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Microplastic contamination in the agri-food chain: The case of honeybees and beehive products

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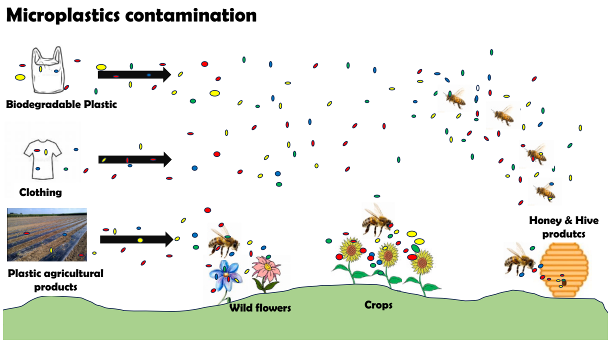
HIGHLIGHTS

- Honeybees are able to capture microplastics and microfibers from air and to transfer them to honey.
- Microplastics and microfibers are present as air contaminants in high and low urbanized areas.
- Natural, artificial and synthetic microfibers are the main contaminants of honeybees and honey.
- The occurrence of PE based microplastics in honey samples reflect the PE usage in agriculture.
- The detection of PCL microparticles in honey alerts on the management of biodegradable materials.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics, MPs, plastic fragments with a dimension lower than 5 mm, and microfibers, MFs, synthetic and natural/artificial fibrous fragments with a diameter lower than 50 μm, are ubiquitous pollutants identified in different environmental compartments. In this work the occurrence of MPs and MFs on honeybees, *Apis mellifera*, and beehive products was evaluated, using Fourier transform infrared microspectroscopy, confirming that MPs and MFs are widely present as air contaminants in all the apiary's areas (high and low urbanized areas) in Southern Italy.

Results indicated that independently from the site, both honeybees and honey samples, are contaminated by MFs with non-natural color. The majority of MFs were of natural origin followed by artificial MFs and synthetic MFs. Moreover, the chemical composition of MFs isolated from honeybees reflect that used in synthetic fabrics,

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[1] These authors share first authorship M.E. Schiano contributed to microparticles isolation and identification, L.J. D'Auria contributed to sampling.

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leading to the hypothesis that they are released from textile to air where are captured by bees. Results highlight that MFs represent a class of ubiquitous airborne anthropogenic pollutants. The identification of polytetrafluoroethylene, PTFE, MPs in honeybees confirm the recent findings that PTFE MPs are diffuse soil and air contaminants while the identification of polyethylene, PE, based MPs in honey samples, from low density urban sites, could be correlated to the large use of PE in agriculture. In the honey samples, also polycaprolactone, PCL, MPs were identified, mainly in high density urban sites, confirming that biodegradable materials could be further pollutants in the environments. The results indicate that honeybees are contaminated by MPs and MFs during their flights or picking up from the hive components, flowers, from other nest mates, from the clothes of the beekeeper, among others and some of them could be transferred to honey samples that could be also affected by soil contamination.

1. Introduction

Microplastics (MPs) accumulation in the environment is currently of great concern due to the negative impact on the ecosystems (Li et al., 2024; Du et al., 2021; Yu et al., 2022). Several authors have published the results of their investigations assessing the distribution and composition of MPs in aquatic environments (Enders et al., 2015; Shim et al., 2018; Yan et al., 2019; He et al., 2020), on identifying biological indicators to assess MP levels in aquatic environments (Palazzo et al., 2021; Alfonso et al., 2023; Wang et al., 2024) and on the effects of MPs on aquatic organisms (Biamis et al., 2021. Hartmann et al., 2017;. Huang et al., 2021).

Beginning with the initial isolation and identification of MPs as atmospheric particulates in France in 2015 (Dris et al., 2015), studies regarding MPs in the atmosphere has grown and escalated in interest over recent years (Liu et al., 2020). These studies have revealed the presence of MPs and particularly MPs of fibrous shape released from textiles (i.e microfibers) in the atmospheric fallout of urban and remote areas (Allen et al., 2019; Roblin et al., 2020) as well as indoor environments (Valdiviezo-Gonzales et al., 2024; De Falco et al., 2020). MPs released from synthetic fabrics have been identified as the main contributor to primary MP in oceans (Boucher and Friot, 2017). Textile fibers are classified in natural, fiber of animal, vegetable, or mineral source, and man-made fibers divided in artificial and synthetic fibers (J Wilson, 2001). Artificial fibers, such as viscose, are obtained from natural products through different physical and chemical treatments. Synthetic fibers are obtained from the spinning of synthetic polymers. In relation to fiber fragments, the term MPs of fibrous shape is referred to synthetic microfiber, representing the most abundant form of MPs found in environmental samples (Avio et al., 2020). However, the occurrence of natural MFs, such as cotton, and artificial MFs, such as rayon, was widely reported in literature (Mateos-Cárdenas et al., 2021). In this paper, the term microfibers, MFs, is used to indicate synthetic and natural/artificial fibrous fragments with a diameter lower than 50 μm, and length ranging from 1 μm to 5 mm (Li et al., 2023).

MPs can be transported across different environmental compartments such as oceans, air, land, freshwater and sediments (Rillig and Lehmann, 1979; Xiao et al., 2023). These pollutants can reach soil environment through agricultural activities involving the usage of plastic mulching, sludge application, sewage irrigation (Briassoulis, 2023) and/or the process of atmospheric deposition (Zhu et al., 2023). In general, their diffusion routes are interconnected and not exclusive (Zhu et al., 2023).

Notwithstanding MPs are ubiquitous in all the environmental compartments and ecosystems their effects to humans and other animals are still not well known (Vethaak and Legler, 2021; Prata et al., 2021).

To contribute to clarify the risks related to MPs presence in the environments it is fundamental to evaluate the occurrence and distribution of MPs in urban and remote environments and to identify their accumulation in living organisms and foods. In this respect, living organisms have been proven to be effective indicators of environmental pollution. Several studies have investigated the occurrence of MPs on insects as on honeybee *Apis mellifera* (Buteler et al., 2022a, 2022b; Wang et al., 2021; Deng et al., 2021; Edo et al., 2021).

The honeybee is a very good biological indicator of environmental contamination because it is ubiquitous, covered by hairs that capture contaminants and particles present in the air, sensitive to pollutants, and has great mobility and wide flying range, among others (Porrini et al., 2003).

Honeybees could transfer contaminants to honey, pollen and wax for human consumption that could thus entering the food web. They have been used as sentinel species of environmental contamination, particularly by heavy metals and pesticides (Porrini et al., 2016). In fact, they are able to cover a large area for the pollination and to accumulate environmental pollutants on body surface due to their body characteristics. Indeed, during flight, their body surface becomes positively charged with static electricity, so that, like pollen particles, substances or other microparticles in the environment are retained on their hairs and bristles (Negri et al., 2015). MPs have been found in commercial honey (Diaz-Basantes et al., 2020), in inflorescences of different species (Liebezeit and Liebezeit, 2015) and on honeybees (Edo et al., 2021). Most recently, Buteler et al. (Buteler et al., 2022b) showed honeybees are able to incorporate MPs from the environment and transport them to different matrices in the hive. Therefore, honeybees and beehive products could represent a mirror for contamination by MPs of the environment in which they live.

