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Presence of Microplastics in Workers of *Apis mellifera* (Linnaeus, 1758) in Different Landscapes in Brazil

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Abstract Microplastics (MPs) are pervasive contaminants across all environmental compartments, with the atmosphere garnering significant attention in recent years due to its role as a crucial receptor and transporter of numerous pollutants. Deposition of these particles can occur via dry or humid processes, and their presence has been documented in areas far removed from urban and industrial centers, suggesting long-distance transport of MPs. Much of this transport is facilitated by air masses, which move in tandem with prevailing winds; however, pollinating insects, notably bees, also contribute to this dissemination as they encounter these particles during flight and foraging activities. Thus, the present study aimed to implement an established protocol for MP analysis in bees of the species *Apis mellifera*, given the limited research in this area within Brazil, and to ascertain whether a contamination gradient exists across three sampling sites (urban, periurban, and rural areas). A total of 505 particles were discovered across the three areas, comprising 307 fibers, 137 fragments, and sixty-one filaments. The PCA analysis indicated that the bees of the four hives located in the urban area and the bees of the two hives located in the peri-urban area had a higher amount of internal microplastic particles. Through the chemical analysis of the particles, 30 spectra were obtained, 13 polyethylene terephthalate (PET), 11 polyamide (PA), 4 polyethylene (PE), 1 polyester (PL) and 1 polyvinyl chloride (PVC) were identified.

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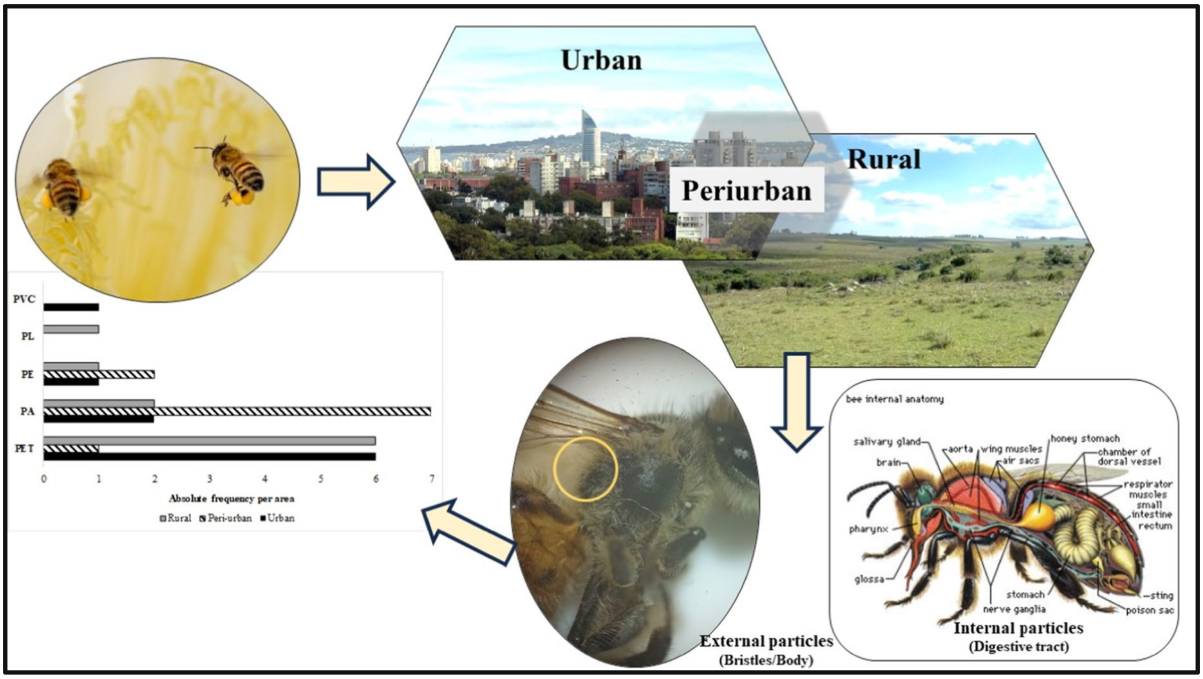
Graphical Abstract



Keywords Pollinators · Contamination · Bio sampling · Aero porters

1 Introduction

Microplastics (MPs) are organic polymers resulting from the degradation of improperly discarded plastic debris, they pose significant risks to the environment and living organisms due to their persistence in ecosystems over long periods; can also bind to other pollutants, such as heavy metals, leading to combined pollution; additionally, they can release harmful chemicals, such as phthalates and bisphenol, which further endanger the environment and organisms (Hakim et al., 2023).

Currently, studies related to MPs have increased, particularly concerning their impact on human health; Luqman et al. (2021) report an average detection of 3.33 to 13.99 grams of MPs per gram of feces in residents of the coastal area of Surabaya, Indonesia; additionally, Yang et al. (2023) have documented the presence of MPs in the hearts of patients undergoing cardiac surgeries.

The MPs are regarded as ubiquitous contaminants across all environmental compartments, with the atmosphere gaining significant attention in recent years as a vital receptor and transporter of numerous pollutants (Büks & Kaupenjohann, 2020; Zhang et al., 2020). Studies suggest that various forms of MPs exist in the atmosphere, with fibers derived from textiles, automotive sources, and industrial materials (Allen et al., 2019; Roblin et al., 2020).

Research, such as that conducted by Abbasi & Turner (2021), indicates that both passive and active sampling methods reveal that deposition of plastic microparticles occurs through dry and wet processes. These contaminants have been detected in areas distant from urban, industrial, or agricultural centers, such as the Alps and the Arctic (Bergmann et al., 2019), underscoring the propensity of these particles to undergo long-distance transport (approximately 1000km) facilitated by air masses. Liu et al. (2019b) and Szewc et al. (2021) suggest that this ability to traverse long distances facilitates the direct entry of these particles into the oceans. Moreover, there is evidence indicating that oceans may indirectly contribute to the emission of MPs into the atmosphere, potentially through wave action (Allen et al., 2020).

Abbasi et al. (2023) conducted suspended sampling from two distinct locations—an industrial area and an urban region—under varying conditions and precipitation-free scenarios during both winter and summer periods. Alongside sample collection, the potential pathways of particle displacement were traced. The authors concluded that there were no significant differences in the concentration and size of particles between the two areas. Furthermore, trajectory modeling (HYSPLIT) indicated that MPs are transported in tandem with wind direction and speed without significant deposition, suggesting multiple potential source regions from which these particles may have originated before being transported to the collection sites.

Some pollinators, such as bees, have been utilized as bioindicators of various terrestrial and atmospheric pollutants, primarily due to their significant socioeconomic impact and their role in maintaining ecological balance through the ecosystem service of pollination (Pedro, 2014). Bees belong to the Order Hymenoptera, and their bodies are anatomically divided into three parts: (I) - Head: housing sensory organs such as eyes and antennae, which aid in sight, hearing, touch, and smell; (II) - Thorax: containing locomotor organs comprising three pairs of legs and one pair of wings, along with a bristle-rich area that electrostatically aids in pollen grain adherence to the animal's body; (III) - Abdomen: housing most of the bees' organs, notably the digestive tract that accommodates the honey bladder (where the conversion of collected nectar into honey and water transport to the colony occur), the stomach, and intestine. In this region, the presence of cerumen glands is noteworthy; these glands, in young workers, secrete liquefied wax, which upon contact with air, forms thin wax scales used in nest architecture (Roubik 1951).

