, such as blue, black, or red. As illustrated in Fig. 3, most of the coloured fibres detected were identified as cellulose-based materials, with an average of 42%. This was followed by rayon (RY) at an average of 7%, and cotton at around 3%. Additionally, approximately 26% of the fragments and 29% of the films were identified as cellulose. RY and cotton, however, were less commonly observed, constituting less than 4% of all films and fragments detected in APITrap. The presence of these materials, often associated with textile or industrial sources, also signifies anthropogenic pollution, aligning with findings from other researchers such as González-Pleiter et al. (2021). In honeybees, PET was the dominant synthetic fibre detected in all analyzed samples, accounted for 32% of all items observed, followed by PP (7%), PAN (6%), and PE (3%). Again, cellulose was the majority natural fibre, with an average abundance of 45%, followed by cotton (6%). The fragments were mainly PET (48%), followed by PP (30%), and PE (17%), while films were mainly PP (94%) in honeybees.

Cellulose comprised nearly half (43%) of the fibres found in pollen samples, followed by PET, PP and PE with average abundance values of

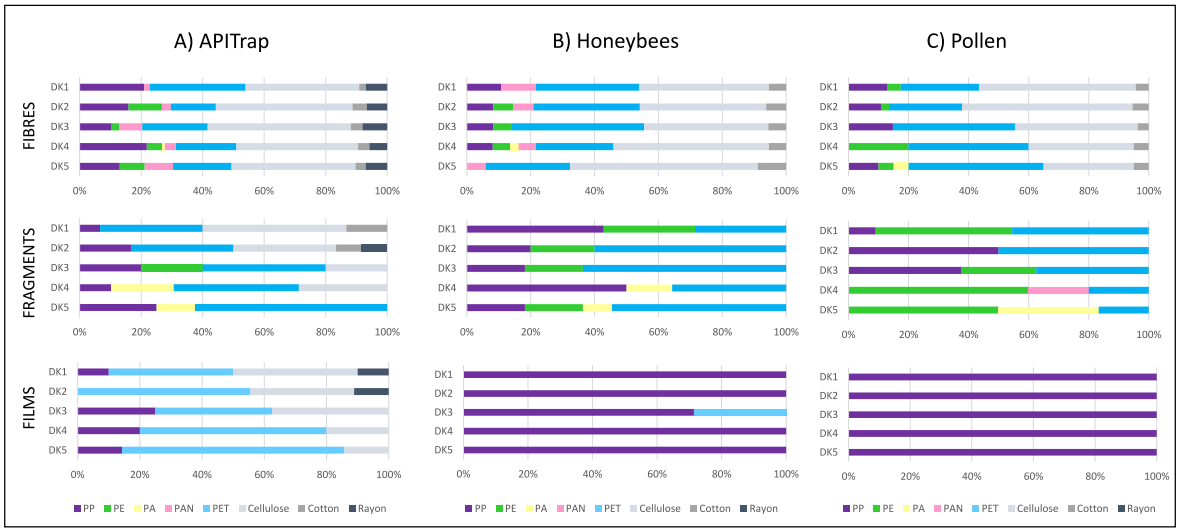

Fig. 3. Average abundance (%) of the different types of MPs identified over four monitoring studies in APITrap, honeybees and pollen according to the shape.

35%, 10%, and 6%, respectively. Cotton fibres were also observed, accounted for 5% of the total of microparticles detected. Fragments were identified as PE, PET, PP, PA, and PAN accounting for 36%, 34%,19%, 7% and 4% of the total items observed in pollen, respectively. All films were identified as PP. As can be seen in Fig. 3, no natural or regenerated materials were identified in any fragments or films found on honeybees or pollen samples.