The main objectives of this work were to determine whether (1) MPs and MFs are contaminants for honeybees, *Apis mellifera,* and these pollutants are present into other apiary matrices and if (2) the contamination is influenced by sampling sites. For this purpose, in this work, we evaluate the occurrence of MPs on honeybees and beehive products to monitor and assess the MP air contamination in the apiary's areas. 10 sampling sites in Campania region, Sothern Italy, have been identified as representing site of high and low urbanized areas. From each site, honeybees, honey and pollen samples have been recovered. Then, isolation and chemical identification of micro-fragments have been performed using Fourier transform infrared spectroscopy (FTIR). Results have indicated that honey samples are contaminated by microplastics with different shape including fragments and MFs. Apart from synthetic MFs, MFs of both natural and artificial origin have been found with no natural colors indicating the widespread dispersion of MFs in the air. Furthermore, the results show that the presence of MPs and MFs in honey samples could be due to the contamination induced by the transfer of these pollutants from bees, but also by soil contamination since certain type of polymers are found as soil contaminants. Due to the different type of polymers and the different MP sources, an unambiguous conclusion cannot be performed. For the first time MPs derived from polycaprolactone, PCL, have been identified as contaminant of agri-food products.

2. Materials and method

2.1. Materials

Potassium hydroxide, KOH, was purchased from Carlo Erba. Hydrogen Peroxide solution 30 % solution, H_2O_2, was purchased from Merck. Nylon filters with 20 and 10 μm pore size and 47 mm diameter were purchased from Merck Millipore. Macroporous silicon filters with

5 μm pore size, MakroPor, was purchased from Thermo Fisher Scientific.

2.2. Site selection for sampling

An environmental index was specifically developed to accurately identify the most representative and significant sampling locations in Southern Italy within the Campania region and it was used to select sampling sites. The creation of the index was carried out through an approach based on the Analytic Hierarchy Process (AHP), a multicriteria decision-making methodology developed by Thomas L. Saaty (Saaty, 1990). This method was chosen for its ability to handle decision complexity involving multiple criteria and subcriteria, allowing for a structured and weighted evaluation of the various environmental variables relevant to beekeeping and the interaction of bees with microplastics. The environmental index is structured into five risk classes, each associated with a numerical value ranging from a low-risk level (with a value of 1), to a high-risk level (with a value of 5). These classes allow for a precise graduation of environmental risk levels and provide a clear picture of the environmental conditions present in the various sampling sites. The index has been designed considering several environmental factors relevant to beekeeping and the interaction of bees with microplastics, in order to ensure accurate selection of study sites and to assess the degree of environmental risk in different areas. A set of key indicators, related to land use, air quality, surface water quality, and potential soil hazards, were used and each factor was also weighted in terms of its capacity to influence the surrounding environment and ecosystems. In particular, land use has been considered a critical factor as it can directly affect the availability of food resources for bees and the presence of potential sources of pollution; the Corine Land Cover data were used to create an index where different land use types were classified with numerical values on a 9-point scale. This classification, based on the main impacts on bees and beekeeping products, was developed using the Delphi method by consulting a team of experts. Air quality has been assessed in relation to the presence of pollutants that could be harmful to bees and other organisms in the environment using products from the European Copernicus Earth Observation program. Surface water quality has been evaluated based on the presence of contaminants that could contaminate the pollen and honey produced by bees using geostatistical techniques applied to water sampling data provided by the Regional Agency for Environmental Protection of Campania (ARPAC). Finally, soil hazard has been considered in relation to the presence of toxic or contaminating substances in the soil that could affect the health and well-being of honeybees was made using topsoil data and geostatistical approach (Signorelli et al., 2023). To facilitate a deeper understanding of the environmental impact, the selected areas were divided into two distinct categories: those with a high environmental impact and those with a low environmental impact.

2.3. Sampling

The sampling of different matrices (honeybees, honey, and pollen) was conducted during the nectar storage phase in the beekeeping seasons (spring-summer) of 2022 and 2023, allowing for the simultaneous collection of honey and pollen samples to comprehensively assess the surrounding environment. Honeybees were captured using special cages, commonly known as under basket cages, which allow for the safe and efficient sample collection without harming the insects. The under-basket cages are used to study and assess bee mortality inside and around the hive. The primary function of under-basket cages is to collect dead bees that fall from the hive. This allows for systematic monitoring of bee mortality and gathering precise data on the number of dead bees over a specific period. Additionally, they enable long-term monitoring of hive health, aiding in identifying potential issues such as diseases, parasites, pesticide exposure, or emerging contaminants. Honey was collected directly from the combs inside the beehives, ensuring maximum freshness and integrity of the sample. The honey samples were extracted directly from the hive frames and subsequently stored in glass containers. The decision to extract honey directly from the frames was made to preserve the honey's composition and to minimize potential accidental contaminations that could occur during the extraction process of honey. This was necessary to demonstrate that any contamination from microplastics was solely attributable to environmental pollution rather than the extraction process. Moreover, the garments and materials used in the monitored apiaries did not contain plastic or synthetic substances. Similarly, pollen was obtained by using pollen traps, a devices used in beekeeping to collect pollen from bees as they return to the hive. Installed at the hive entrance, these traps feature a metal grid that forces bees entering the hive to pass through, removing pollen from their hind legs and collecting it in a container below. A total of 10 sampling sites were selected across the Campania region. Five of these sites were located in areas considered to have low to medium-low environmental impact (LIA), while the remaining five sites were chosen in areas with medium to high environmental impact (HIA). This balanced distribution allowed for covering a wide range of environmental conditions and assessing the interaction between bees and microplastics in diverse and representative contexts of the region. The complete list of apiaries and their respective locations was thoroughly documented and presented through Fig. 1. to provide a clear and comprehensive overview of the sampling sites. In compliance with the provisions of the GDPR (General Data Protection Regulation), to ensure the security and privacy of sensitive information, advanced geomasking techniques have been adopted for representing sampling points on maps.

This approach ensured a complete and representative collection of all three matrices, enabling accurate and exhaustive analysis of their characteristics and compositions. The samples were placed directly into 500 mL glass jars, previously labeled and carefully sealed in bags to prevent any external contamination. Special attention was given to freezing the honeybees directly in the jars, without the addition of any solution, in order to preserve the quality of the samples and prevent any plastic degradation or microbial growth phenomena. The transportation of the samples was carried out with the utmost speed and care, maintaining a constant storage temperature of −20 °C to ensure their integrity.