Regarding the consequences of bees' exposure to MPs, several studies highlight the presence of changes in the intestinal microbiota of these animals upon contact with microparticles, as well as alterations in metabolism, immune function, and neuroendocrine crosstalk (Wang et al., 2021). Despite the increasing apprehension regarding the repercussions of MP contamination on bees, research efforts have primarily been concentrated in countries such as China, Italy, Argentina, Canada, and Denmark. Bees of the genus Apis are utilized as bioindicator organisms due to their widespread use in other toxicity investigations and the existence of numerous well-established laboratory protocols (Balzani et al. 2022; Buteler et al., 2022; Deng et al. 2021; Edo et al., 2021; Wang et al., 2022).

In Brazil, there is only one documented study correlating bees and microplastics, which investigates the incorporation of MPs and metal oxide nanoparticles through larval ingestion in *Partamona helleri* (Viana et al., 2023). Therefore, the present study aimed to apply the existing protocol for *Apis mellifera* in colonies located in three distinct areas (urban, periurban, and rural) within the municipality of Piedade, in the interior of the State of São Paulo, Brazil. The objective was to determine whether workers of the species can serve as MP carriers during their foraging activities and to ascertain the presence of these particles in the digestive tract of these animals. These three categories of areas were selected because studies have indicated urban centers as significant contributors to the formation of fibers from synthetic textile wear, and they also play a crucial role in dispersing these contaminants to other areas through air masses (Liu et al., 2019a).

2 Materials and Methods

2.1 Species

The honeybee *Apis mellifera* (Linnaeus, 1758) was selected as the bioindicator organism for MP contamination due to its significant economic importance and the existence of an established experimental protocol for MP analysis (Edo et al., 2021) (Figure 1). This species was chosen as a bioindicator due to its high occurrence in Brazil and its significant contribution to the national economy through the export of its products, especially honey. Additionally, there are several well-established study protocols that utilize these individuals as bioindicators of various environmental contaminants.

Traditionally, the species is divided into four evolutionary lineages: A (Africa), M (western and northern Europe), C (southern and eastern Europe), and O (Caucasus, Turkey, the Middle East, Cyprus, and Crete), based on morphometric and molecular studies

Fig. 1 *Apis mellifera* (photo courtesy of Wilson Gamboa)

(Ruttner, 1988; Franck et al., 2000; Whitfield et al., 2006). However, this species demonstrates a high capacity to adapt and colonize nearly all habitable biomes on the planet, exhibiting remarkable resilience to various bioclimatic conditions; this adaptability indicates significant morphological and behavioral flexibility This adaptability indicates significant morphological and behavioral flexibility, thus giving rise to several subspecies without easily identified specific characteristics (Tihelka et al., 2020).

The relationship with Brazil began in 1956 when Brazilian researchers attempted to produce a bee adapted to subtropical conditions by crossing commercial individuals (a mixed population originating from subspecies of lineage M - *Apis mellifera mellifera* and *Apis mellifera iberiensis*, and lineage C - *Apis mellifera ligustica* and *Apis mellifera carnica*) with South African individuals of *Apis mellifera scutellata* (lineage A) (Kerr, 1957; Nogueira-Neto, 1964; Winston, 1987, 1992; Sheppard, 1989; Crane, 1999). However, the swarms of *A. m. scutellata* reproduced rapidly and swarmed together, leading to hybridization with local individuals. These hybrids quickly spread throughout Brazil and South America, adapting remarkably well (Michener, 1975; Harpur et al., 2020).

2.2 Place of Study

Sampling was carried out in collaboration with beekeepers in the municipality of Piedade, São Paulo, Brazil. The colonies were managed by their respective owners during the collection period. The six sampling points were categorized into three areas: urban, peri-urban, and rural, with two collection points designated for each area. The selection of these areas was based on the Zoning Plan provided by municipal agencies, with PT_01 and PT_02 representing the urban area, PT_03 and PT_04 the peri-urban area, and PT_05 and PT_06 the rural area. It should be noted that point PT_06 is outside the municipal boundaries, as it is classified as a district, but it still falls under the jurisdiction of the municipality of Piedade (Fig. 2).

To complement the zoning approach, the landscape was characterized for each sampling point to understand the prevailing activities and land use in these locations (see Table 1). Each map considered a 5 km radius around the hive's location where the workers were collected. This radius was determined based on the distances traveled by bees for foraging and reproduction; Utaipanon et al. (2019) suggested that bees could range up to 3.75 km. Hence, the radius selected for this study aimed to extend this distance for a better understanding of the land use surrounding the hives (Fig. 3).

The characterization maps confirmed that Points 01 and 02 are indeed Urban Areas, characterized by elevated levels of urbanization and lower percentages of forest formation and restoration. These areas are highly degraded and directly influenced by various pollutant emissions, as noted in previous studies (Liu et al., 2019a).

Points 03 and 04 were identified as transition points between urban centers and less urbanized areas. They exhibit intermediate levels of urbanization, higher percentages of forest formation, and greater percentages of forest restoration compared to Points 01 and 02, suggesting a focus on vegetation maintenance and restoration efforts. Points 05 and 06, classified as Rural Areas, showed a significant decrease in urbanization percentages compared to the other points. However, all points displayed similar percentages in the category of agricultural and pasture mosaics, reflecting the predominant economic activities in the municipality.