Overall, the MPs recovered from agricultural or rural areas (location 1, 2 and 3) showed a chemical composition not too different from those subject to higher urban/industrial pressure (location 4 and 5). Our results revealed that PET was the most frequently observed synthetic polymer in both fibres, fragments, and films and in all sampling methods. The only exception were microfilms found in honeybees and pollen, in which PP was the main synthetic polymer identified. PET is commonly used in the manufacturing of beverage, food, and various product packaging, but also in the textile industry to produce synthetic fibres and fabrics. Its versatility, transparency, strength, and recyclability make PET a widely used material in various industrial and consumer applications. The chemical composition of the MPs observed in this work are in line with other studies on atmospheric MPs in suspension. Liu et al. (2019a) reported that PET, PE, and PAN were the mainly synthetic polymer fibres detected in Shanghai air samples. In another recent work carried out in Denmark, Edo et al., 2021 reported that polyester (PET) was the most frequently detected synthetic polymer in honeybees, similar to our findings.

Finally, approximately half of the total fibres identified by the three sampling methods assessed were composed of cellulose, with average abundance values ranged from 42% to 45%.

To identify microplastic particles with maximum reliability, the obtained spectra were compared to a comprehensive polymer and non-polymeric material libraries. Examples of images and μFTIR spectra of some of the most frequently detected MPs in the samples are presented in Fig. 4. Spectral matches obtained from spectra libraries were also included. In all cases, the matching was higher than 75%. It is important to note that the identification of MPs is a complex process, especially the spectral data interpretation. Blends of polymeric materials are common in industry to improve specific properties or combine desirable characteristics of different polymers. In addition, the degradation of MPs is

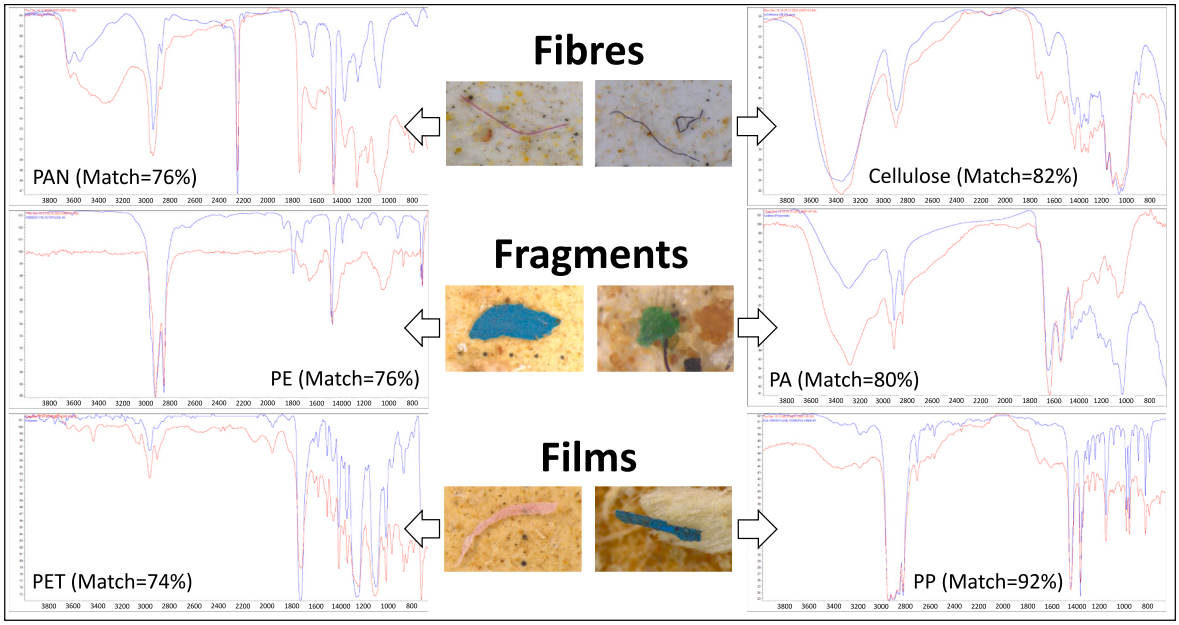

Fig. 4. Examples of micro-FTIR spectra and images of the most representative MPs identified in this work.