2.4. Matrix inspection

For each apiary honeybees and pollens were observed under optical microscopy using a LEICA M205C light microscope, equipped with a camera and ImageFocus 4 software, with magnification ranging from 0.78× to 16× (Leica Corporation, Germany).

In detail, for each apiary, 9 honeybees, and 5 g of pollens were observed, for a total of 84 honeybees and 50 g of pollens. All particles of size <5 mm along their largest dimension were observed and classified according to morphological characteristics: size, shape and color, using the Leica microscope. The observed particles were classified into fragments and MFs. Fragments were defined as particles with irregular shape and edges, probably originating from the fragmentation of larger particles. Particles characterized by a major dimension (length) significantly larger than the second largest dimension of the projected area (width) were classified as MFs. The acquired micrographs were processed with ImageJ software to calculate particle dimensions (length and width) (Volgare et al., 2022a; Volgare et al., 2022b).

2.5. Honeybees processing

For each sampling site area 30 defrosted honeybees were taken and placed in glass beakers. All the 30 honeybees from the same sample were washed together using 30 mL of a 10 % KOH solution. The honeybees were left in contact with the solution for 5 min and then removed, after meticulous shaking of each body inside the solution. This approach allowed the material to be recovered from the honeybees' bodies without

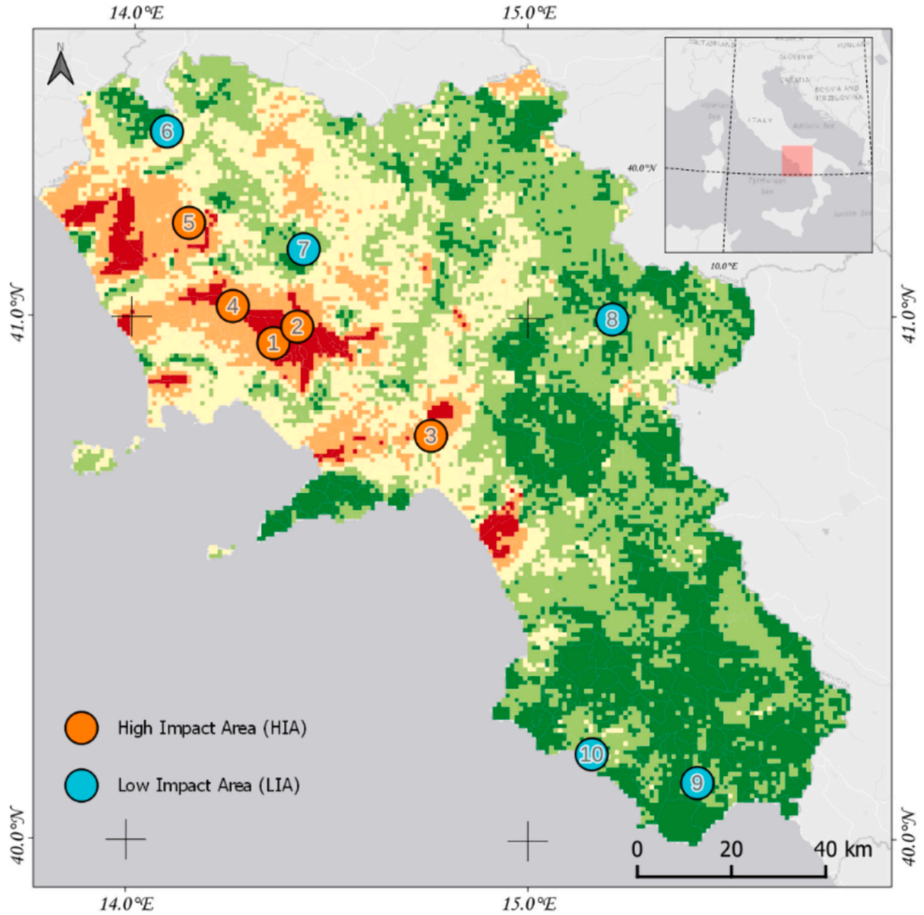

Fig. 1. Representation of the sample points overlaid on the index.

affecting their integrity. The resulting suspension was stirred at 100 rpm and heated at 50 °C for 15 min. Then, 10 mL of 30 % H_20_2 was added to the dispersion and stirred at 200 rpm and 50 °C overnight. The dispersion was filtered under vacuum through 10 μm nylon filter. The filter was placed in a glass beaker with 10 mL of MilliQ water and sonicated using a Branson 2210R-MT Ultrasonic cleaner bath (Branson UltrasonicsCorp., Connecticut, USA), at a frequency of 40 kHz for 30 min. By means of this procedure, the MPs deposited on the surface of the nylon filter were recovered, resulting in a suspension that was further filtered through MakroPor silicon filters having a pore size of 5 μm. During this second filtration step, the silicon filters were continuously washed with 50 mL of MilliQ water at 100 °C and 50 mL of ethanol (EtOH) to remove any organic residue (especially wax) that could clog pores and create a yellowish film hindering subsequent analyses. After filtration, filter was recovered, placed in glass Petri dishes and dried in an oven at 40 °C.

2.6. Honey processing

Defrosted honey, from each apiary, was manually extracted from honeycomb and filtered through a 600 μm mesh to limit the presence of wax in honey samples. 5 g of each honey sample was dissolved in 5 mL of MilliQ water previously heated at 70 °C. Then, 20 mL of 30 % H_20_2 was added to the dispersion and stirred at 200 rpm and 50 °C for 48 h. The obtained dispersion was filtered under vacuum through 20 μm nylon filter. Throughout filtration the surface of the filter was continuously washed with 50 mL of MilliQ water at 100 °C and 50 mL of EtOH to remove sugar and organic residue. The nylon filter was placed in a glass beaker with 10 mL of MilliQ water and sonicated using a Branson 2210R-MT Ultrasonic cleaner bath (Branson UltrasonicsCorp., Connecticut, USA), at a frequency of 40 kHz for 30 min, to recover fragments and MFs. The recovered suspension was filtered again through MakroPor silicon filter having a pore size of 5 μm. Also in this case, the filter was continuously washed with 50 mL of MilliQ water at 100 °C and 50 mL of EtOH. The recovered filter was placed in glass Petri dishes and dried in an oven at 40 °C.

2.7. Quantification and identification of microplastics

To identify the chemical nature of the isolated microfragments, MakroPro silicon filters were analysed with a Nicolet iMX 10™ 10 Micro Fourier Transform Infrared Spectroscopy (micro-FTIR) (Thermo Fisher Scientific), equipped with an ultrafast motorised stage and a liquid nitrogen-cooled MCT (mercury cadmium telluride detector).

FTIR spectra were acquired in transmission mode, performing 64 scans with a resolution of 4 cm^{-1} in the range 4000–650 cm^{-1}.