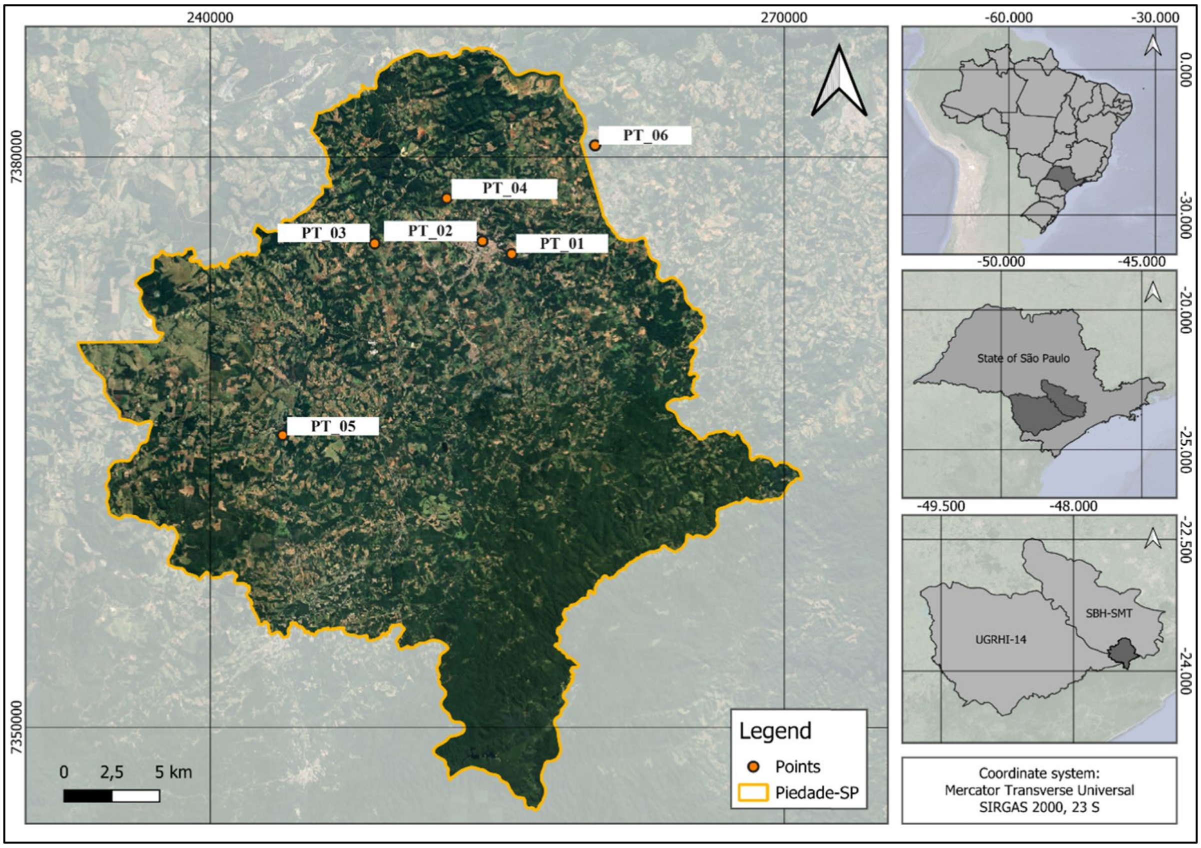


Fig. 2 Sampling points were categorized into three areas: urban (PT_01 and PT_02), peri-urban (PT_03 and PT_04), and rural (PT_05 and PT_06), with two collection hives designated for each point (4 hives per area)

2.3 Capture Procedures

At each sampling point, two separate hives were chosen, from which 40 to 50 workers of *A. mellifera* were collected per hive. Workers were collected near the colony's brood area, where they fed the larvae and stored surplus food. Workers were captured using 50mL glass vials, which were labeled and stored in zip-lock bags. The samples were then frozen without the addition of a solution to prevent plastic degradation and microbial growth and were transported to the laboratory to undergo the protocol established by Edo et al. (2021).

2.4 Laboratory Procedures

For Phase I of the experiment (analysis of external particles – bristles/body), the samples were thawed and placed in a beaker containing a solution of 150mL of ultra-pure water (Milli-Q) and 50mL of 70% ethanol (p.a). The beaker was placed on a stirring table and stirred lightly for 15 minutes. Subsequently, the solution was filtered through a 47 mm diameter fiberglass pre-filter with a 0.5µm particle retention capacity using a porcelain funnel. The pre-filters were then transferred to Petri dishes and sealed.

For Phase II of the experiment (analysis of internal particles – digestive tract), the whole body of the honey bees from Phase I were placed in a test tube and submerged in 33% H2O2. The tubes were then placed in a greenhouse at 60ºC for 24 hours. After this period, the liquid was filtered through another fiberglass pre-filter, 47mm in diameter, and with a 0.5µm particle retention capacity, using a porcelain funnel. The filtered liquid was then transferred to another Petri dish, which was subsequently sealed (Edo et al., 2021). This phase of the experiment aimed to digest the viscera of the workers so that any organic matter was dissolved, leaving only inorganic particles, such as MPs.

Glass, porcelain, or metal materials were used throughout all stages to prevent contamination via utensils, and all necessary measures were taken to prevent atmospheric contamination during the procedures.

As a form of control, during the sample analysis, both steps were conducted without the presence of test organisms to identify contaminant particles in the materials, solutions, or even in the laboratory environment during the procedures.

2.5 Quantification and Identification of MPs

The Petri dishes containing the filters were examined under a stereomicroscope equipped with a camera, and the images were processed using ImageJ software, which facilitated the measurement of particle length and width. All particles measuring <5mm were categorized based on morphological characteristics such as size, shape, and color, into fragments, fibers, and filaments.

2.6 Chemical Characterization of MPs

After quantification and classification based on the morphology of the particles discovered, approximately 25 to 30 particles were randomly chosen from the 8 samples (four internal and four external) for each of the three sampling areas. In total, 88 particles were selected and transferred with the aid of tweezers and stereomicroscope to a pre-cleaned fiberglass filter of 47 mm in diameter and 0.5µm, specific for each area. These filters were transported to USP's Institute of Chemistry, which was responsible for carrying out the chemical analyses.

A total of 30 Raman spectra were obtained, with 10 spectra collected from each of the areas. These spectra were acquired using the Renishaw inVia Micro-Raman spectrometer (785 nm) within the spectral range of 1800-400 cm^{-1}, with a resolution of 4 cm^{-1}, an exposure time of 10 s, and 4 accumulations.

2.7 Location of MPs in Individuals

A total of ten individuals were randomly collected from the three areas for stereomicroscope analysis to identify the specific locations where the

Table 1 Characterization of the sampling points, within a radius of 5km, about land use and occupation

Point	Area (km^2)	Mosaic of Agriculture and Pasture (%)	Forest Formation (%)	Mosaic of Crops (%)	Pasture (%)	Urban Infrastructure (%)	Soybeans (%)	Forest Plantation (%)	River, Lake (%)	Other Non-Vegetated Area (%)	Coffee (%)	Other Perennial Crops (%)	Sugar Cane
PT_01	70.392,231	33.07	25.81	14.15	12.10	9.34	3.04	2.15	0.14	0.12	0.06	0.01	-
PT_02	70.395,568	33.22	27.95	12.58	11.5	9.78	2.61	1.94	0.14	0.22	0.05	0.01	-
PT_03	68.285,294	33.35	29.48	18.6	8.8	3.77	1.7	3.8	0.24	0.22	0.02	-	0.02
PT_04	70.443,312	31.96	32.25	9.62	10.88	8.64	1.34	4.96	0.1	0.21	0.01	-	0.03
PT_05	70.318,998	29.04	25.67	24.82	6.76	0.11	4.63	8.47	0.36	0.1	-	0.01	0.03
PT_06	70.234,979	35.02	27.36	15.44	11.3	4.2	5.48	0.87	0.12	0.2	-	-	0.01