closely related to environmental conditions and therefore their physic-chemical properties and their characteristic spectrum. Uheida et al. (2021) reported that increased light exposure to MPs continuously increased their carbonyl (C═O) absorption. In other recent work, the authors found that PET, PP and PE exhibited significant variations in the characteristic peaks of the FTIR spectrum, suggesting possible degradation and proliferation of surface functional groups, as well as the formation of new characteristic peaks due to environmental exposure (Hsu et al., 2024). Some details about the characteristics bands of each material identified are comment below. Some of the characteristic bands in the IR spectrum of PET observed were C═O stretch band (1710-1735 cm^{-1}), C–O–C stretch band (1260-1240 cm^{-1}), C–H stretch band in the aromatic ring (3100-3000 cm^{-1}) and the C–H bending band in the aromatic ring (860-700 cm^{-1}). In the IR spectrum of PP were observed the aliphatic asymmetric and symmetric C–H stretch band (2990-2960 cm^{-1} and 2860-2840 cm^{-1}, respectively), C═C stretch band in unsaturated polypropylene (977 cm^{-1}) and the C–H strain band in isotactic polypropylene (1375 cm^{-1}). The PAN polymer spectrum showed the C≡N (nitrile) stretch band (2240-2260 cm^{-1}), C–H stretching band in methyl and methylene groups (2950-2850 cm^{-1}), C═C stretching band in the aromatic ring (1600-1580 cm^{-1}) and the C═N stretching band in the aromatic ring1 (1490-1465 cm^{-1}). Some of the characteristic bands of the PA's spectrum found were the amide I band (C═O stretching) (1650-1630 cm^{-1}), amide band II (N–H deformation and C–N stretching) (1550-1540 cm^{-1}) and the C–H stretching band in methyl and methylene groups (2980-2940 cm^{-1}). PE showed the following bands due to its specific molecular vibrations: aliphatic asymmetric and symmetric C–H stretch band (2917-2852 cm^{-1} and 1470-1462 cm^{-1}, respectively) and the C–C stretch band in the polyethylene skeleton (1462-1436 cm^{-1}). C–O–C stretch band (cellulose and glucose groups, 1100-1150 cm^{-1}), C–H stretch band in methyl and methylene groups (2900-2800 cm^{-1}) and the O–H stretching band (3300-3400 cm^{-1}) characteristic of cellulose-based materials were also found.

4. Conclusion

The newly developed non-invasive passive sampler has shown good performance in evaluating MPs pollution through bee colonies. Its effectiveness is further enhanced by the ability to extend the sampling period, thus avoiding the limitations associated with collecting bees or pollen. The study's results indicated a higher level of reproducibility when using the APITrap compared to honeybees or pollen for monitoring MPs. For APITrap, the variation of results within the same apiary was less pronounced compared to other procedures studied. Fibres had values of less than 40% (average 27%), fragments had 71% (average 52%), and films had 55% (average 40%). These higher last two values are acceptable considering the low total number collected. No significant differences were observed in the total number of MPs across various sampled locations, probably due to the similar proximity of the populated areas in all cases. Additionally, a wider range of polymer types was detected using APITrap compared to that using the other sampling procedures.

The proportion of MPs detected varied depending on their shape, sampling method, and the location, except for fibres, which showed no significant variations. Predominantly, polyethylene terephthalate (PET) was the synthetic polymer that was detected most frequently in all instances, followed by polypropylene (PP) and polyethylene (PE). Polyacrylonitrile (PAN) was identified solely in fibres. Most of the coloured fibres were found to be natural-based materials. Furthermore, in this study, we developed a straightforward methodology for extracting MPs from honeybees and, for the first time, from pollen samples. This procedure avoids the use of oxidizing agents and lengthy processes for digesting organic matter, thus reducing the risk of sample contamination. These results cannot be directly applied to bee contamination as the relationships for this are not yet established; however, further experimental work could potentially make this possible.

CRediT authorship contribution statement

Laura Cortés-Corrales: Writing – original draft, Methodology, Investigation. **Jose Javier Flores:** Methodology, Investigation. **Adrian Rosa:** Methodology, Investigation. **Jozef J.M. Van der Steen:** Methodology, Investigation, Funding acquisition, Conceptualization. **Flemming Vejsnæs:** Methodology, Investigation, Conceptualization. **Ivo Roessink:** Writing – review & editing, Methodology. **Maria Jesús Martínez-Bueno:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Amadeo R. Fernández-Alba:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124046.

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