Using the OMNIC Picta software's area tool, it was possible to analyse the entire filter area and create a mosaic of this area, resulting in a total image of the filter, reported in Fig. S1 of the supporting information, which was compared with the image obtained with the Leica microscope, to make positioning and spectra acquisition easier and more immediate. The resulting spectra were processed with OMNIC™ Specta Software and were compared with specific polymer reference libraries. The identification of a spectrum was indicated as a percentage match (match %). A match >70 % was considered sufficient for positive identification of plastic materials (Corami et al., 2022); (Liu et al., 2019).

2.8. Quality control

Several precautions were taken to reduce potential laboratory contamination. Firstly, plastic materials such as polyester or acrylic were avoided in both sampling and laboratory procedures. If plastic materials had to be used, they were rinsed at least three times with Milli-Q water to reduce possible contamination. In addition, 100 % cotton lab coats and nitrile gloves were worn. Glass material was used to store the samples and all glass containers (including filtration apparatus) were rinsed previously with pure water at least three times. The samples were covered with aluminum foil during the digestion, stirring and filtration processes. Blank samples, which undertook all the steps of sampling analyses, was performed for every batch of honeybee and honey. No contamination was detected during the analyses since the blank samples exhibited a number of MP ≤ 1 and MF ≤ 3.

2.9. Statistical analysis

A one-way ANOVA was used to assess statistically significant differences between the results of the analysis of honeybee samples from the different geographical areas (high and low impact areas). The significance level was set at $p < 0.05$.A commercially available statistical package for personal computer (Microsoft Excel 2021) was used for the ANOVA.

analysis.

3. Results and discussion

An environmental index specifically developed to accurately identify the most representative and significant sampling locations within the Campania region was applied to select sampling sites.

The adopted approach has allowed for exploring a wide range of environmental conditions and assessing any disparities in interactions between bees and microplastics in different contexts.

Sampling apiaries and locations together with the site description are reported in Table 1,

This initial inspection of honeybees and pollens under optical microscope allowed to evaluate the presence of fragments and MFs on them and to determine the most frequent areas of deposition on the animal's body.

From the preliminary investigation conducted on 9 honeybees from each apiary it can be seen that at least one sample of the 9 honeybees observed was found to be contaminated with different fragments. The fragments appear homogeneously distributed on the body of honeybees but the main sites of deposition are above all the wings and the ligula (i.e. the proboscis that the bee inserts into the floral calyx during the collection of the nectar). Some micrographs obtained during the honeybees' inspection are reported in Fig. 2a.

Pollen grains is a complex matrix that contains >200 substances, including proteins, amino acids, carbohydrates, fatty acids, phenolic compounds, enzymes, and vitamins and bioelements, depending from the plant species from which they derive (Komosinska-Vassev et al., 2015). Therefore, only the preliminary inspection under optical microscope was performed. Pollen grains appeared mainly contaminated by MFs (see Fig. 2b).

Table 1
Detail of sampling points.

Apiary	Location	Site description
1	Acerra, Napoli	High density urban
2	San Felice a Cancello, Caserta	High density urban
3	Mercato San Severino, Salerno	High density urban
4	Marcianise, Caserta	High density urban
5	Pignataro Maggiore, Caserta	High density urban
6	Presenzano, Caserta	Low density urban
7	Dugenta, Benevento	Low density urban
8	Guardia dei Lombardi, Avellino	Low density urban
9	Roccagloriosa, Salerno	Low density urban
10	Ascea, Salerno	Low density urban

As concerning honeybees, it is possible to hypothesize the presence of further fragments not visible under the microscope, either because of the magnification used or because they are hidden in the layer of hair. For this reason in our study, we developed a procedure for washing honeybees that allowed us to recover all the residues present on their body and then quantify and identify them. The filter surfaces obtained, as described above, were first observed using an optical microscope. A total of 178 particles with a dimension lower than 5 mm were analysed. Fig. 3a shows the size distribution and relative abundance of fragments and MFs among the observed microparticles.

The dominant shapes of isolated particles were MFs (78 %) and fragments (22 %). The size ranges for MFs and fragments were between 97 and 2632 μm and 257–819 μm, respectively. The size range 350–650 μm contained the most MPs, 30 % MFs and 60 % fragments. The chemical compositions of 178 microparticles were determined by means of microFTIR spectroscopy. Fig. 4 shows the FTIR spectra of three MFs of natural, artificial and synthetic origin and two microfragments of natural and synthetic origin.

Results indicated that on 137 MFs 93 were natural, 18 were synthetic and 26 artificial. As concerning the results achieved on the 40 fragments 6 were identified as natural and 34 as synthetic. The analysis of MFs of natural origin allowed to determine that all the MFs were of cellulosic nature. All the MFs of artificial origin were identified as rayon. Finally, the synthetic MFs, classifiable as MPs, were polyester (PET) 60 %, polyamide (PA) 20 %, polyacrylonitrile (PAN) 15 %, polyurethane (PU) 5 %. The fragments of natural origin were identified as wood and chitin (50 % and 50 %), the latter derived from the exoskeleton of honeybees. In contrast, the fragments of synthetic origin, classifiable as MPs, were polyamide (PA) 19 %, polytetrafluoroethylene (PTFE) 69 %, acrylonitrile and butadiene (AB) 3 % and epoxy resin (EP) 3 %. The abundance % of the different type of MPs (MFs and fragments) is shown in Fig. 3 b, c.

The analysis of the color of synthetic MFs and fragments indicates that MPs (both MFs and fragments) were predominantly blue, black or transparent. With regard to the natural MFs, 48 were transparent, 2 were brown, 25 black, 15 blue, 1 green, 1 gray and 1 red. These colored MFs could derive from textile substrates. It has been amply demonstrated that natural MFs (e.g., cotton) released from textile substrate could represents an environmental problem due to the chemical treatments they have been subject during manufacturing steps (De Falco et al., 2019) and due to their ability of absorbing chemical pollutants. In this respect, they also represent a class of ubiquitous airborne anthropogenic pollutants that have received limited attention to date (Ladewig et al., 2015).

These results are consistent with those already published by Rosal et al. that account for microparticles isolated from honeybees by apiaries located in urban, semi-urban and rural areas in and around Copenhagen. They found MPs in all the locations sampled and mainly in the form of fragments and fibers. As in our study, the micro-FTIR analysis confirmed the presence of several synthetic polymers, of with polyester being the most abundant. The MPs were identified to be made, in addition to polyester (PET), by 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). Moreover, they evaluated the presence of natural MFs, i.e. cotton, with non-natural colors, considered as tracer of anthropogenic pollution (Edo et al., 2021).

Instead, MPs with different composition were isolated and identified from honeybees sampled in China, where the majority of MPs were made by polycarbonate (PC) and PET, followed by mixture PE/PS and by PS. In addition to MPs the occurrence of graphite and fiber was reported without any identification of the fiber chemical composition (Deng et al., 2021).