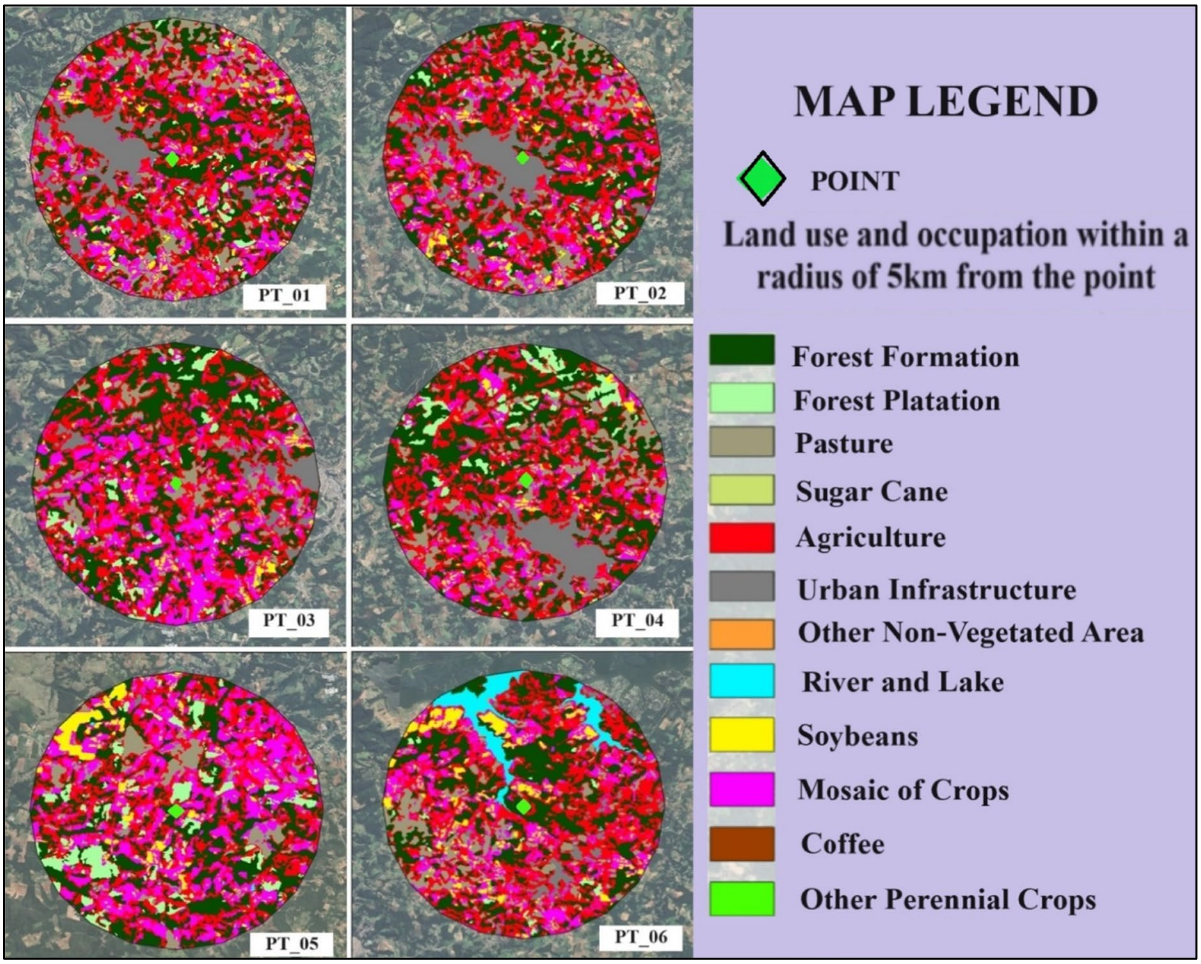


Fig. 3 Characterization of the collection points within a radius of 5 km, considering land use activities

particles accumulate and are transported within the bees' bodies.

2.8 Statistical Analysis

For this study, twelve hives were sampled and distributed across three study areas, with each area consisting of four hives. Normality tests, such as the Shapiro-Wilk test, were conducted. If normality was confirmed, the two-way ANOVA test was applied, followed by the Tukey mean comparison test. Subsequently, Principal Component Analysis (PCA) was performed to further analyze the data.

3 Results

Three categories of MPs were identified across the three sampling areas: fibers, fragments, and filaments, with frequencies decreasing in that order. Figure 4 illustrates the morphological distinctions among these particles, enabling their classification within the respective categories.

For the control samples, a few contaminations were identified: eight fibers for Phase I of the experiment and two filaments for Phase II. These particles may have accompanied the solutions used in the experiments or been incorporated during the filtration process of these solutions. However, given their

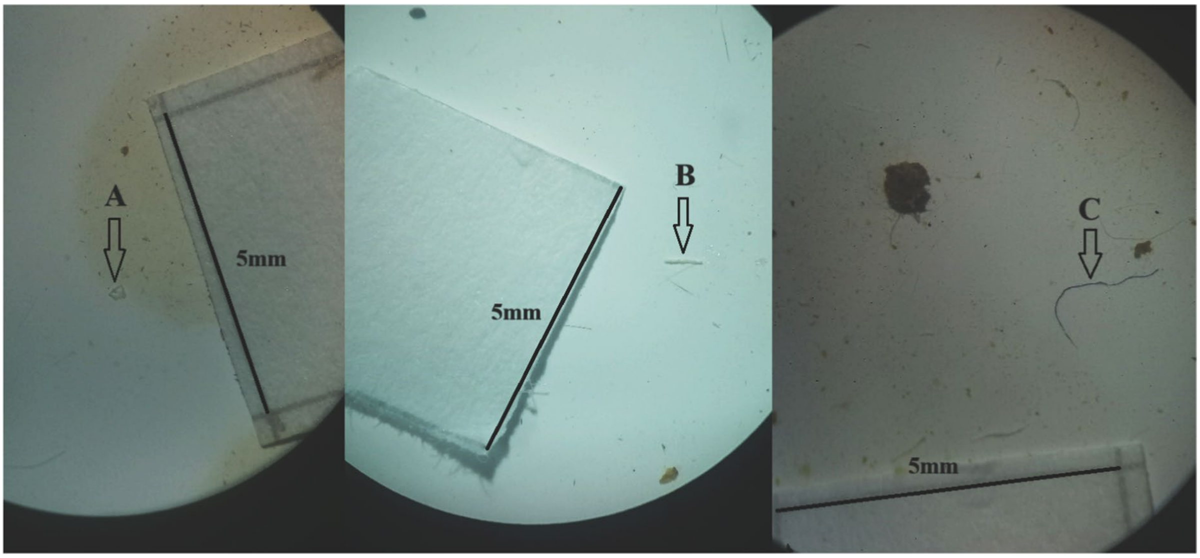


Fig. 4 Categories of MPs found (**A**: fragment; **B**: Filament; **C**: Fibre)

minimal amounts (8 MPs/200 mL of solution in Phase I and 2 MPs/10 mL of solution in Phase II), it was determined that these values do not compromise the validity of the experiments.

Regarding the data obtained for the three areas, out of the 307 fibers found, transparent fibers were predominant with ninety-eight, followed by white with seventy, blue with fifty-eight, black with thirty, red with sixteen, silver with ten, gray with five, and orange and white with red with four each. Green, gold, and blue with white each had three, while pink, yellow, and red with blue each had 1 (see Figure 5). Of the 137 fragments found, white fragments were predominant with eighty-three, followed by transparent with forty-seven, yellow with five, and black with 2 (see Figure 5). Regarding the sixty-one filaments found, white filaments were predominant with thirty-one, followed by transparent with twenty-three, and orange, gold, and yellow with two each, while brown had 1 (Figure 5).

In the urban area, a total of seventy-six particles were discovered, with thirty-four originating from PT_01 and forty-two from PT_02. Among these, fifty-six were fibers, fifteen were fragments, and five were filaments. For the peri-urban area, a total of 190 particles were identified, with 40 found at PT_03 and 150 at PT_04. Of this total, 103 were fibers, seventy were fragments, and seventeen were filaments. In the rural area, a total of 241 particles were observed, with 103 located at PT_05 and 138 at PT_06. Among these, 151 were fibers, fifty-one were fragments, and thirty-nine were filaments (Table 2).

Considering the data obtained for points PT_01 and PT_02 in the urban area, the average equivalent length of fibers was 1,705 ± 1,325 mm, with an average equivalent width of 0.035 ± 0.029 mm. The mean diameter of fragments was 0.078 ± 0.081 mm, while the average diameter of filaments was 0.087 ± 0.030 mm.

For points PT_03 and PT_04 in the peri-urban area, the average equivalent length of fibers was 1,819 ± 1,369 mm, and the average equivalent width was 0.032 ± 0.032 mm. The mean diameter of fragments was 0.021 ± 0.020 mm, and for filaments, the average diameter was 0.033 ± 0.039 mm.

In the rural area, represented by points PT_05 and PT_06, the average equivalent length of fibers was 1,773 ± 1,471 mm, with an average equivalent width of 0.024 ± 0.021 mm. The mean diameter of fragments was 0.039 ± 0.052 mm, and for filaments, the average diameter was 0.040 ± 0.030 mm.

Regarding the location of MPs in the individuals, particles were found in the thorax, abdomen, and legs, with a predominance of bristles along the body (Figure 6).

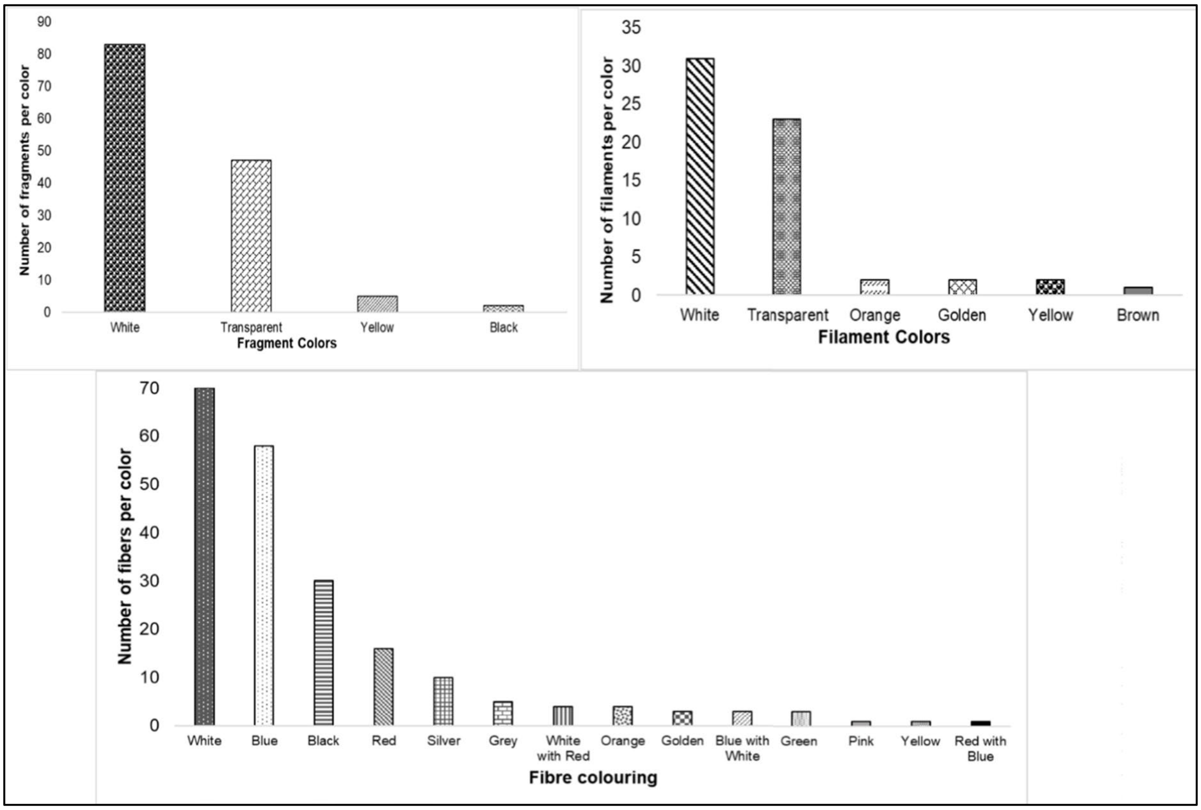


Fig. 5 Staining of filaments, fragments, and fibers found

Table 2 Total MPs found per point and hive, and distribution of these throughout the Body/Bristles and Digestive Tract

Point	Total of MPs	Hive	Nº of MPs on Body/Bristles	Nº of Fibers	Nº of Fragments	Nº of Filaments	Nº of MPs on the Digestive Tract	Nº of Fibers	Nº of Fragments	Nº of Filaments
PT_01	34	A	12	12	0	0	11	8	2	1
		B	6	4	2	0	5	2	3	0
PT_02	42	A	14	11	2	1	19	15	2	2
		B	3	3	0	0	6	1	4	1
PT_03	40	A	22	16	6	0	4	0	4	0
		B	10	10	0	0	4	2	1	1
PT_04	150	A	63	23	34	6	9	4	3	2
		B	56	33	18	5	22	15	4	3
PT_05	103	A	26	9	17	0	11	6	5	0
		B	54	37	14	3	12	8	3	1
PT_06	138	A	89	60	7	22	3	3	0	0
		B	39	21	5	13	7	7	0	0