A total of 104 particles with a dimension lower than 5 mm were isolated and identified in honey samples. Fig. 5a shows the size distribution and relative abundance of fragments and MFs isolated in honey

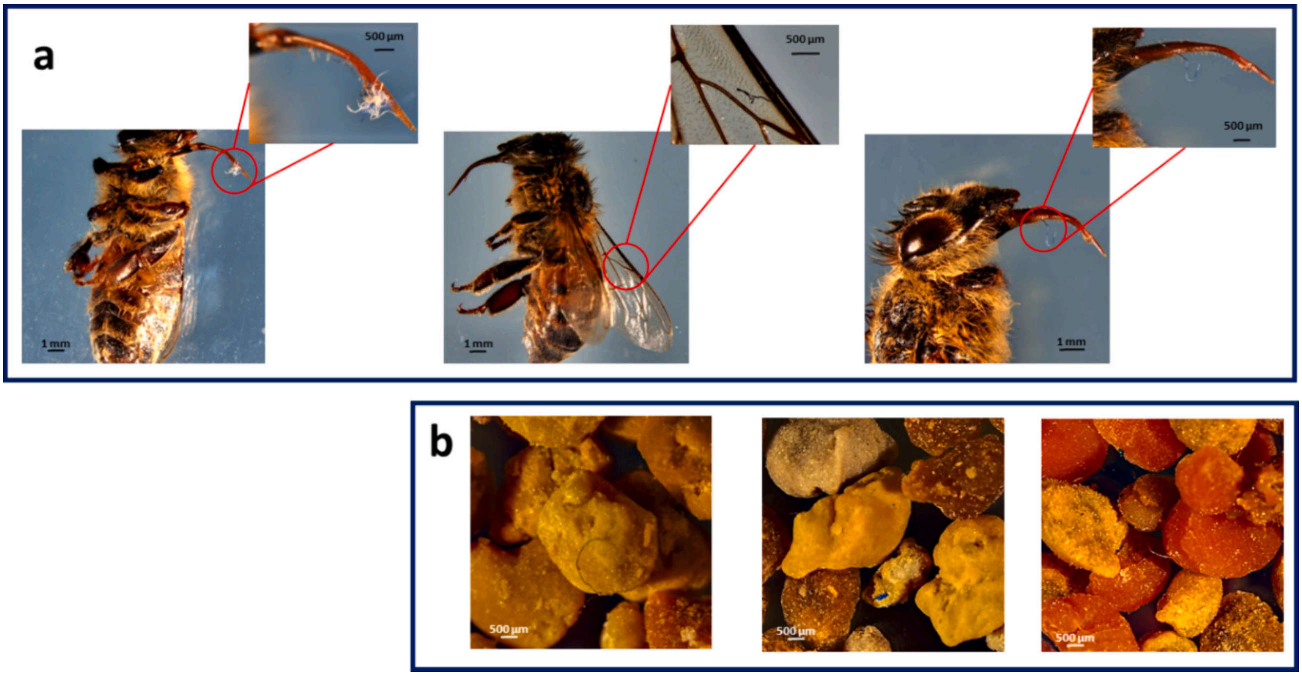

Fig. 2. Optical micrographs highlighting the presence of MFs and microfragments on a) honeybees and b) pollen grains.

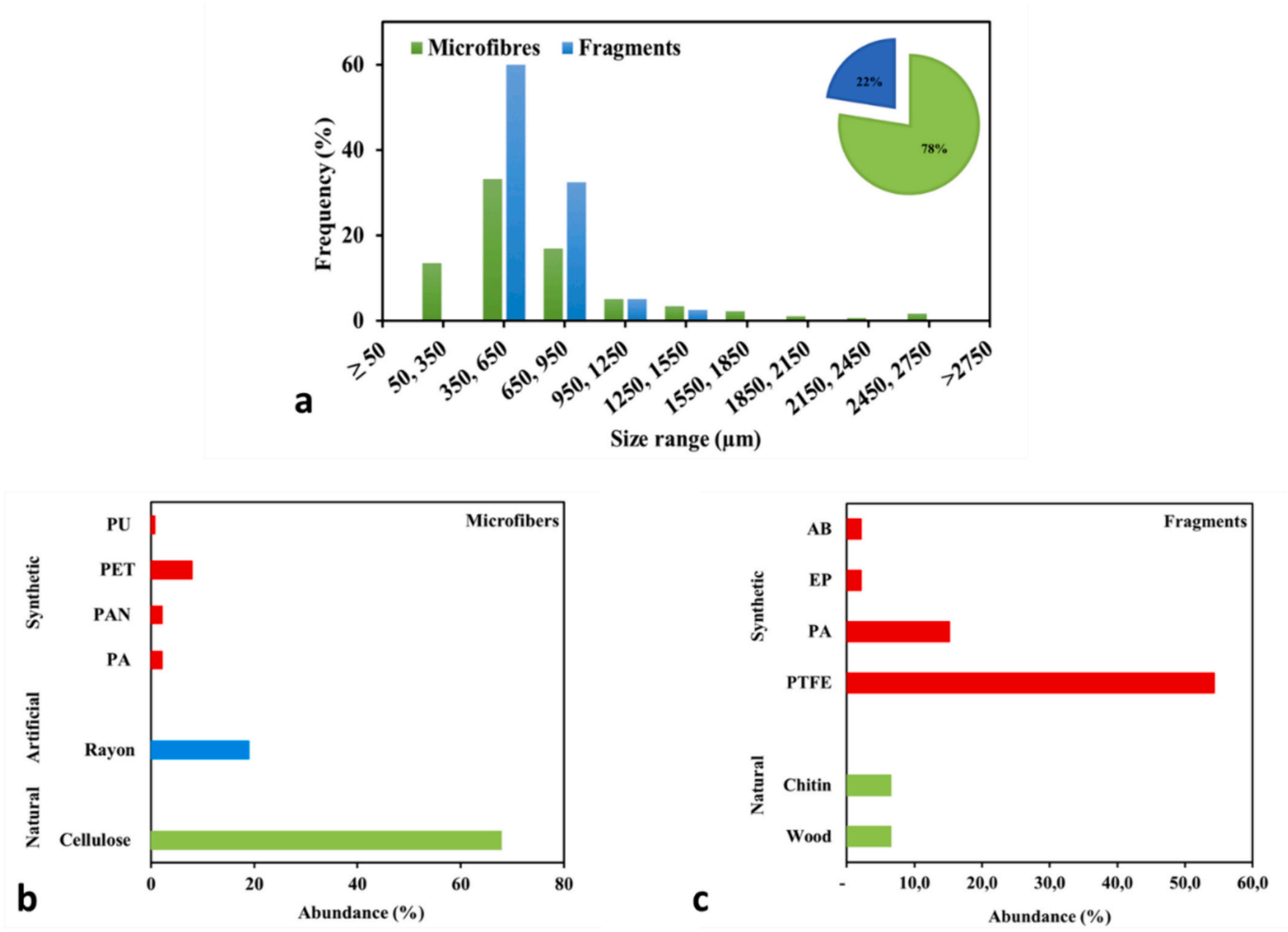

Fig. 3. a) Size distribution of the microparticles isolated from honeybees, the inset shows the distribution of the two different classes of microparticles: microfibres and fragments; b) abundance % of the different type of MFs; c) and abundance % of the different type of fragments.

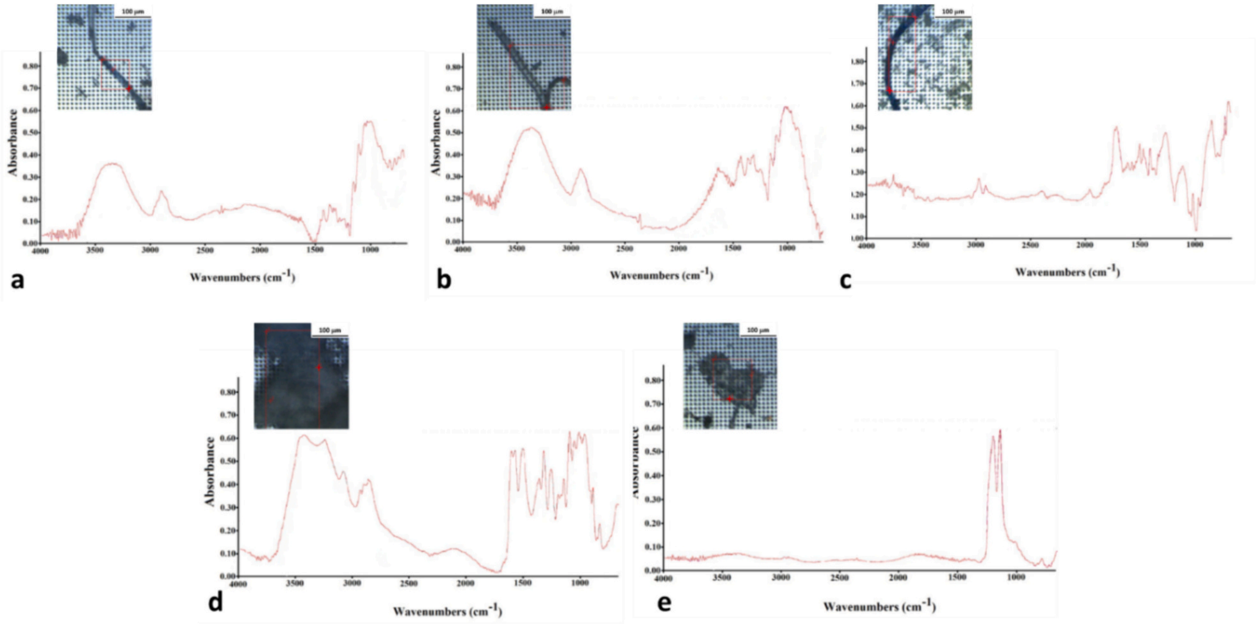

Fig. 4. FTIR spectra for the microparticles identified in honeybees: a) cellulose MFs; b) rayon MFs; c) PET MFs; d) chitin fragment; e) PTFE fragment.

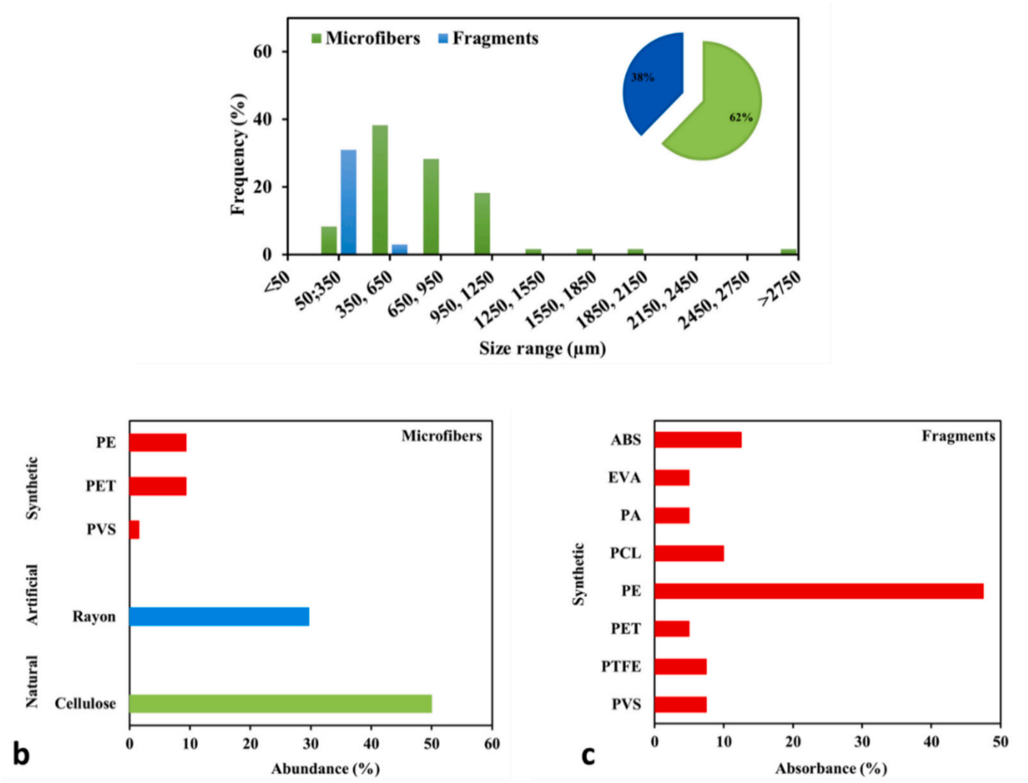

Fig. 5. a) Distribution of the two different classes of microparticles: MFs and fragments isolated in honey samples; b) abundance % of the different type of MFs; c) and abundance % of the different type of fragments.

samples. As for honeybees the majority of the isolated particles were MFs (62 %) and fragments (38 %). The size ranges for MFs and fragments isolated in honey samples were between 190 and 3525 μm and 68–779 μm, respectively. The chemical compositions of the isolated microparticles are reported in Fig. 5b-c. Out of 64 MFs isolated in honey samples, 32 were of cellulose, 19 were of rayon and 13 were of synthetic origin (Fig. 5b). All the 40 fragments isolated were identified as synthetic. Synthetic MFs were polyvinyl stearate (PVS) 1 %, polyester (PET) 9 %, polyethylene based MFs (PE) 9 %, while synthetic fragments were polyethylene (PE) 48 %, acrylonitrile butadiene styrene (ABS) 13 %, polycaprolactone (PCL) 10 %, polytetrafluoroethylene (PTFE) 7 %, polyvinyl stearate (PVS) 7 %, polyamide (PA) 5 %, ethylene vinyl acetate (EVA) 5 %, polyester (PET) 5 %. Among the isolated MPs 10 % are biodegradable polymers confirming that biodegradable materials could represent a problem for the environment if not correctly managed at the end of life (De Falco et al., 2021; Manfra et al., 2021).