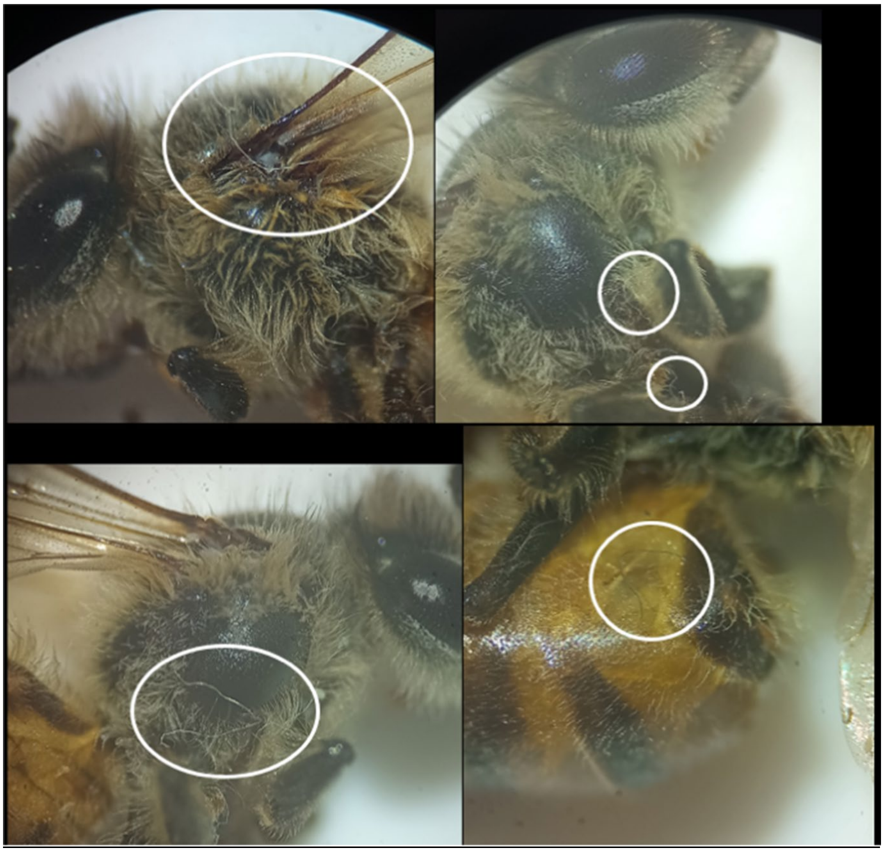


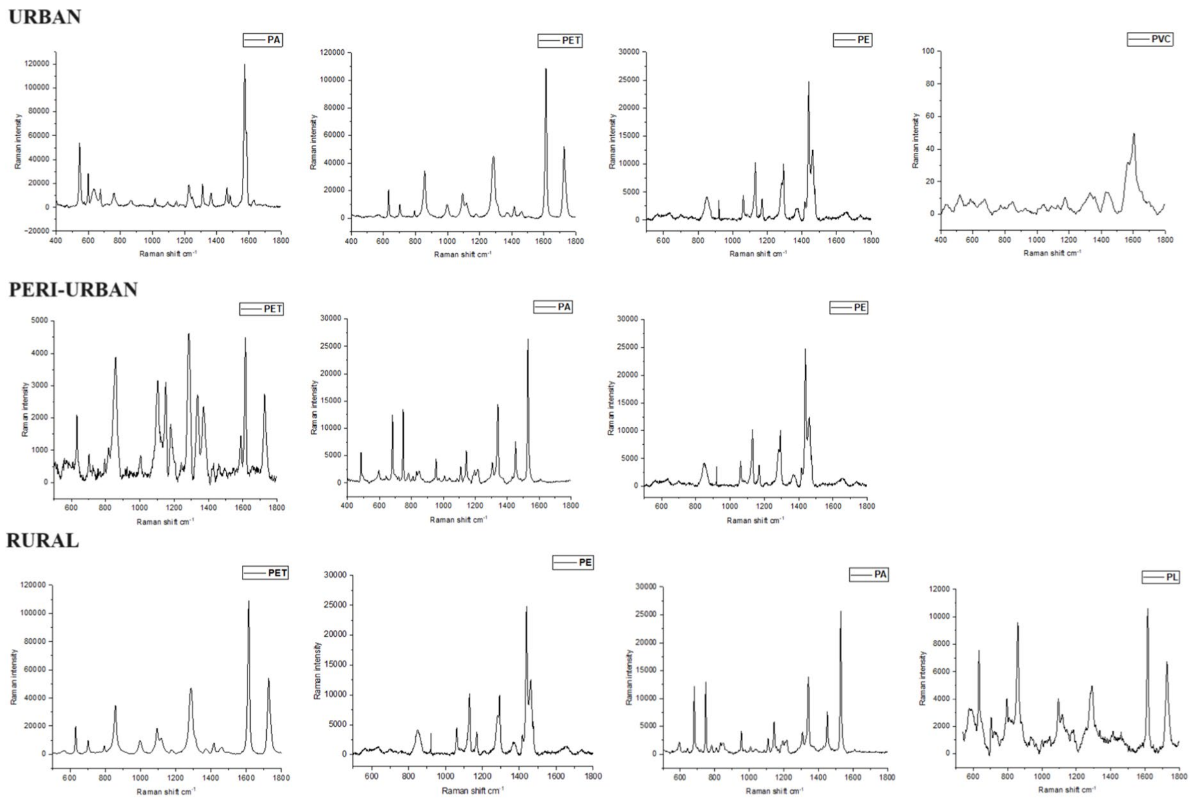
Fig. 6 Location of MPs in *Apis mellifera*

In the Chemical Characterization of the MPs, out of a total of 30 spectra obtained, 13 were identified as Polyethylene terephthalate (PET), 11 as Polyamide (PA), 4 as Polyethylene (PE), 1 as Polyester (PL), and 1 as Polyvinyl Chloride (PVC). For the urban area, 6 spectra were identified as PET, 2 as PA, 1 as PE, and 1 as PVC. In the peri-urban area, 7 spectra were identified as PA, 2 as PE, and 1 as PET. As for the rural area, 6 spectra were identified as PET, 2 as PA, 1 as PE, and 1 as PL (Figure 7).

Regarding the statistical analyses, the results obtained for the groups of internal and external microplastic particles in bees from hives located in urban, peri-urban, and rural areas were found to be within the normal range based on the Shapiro-Wilk test. All groups conformed to the normal distribution, confirming the null hypothesis of similarity to the expected Gaussian distribution, except only two groups: fragments and external filaments from collections in urban locations. No outliers were detected, as determined by the ROUT model (Q5%).

Based on the results obtained from the classification and counting of microplastic particles in terms of quantity and type, a two-way ANOVA was conducted followed by Tukey's subsequent test of comparison of means, as illustrated in Figure 8, for samples from the four colonies in each area. The variable "microplastic particles" was found to account for 36.16% of the total variance (F (5, 54) = 9.44; $p < 0.0001$), signifying an extremely significant effect. The variable "location" contributed to 7.607% of the total variance (F (2, 54) = 4.97; $p = 0.0105$) and was considered significant. Conversely, the interaction between these two variables represented 14.88% of the total variance (F (10, 54) = 1.94; $p = 0.0589$) and was not deemed significant.

It was observed that the number of external fibers was lower in urban areas compared to rural bees ($p = 0.0001$), and the number of external fragments in urban bees was lower than that found in peri-urban bees ($p = 0.043$). Concerning the localities, bees from peri-urban hives exhibited a higher number of



	PET	PA	PE	PL	PVC
URBAN	6	2	1	-	1
PERI-URBAN	1	7	2	-	-
RURAL	6	2	1	1	-

Fig. 7 Spectra of MPs found. PET: polyethylene terephthalate; PA: Polyamide; PE: Polyethylene; PL: Polyester; PVC: Polyvinyl chloride

external fibers than external filaments ($p = 0.023$), fragments ($p = 0.026$), and internal filaments ($p = 0.012$). Bees from rural hives displayed a similar profile, with the number of external fibers being higher than external fragments ($p = 0.004$), external filaments ($p = 0.002$), internal fibers ($p = 0.0003$), internal fragments ($p < 0.0001$), and internal fragments ($p < 0.0001$).

To comprehensively understand the overall distribution of the data, Principal Component Analysis (PCA) was performed, considering hives from distinct locations as "cases" and different microplastic particles as "variables." The PCA revealed a cumulative percentage of 91.413% in the second component (axis) and an Eigenvalue of 93.74 in the second axis. It was observed that the hives in the urban area were grouped under the influence of the vectors related to the variable "Internal fragments" and to a lesser extent by the variable "Internal filaments." The bees from the four hives located in the urban area and the bees from two hives located in the peri-urban area exhibited a higher amount of internal microplastic particles, as highlighted in red in Figure 9.