As reported in literature, fiber and fragments were found in commercial honey samples in Germany, with a range of 40–660 fibers/kg and 0–38 fragments/kg of honey, and in homogeneously distributed in the packaged product (Liebezeit and Liebezeit, 2015). PE and PP MPs, with shapes of fragment or fiber, have been identified in artisanal and industrial honeys produced in Ecuador but the MPs results could be amplified by the human activity and packaging (Diaz-Basantes et al., 2020). In this respect, the present work represents the first paper reporting the occurrence of MPs and MFs in natural honey.

Comparing the chemical composition of the MFs isolated in honeybees and in honey samples it is possible to observe differences in the chemical compositions. Honeybees are mainly contaminated by PET, PA, PAN and PU MFs as well as by rayon and cellulosic fibers with non-natural colors. The chemical composition of MFs found on bees matches that of synthetic fabrics, leading to the hypothesis that MFs isolated from bees may represent air pollutants released from synthetic fabrics into the environment. Honey samples are contaminated by PE, PET and PVS MFs as well as by rayon and cellulosic fibers, see Fig. 6.

PET is the most widely used synthetic fiber in textile industry (Weis et al., 2022), PE is one of the most commonly used material for agrotextile (Dorugade et al., 2023) and PVS is used as finishing agent to impart water repellency and abrasion resistance to cellulosic fabrics (Gonzales et al., 1964). The differences in the chemical composition of the MFs identified in the two matrices (honeybees and honey samples) indicated that MFs released to air from synthetic textile (PET, PA, etc) can be captured by honeybees during their foraging flights but also picked up from flowers, from other nest mates, from the clothes of the beekeeper, among others. In addition to some MFs that could be transferred from honeybees, honey is contaminated by MFs that could be derived from the agricultural activities. Similarly for fragments, honeybees were contaminated by PTFE, PA, AB and EP while honey samples were contaminated by PE, ABS, PCL, PTFE, PVS, PA, EVA, PET. Also, for these MPs it is possible to highlight that part of the fragments could be transferred to honey by honeybees but many fragments are transferred to honey by other ways.

In Fig. 7, the abundance % of microparticles identified in honeybees and honey samples calculated normalizing the number of each type for the total number of particles (MFs or fragments) in each sampling sites is reported.

Results indicated that in all the sites, both honeybees and honey samples, are contaminated by MFs. In high density urban sites (from 1 to 5), apart from natural (cellulosic MFs) and artificial (rayon MFs), PET MFs are the most abundant contaminants of both honeybees and honey samples (Figs. 6 b-d). The matrices (honeybees and honey) sampled in low density urban sites (from 6 to10) present high level of artificial and synthetic MFs, these last having a different chemical composition. In low density urban sites, honeybees are mainly contaminated by PA MFs while honey mainly by MFs containing PE. MFs compositions were similar to that used for clothing, such as cotton, ramie, polyester and nylon, while PE MFs could be released by specific textile used for agriculture activities and captured by honeybees.

The abundance of fragments isolated from honeybees, see Fig. 6b, indicates that, independently from the site, the main abundant contaminants were PTFE MPs. Recently different papers have reported the presence of PTFE microparticles as soil and air contaminants. Different microparticles such as PE, polypropylene (PP), polyvinyl chloride (PVC), PA, and PTFE were identified in soil of farmland with long–term agricultural activities (Jia et al., 2022). Large amount of PTFE MPs was identified as air contaminants of a highly populated area (Mestre) on the Venice Lagoon strongly affected by anthropogenic activities (Rosso et al., 2023).

PTFE is used in a variety of industrial applications but also for outdoor application. PTFE MPs are directly released to the atmosphere during usage of the object and can be transported in different areas when favourable conditions of wind speeds and trajectories occur, as previously reported by Brahney et al. (Brahney et al., 2020). PTFE MPs should be considered mainly as air contaminants captured by honeybees since PTFE MPs were found in honey samples only in two of the five high density urban sites. In the honey samples, also PCL MPs were identified, mainly in high density urban sites. Up to now PCL MPs were identified in sludge (Edo et al., 2020), in Mediterranean surface waters (Suaria et al., 2016) and here for the first time were identified in agrifood samples. In low density urban site honey samples were contaminated mainly by PE MPs reflecting probably the large use of PE in agriculture.

Specifically, a statistical comparison was conducted using ANOVA analysis to compare the number of synthetic MFs and fragments

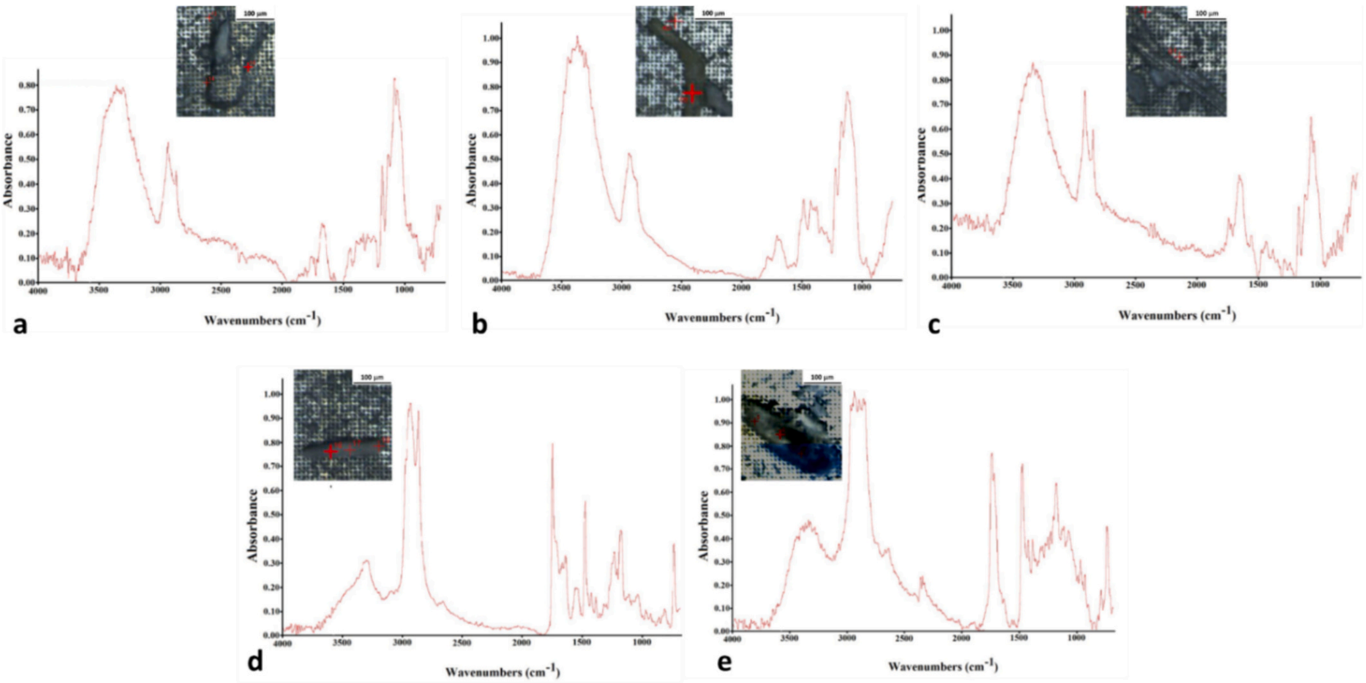

Fig. 6. FTIR spectra for the microparticles identified in honey samples: a) cellulose MFs; b) rayon MFs; c) PE based MFs; d) PE fragment; e) PCL fragment.

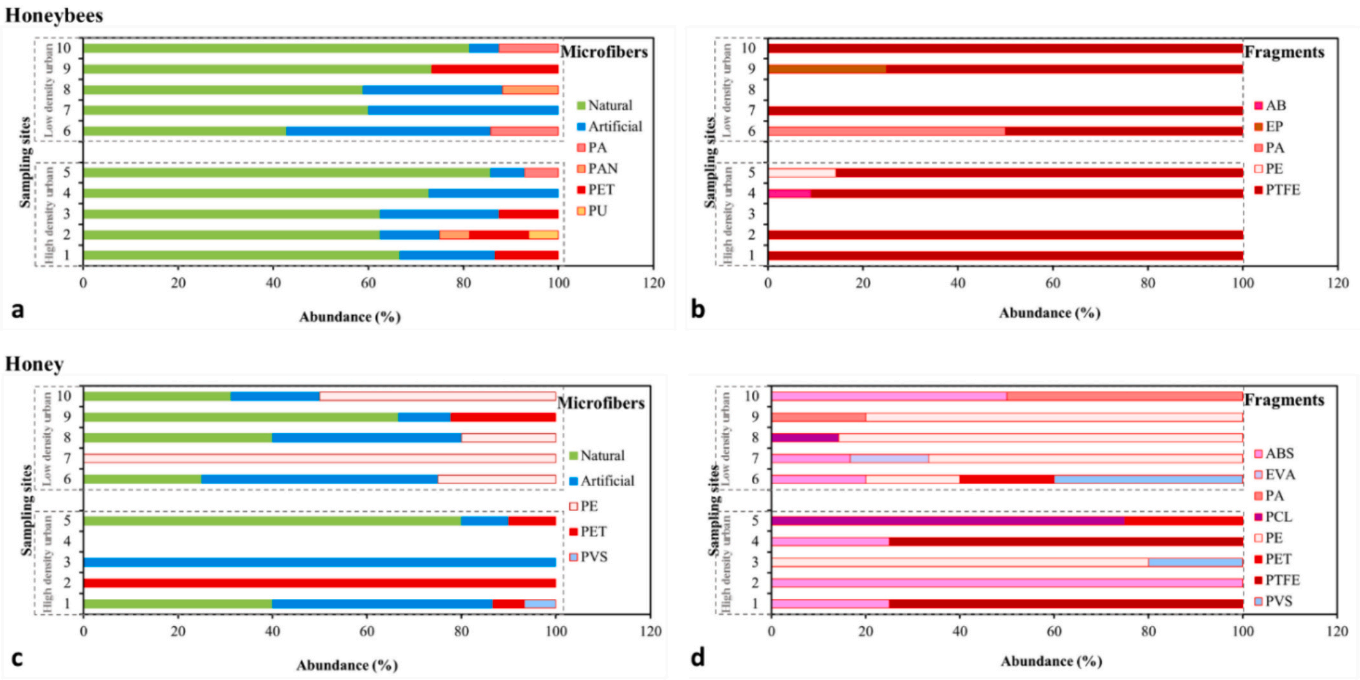

Fig. 7. Abundance of microparticle type for each sampling sites: a) MFs and b) fragments identified on honeybees; c) MFs and s) fragments identified on honey samples.

identified in honeybee and honey samples collected from low-impact environmental areas with those collected from high-impact areas. Despite initial expectations of significant differences between the two groups, the ANOVA analysis yielded a *p*-value >0.05, indicating the absence of statistically significant differences between the two groups. Therefore, it was not possible to establish a correlation between the type of area and the presence of synthetic MFs and fragments in honeybee and honey samples.

4. Conclusion

Honeybees, honey, and pollen samples were collected from ten different apiaries, selected across the Campania Region, Southern Italy to include five sites having low to medium-low environmental impact (LIA) and five sites with medium to high environmental impact (HIA). Results indicate the occurrence of MFs of natural (cellulose), artificial (rayon) and synthetic origin in all the samples from all the sites. The chemical composition of the synthetic MFs isolated form honeybees and honey samples suggests that MFs released to air from synthetic textile (PET, PA, etc) can be captured by honeybees during their foraging flights as well as recovered from flowers, from other nest mates, from the clothes of the beekeeper, among others and can be transferred to honey. Honey, in addition, is contaminated by MFs, this contamination could be correlated to the agricultural activities, too.

Independently from the apiary area, honeybees are contaminated by PTFE MPs. These results are in agreement with recent literature data reporting the wide diffusion of PTFE MPs in soil and air compartments. Biodegradable MPs, i.e. PCL MPs, contaminate honey samples collected in high density urban sites. This work reports for the first time the occurrence of PCL MPs in agrifood samples. Furthermore, due to the different type of polymers and the different MP sources, an unambiguous conclusion cannot be performed.

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CRediT authorship contribution statement

Marica Erminia Schiano: Writing – original draft, Methodology, Investigation, Formal analysis. **Luigi Jacopo D'Auria:** Writing – original draft, Methodology, Funding acquisition. **Roberta D'Auria:** Methodology, Investigation, Formal analysis. **Serenella Seccia:** Writing – original draft, Methodology. **Giuseppe Rofrano:** Writing – original draft, Methodology. **Daniel Signorelli:** Writing – original draft, Methodology. **Donato Sansone:** Writing – original draft, Methodology. **Emilio Caprio:** Writing – original draft, Methodology. **Stefania Albrizio:** Writing – original draft, Data curation. **Mariacristina Cocca:** Writing – original draft, Project administration, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jacopo Luigi D'Auria and Mariacristina Cocca reports financial support was provided by Italian Minister of Health. If there are other authors, they 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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