4 Discussion

Contrary to the initial hypothesis, the rural area exhibited a higher particle index, contradicting the assumption that it would be the least contaminated

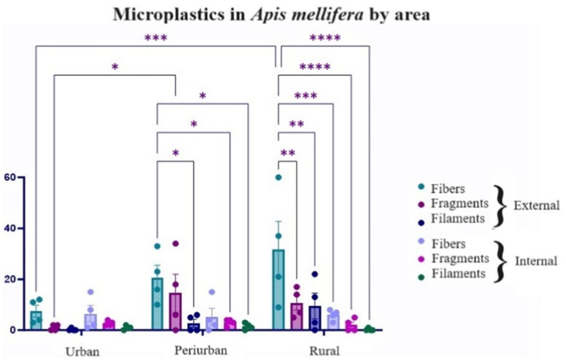


Fig. 8 Analysis of the amount of microplastic particles quantified from the external and internal parts of *Apis mellifera* collected from hives located in urban, peri-urban, and rural areas. The analysis was performed by two-way ANOVA, with Tukey's test of comparison of means, considering a significance of a<0.05 for all analyses. * = $p<0.05$; **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

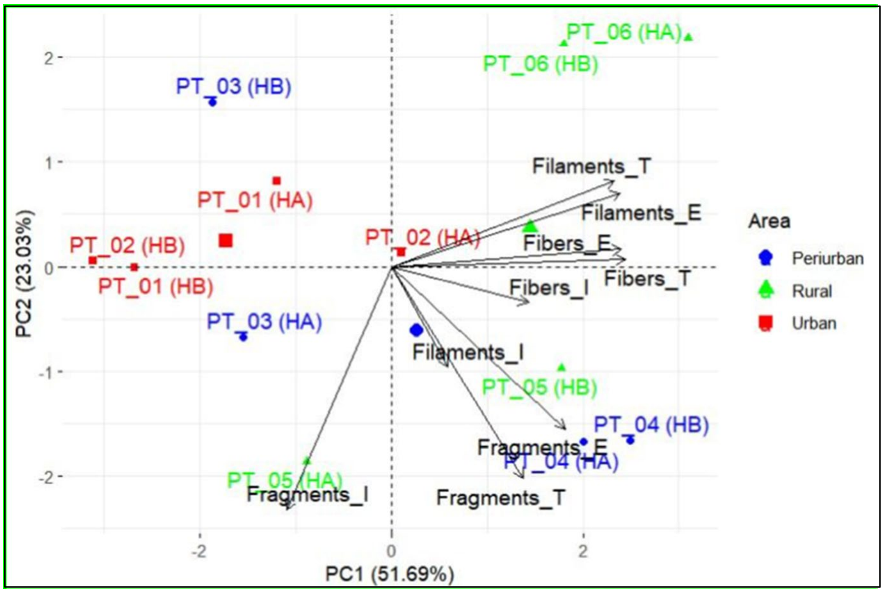
due to lower environmental degradation levels. This discrepancy can be attributed to the presence of urban settlements in more isolated areas, extensive foraging radius of worker bees, and wind drift facilitating particle displacement.

Guerrero-Pineda et al. (2022) further support this argument, suggesting that municipalities relying heavily on agriculture, as observed in the study area, pose significant implications for sustainable development and biodiversity conservation. The expansion of agricultural areas often leads to the degradation of native forests. Additionally, rural, and peri-urban areas, despite being less urbanized, face challenges such as improper waste disposal and accumulation due to long distances from urban centers. In summary, the study highlights the complex interplay between urbanization, agricultural activities, and microplastic pollution. It underscores the need for comprehensive waste management strategies and emphasizes the importance of considering environmental factors in urban planning and development initiatives to mitigate pollution and preserve biodiversity.

The abundance of fibers, fragments, and filaments found across all points in the study aligns with previous research by Brahney et al. (2020), Liu et al. (2019b), and Szewc et al. (2021), who attribute their presence to wind drift. These particles can be transported over long distances before being deposited, often in remote locations far from their sources of origin. Through analyses of air mass trajectories, these studies suggest that urban centers serve as primary sources of emission for these particles.

The prevalence of fibers and fragments can be explained by their morphologies, which make them more susceptible to transportation by air masses over long distances before eventual deposition, as noted by Brahney et al. (2020). Large urban centers, according to Liu et al. (2019a), are typically the primary sources of origin and emission for MPs in remote areas.

Fig. 9 Analysis of the amount of microplastic particles quantified from the external and internal parts of *Apis mellifera* collected from hives located in urban, peri-urban, and rural areas. Analyze by PCA



Liu et al. (2023) propose that the high incidence of fibers in the environment stems from their production medium, as their origin is intricately linked to MP filaments. The authors highlight those yarns made of natural fibers or regenerated cellulosic filaments, when cut into short lengths, are prone to releasing microfibers. On the other hand, synthetic fibers are produced as continuous filaments of varying sizes. The difference lies in the fact that natural fibers must be twisted before the weaving process, while filaments can be directly woven. Consequently, the lower frequency of filaments can be attributed to their degradation into fibers over time.

Regarding particle staining, studies by Edo et al. (2021) and Deng et al. (2021) also observed the prevalence of transparent, white, and blue particles, among other unnatural colorations, which are of textile origin; most of the fibers being transparent is also due to the fact that their original colors fade to transparency through degradation processes such as thermodegradation (temperature) and photolysis (ultraviolet radiation) (Hakim et al., 2023). Regarding particle size, Edo et al. (2021) note the challenge of comparing their findings with existing literature due to the novelty of atmospheric transport of MPs and limited data on the subject. However, they reference the work of Klein & Fischer (2019), who conducted an urban study in Germany and reported MP deposition rates ranging from 136.5 to 512.0 m−2/day−1, with particle sizes ranging from <0.063 mm to 5 mm. Although methodological differences make comparisons challenging, existing literature indicates MP depositions in both urban and rural areas, with no significant differences observed between them, and even in very remote areas.

Regarding the ingestion of particles, Deng et al. (2021) emphasized in their experiment that the size of the MPs ingested by bees ranged from 0.001 mm to 0.1 mm, indicating a certain selectivity in the ingestion of these contaminants. Buteler et al. (2022) conducted a study where bees were exposed to sucrose solution and MPs, finding that the length of ingested fibers varied between 0.05 and 1.24 mm, with a mean and standard deviation of 0.42 ± 0.25 mm. Similarly, Alma et al. (2023) subjected bees to sucrose solutions containing MPs, discovering the intake of fibers with a width of 0.04 mm and an average length of 0.39 ± 0.25 mm (mean ± SD). These findings collectively indicate the ingestion of plastic microparticles by bees, although there is no consensus regarding the maximum size of these particles that bees are capable of ingesting.

Regarding the prevalence of particles adhered to the bristles of bees, Negri et al. (2015) noted that during flight, the bodies of bees tend to acquire a positive static charge. Consequently, when landing on flowers, pollen particles adhere to the bristles of their bodies, and a similar effect occurs with other microparticles, including plastic particles.

In the chemical characterization, a prevalence of polyethylene terephthalate (PET) spectra was observed. PET is the most common thermoplastic in the polyester family, known for its high rigidity, mechanical and chemical resistance, and relatively low cost, making it widely used in packaging and synthetic fibers manufacturing (Quan et al., 2022). The increasing world population directly correlates with the rising annual consumption of PET. According to Velásquez et al. (2019), PET production reached 8400 kilotons in 2016, and it is estimated to increase by 6.9% between 2017 and 2025.

The increase in demand for PET production is indeed associated with the rise in improper disposal of this material and its environmental consequences. PET products can take 300 to 450 years to decompose naturally, and during this degradation process, they can break down into microplastics (MPs) (Khoonkari et al., 2015; Singh et al., 2021). Raheem et al. (2019) and Singh et al. (2021) underscore that PET waste currently contributes to 12% of the volume and 8% of the total weight of solid waste globally. Ortega & Cortés-Arriagada note that PET microplastics are present in the atmosphere in two forms: atmospheric and airborne (PET-MPs), and have been detected in Asia and Europe, with concentrations ranging between 1 and 400 particles/m2 per day (Raheem et al., 2019). Sax (2010) suggests that PET bottles may release endocrine disruptors into the liquid they contain, especially when subjected to prolonged storage and high temperatures, posing potential risks to human health upon ingestion.

Polyamide (PA), the second most observed spectrum, is a type of microplastic commonly known as nylon and extensively utilized in the textile and automotive industries for its electrical performance and abrasive resistance (Peng et al., 2020). This polymer is frequently found in various forms in sewage treatment plants, posing a significant concern for aquatic environments (Hamidian et al., 2021). A study by Choi et al. (2023) revealed that acute exposure to PA induced neurotoxicity in fish of the *Carassius carassius* species, resulting in a notable reduction in AChE activity, as well as lysozyme activity and IgM levels, due to the induction of immunotoxicity. Li et al. (2023) suggests that PA, along with other polymers, can adsorb additional substances and contaminants, particularly PA has a higher affinity for antibiotics compared to other polymers because of its specific functional group, amide; the incorporation of PA along with other contaminants can potentially exacerbate its effects on organisms.

Polyethylene (PE) was identified as the third most common spectrum. It is recognized as the first polymer used in the production of disposable plastic bags and has seen increased utilization in agricultural activities, particularly through the application of plastic films for soil cover (Steinmetz et al., 2016). Wang et al. (2022) highlight a nearly four-fold surge in the use of plastic films in agriculture over the past two decades in China, with residual PE film accounting for approximately 10% of the total planting area (Ramos et al., 2015). PE exhibits a certain degree of resistance to degradation, and its particles tend to accumulate in soil, where they can interact with other pollutants deposited in these environments (Wang et al., 2020a).

Polyester (PL) finds extensive usage in the textile industry. It comprises crystalline (high density) and non-crystalline regions, exhibits low moisture adsorption, and has the capability to accumulate electrostatic charges on the surface, while also forming fiber bundles (Grishanov, 2011). Wiśniowska & Włodarczyk-makuła (2022) underscore that the large surface area of these microfibers tends to adsorb other environmental pollutants. Studies such as those conducted by Jemec et al. (2016) demonstrate that such fibers can be toxic to organisms, as evidenced in the case of *Daphnia magna*, where ingestion of PL fibers led to increased mortality rates among individuals. Harrison (2018) indicates that a city with 100,000 inhabitants can produce between 5 to 60 kg of microfibers daily, which ultimately deposit into surface waters. It is also estimated that between 200 to 72,000 microfibers per gram of fabric can be released with every wash.

Polyvinyl chloride (PVC) boasts excellent physicochemical properties, which justify its widespread use in both industrial and domestic settings. The Research Report on China's Polyvinyl Chloride (PVC) Industry (2018) underscores that China has surpassed the US in PVC production since 2006. However, Wang et al. (2020b) report that much of the PVC produced is improperly disposed of, leading to its residual presence in numerous environmental matrices. Browne et al. (2010) highlight that PVC is the most common type of debris found in beach sands, constituting approximately 25% of the MPs present in estuaries. Several studies (Green et al., 2016; Lei et al., 2018) have documented toxicological effects following the ingestion of PVC microparticles. These effects range from reduced food intake to intestinal problems and death in species such as zebrafish (*Danio rerio*), nematodes (*Caenorhabditis elegans*), and annelids (*Arenicola marina*). Green et al. (2016) also emphasize that PVC's composition includes chlorine atoms, a characteristic that can exacerbate its toxicity in certain species.

Indeed, the toxic potential of all types of microplastics (MPs) identified by their spectra is well-documented across various organisms. However, in the case of bees, our understanding is still in its early stages. Studies have already indicated that ingestion of plastic microparticles can lead to changes in the gut microbiota, immune function, metabolism, and neuroendocrine crosstalk in bees (Wang et al., 2021). These findings underscore the importance of further research on this topic, particularly concerning the adsorption of microplastics to other contaminants present in the environment.

The data presented by ANOVA and PCA, indicating the predominance of internal microplastic (MP) particles in the urban area, can be attributed to the loss of habitat and natural resources typically associated with more degraded areas, which are characteristic of large urban centers. In such scenarios, animals often seek alternative means of survival and sources of resources, even if they are not of natural origin. This phenomenon is exemplified in a specific case involving megachilid bees (*Megachile rotundata* and *Megachile campanulae*), as reported by MacIvor and Moore (2013). These bee species have adapted to urban environments by utilizing materials such as polyurethane and polyethylene in nest construction and brood cell closure. These compounds are readily available and bear a resemblance to the plant materials these species traditionally use. This adaptation underscores the flexibility of certain species in urban environments and their ability to utilize anthropogenic materials for survival. Therefore, the predominance of internal MP particles in urban areas may be attributed to the increased availability and prevalence of anthropogenic materials, including plastics, in urban habitats, leading to inadvertent ingestion or incorporation into the bees' nests and bodies.

The predominance of external particles in points situated between peri-urban and rural areas, as opposed to the predominance of internal particles in urban areas, can indeed be linked to the transportation of these particles by air masses. Large urban centers, where these particles originate, emit microplastics into the atmosphere, which are then carried by air currents to more distant locations. Studies, such as those by Brahney et al. (2020), suggest that air masses play a significant role in transporting contaminants like microplastics over long distances. Bees, during their foraging flights, become more susceptible to contamination as they meet these particles suspended in the air. Therefore, the higher prevalence of external particles in peri-urban and rural areas could be attributed to the influx of microplastics from urban centers carried by air masses. This phenomenon underscores the interconnectedness of ecosystems and the far-reaching impact of human activities on environmental pollution.

5 Conclusion

It is concluded that *Apis mellifera* serve as effective bioindicators of MPs contamination across various environmental. The results suggested that individuals from urban areas have a higher number of internal particles when compared to those in peri-urban and rural areas. Consequently, these bees adapt to incorporating non-natural resources into their habits. The study also highlights those bees contributing to this transport by carrying particles during foraging activities and being susceptible to ingestion of these contaminants. Therefore, it is imperative to further investigate the effects of microplastic ingestion on bees, particularly considering the adsorption capacity of MPs to other potential contaminants, which are also ubiquitous in ecosystems. Understanding the interactions between microplastics and bees is crucial not only for safeguarding bee populations but also for protecting ecosystems and agricultural productivity.

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Data Availability All data supporting the findings of this study are available within the paper.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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