Microplastics in the Honey bee (*Apis mellifera*) from Urban Apiaries in Metropolitan Lima, Peru.

Microplásticos en la abeja melífera (*Apis mellifera*) en colmenares urbanos de Lima metropolitana, Perú.

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Highlights

- Contamination by microplastics (MPs) is evidenced in *A. mellifera* collected from urban environments in Lima.
- The colors of MPs (blue 71% and black 20%) reflect potential urban sources such as plastic containers and automotive products.
- Filament-type MPs measuring 40–585 µm were the most prevalent in *A. mellifera*.

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ABSTRACT

Introduction. Microplastics (MPs) are currently an emerging problem due to their slow degradation, and their wide distribution in soil and atmosphere, contributing to an environmental threat. The honey bee (*Apis mellifera*) serves as a crucial pollinator and environmental bioindicator, playing an essential role in agrobiodiversity. **Objective.** This study evaluated the presence of MPs in worker bees from urban hives in Metropolitan Lima, Peru. **Materials and Methods.** Honey bee samples were collected from three urban apiaries in the districts of Puente Piedra (north), La Molina (central), and Pachacamac (south) between October and December 2023. MPs were quantified in particles per 90 bees, considering both external and internal surfaces, and classified by shape, color, and size. **Results and discussions.** The presence of microplastics (MPs) was identified in *Apis mellifera* from all three evaluated apiaries. Similar quantities of MPs were observed in the apiaries located in Pachacamac (41 external particles and 10 internal) and La Molina (38 external particles and 12 internal), with lower quantities found in Puente Piedra (19 external particles and 15 internal). The external surfaces of *A. mellifera* had more MPs than the internal parts. Significant differences were found in the distribution of filamentous MPs across the apiaries. Filamentous MPs were predominant, with colors including blue, black, red, and white, and sizes ranging from 40 to 4400 µm. **Conclusions.** The findings and data suggest that worker *Apis mellifera* bees could serve as potential bioindicators of microplastic contamination in urban apiaries.

RESUMEN

Introducción. Los microplásticos (MPs) son actualmente un problema emergente debido a su lenta degradación, y a su amplia distribución en el suelo y en la atmósfera, lo que contribuye a una amenaza ambiental. La abeja melífera (*Apis mellifera*) es un polinizador y bioindicador ambiental de alta importancia para la agrobiodiversidad. **Objetivo.** El estudio evaluó la presencia de MPs en obreras de *A. mellifera* en colmenares urbanos de Lima Metropolitana, Perú. **Materiales y Métodos.** Se seleccionaron tres colmenares ubicados en los distritos de Puente Piedra (zona norte), La Molina (zona centro) y Pachacamac (zona sur) en Lima, Perú, y se recolectaron abejas melíferas entre octubre y diciembre de 2023. Se cuantificaron los microplásticos (MPs) en números de partículas de MPs/90 abejas, presentes en el exterior e interior corporal de las abejas, clasificándolos según forma, color y tamaño. **Resultados y Discusión.** Se identificó la presencia de MPs en *A. mellifera* en los tres colmenares evaluados. Las cantidades observadas de MPs fueron mayores en Pachacamac (41 partículas externas y 10 internas) y La Molina (38 partículas externas y 12 internas), que en Puente Piedra (19 partículas externas y 15 internas). Se encontraron diferencias significativas en la distribución de MPs filamentosos según el colmenar. Los MPs predominantes fueron filamentosos, con colores azul, negro, rojo y blanco, y tamaños entre 40 y 4400 µm. **Conclusiones.** Los hallazgos y datos sugieren emplear a las obreras de *A. mellifera* como un potencial bioindicador de contaminación por MPs en colmenares urbanos.

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INTRODUCTION

Over the past decade, microplastics (MPs) have drawn increasing attention as an emerging environmental contaminant [1,2,3,4]. These particles are found in a wide range of environmental matrices, including soil, water, air, and biota [4,5,6,7]. Growing concern surrounds their effects on living organisms, as they can lead to population declines and sublethal impacts [8,9].

Significant quantities of these plastic particles are released into the air during the manufacturing and routine use of plastic products [8]. MPs are polymeric particles defined as being no larger than five millimeters in diameter [10,11]. They are further classified into two subgroups: primary and secondary. Most primary MPs are directly manufactured as microscopic materials and are commonly used in consumer products such as cosmetics, detergents, and cleaning agents. Secondary MPs, on the other hand, result from the breakdown of larger plastic materials through natural degradation processes in air or water. Due to the vast amounts of plastic entering the environment, secondary microplastics are believed to be the most prevalent type [9,12,13,14].

MPs can affect critical ecosystem services, such as pollination. This service occurs through wind, water, or animals. It is estimated that nearly 75% of plants worldwide depend on animal pollination [15,16]. The connection between pollinators and plants is mutually beneficial: pollinators feed on nectar and pollen, receiving floral rewards, while improving plant reproductive success by transferring pollen between flowers [9,17]. Pollinating insects maintain healthy and genetically diverse plant ecosystems, are essential for crop pollination, and thus play a vital role in agro-biodiversity and global food security [18,19,20,21,22].

Apis mellifera (Hymenoptera: Apidae), or the honey bee, is a eusocial insect and frequent visitor to crops worldwide. It plays a key role in maintaining species diversity within ecosystems [15,23,24]. These bees actively interact with plants, air, soil, and water near their hives, exposing them to environmental contaminants. Such pollutants can adhere to their body hairs or be ingested, affecting both the bees and hive products [25]. While collecting nectar, honeydew, pollen, and other plant exudates, honey bees come into contact with all environmental compartments [15,26].

Honey bees are particularly suitable for monitoring environmental contamination due to their adaptability and ease of management. They are impacted by pesticides and other pollutants, such as MPs, heavy metals, and radionuclides [15,27]. Their sensitivity and wide foraging range make them potential sentinel insects for detecting trace contaminants in the environment [9,24,28]. Worker bees, in particular, can provide valuable insights into environmental quality, even beyond the immediate surroundings of their hives [29].

Previous research has shown that these contaminants can affect honey bees by altering their body size, gut microbiota, and susceptibility to viral infections. MPs have been found in hives, and honey bees have been used as bioindicators of urban and suburban pollution [4,8,9]. Consequently, they offer a practical means to assess contamination in urban hives within Metropolitan Lima, Peru. Based on these considerations, the present study aimed to evaluate the presence of MP contamination in *A. mellifera* from urban hives in Metropolitan Lima.

MATERIALS AND METHODS

Study Area. Worker bees (*A. mellifera*) were collected from three urban apiaries: (1) Virhuez Santiago (11°49'59"S; 77°05'53"W) located in the district of Puente Piedra (northern zone); (2) Miguel Felipe Dávila Noriega Apiary, part of the Universidad Nacional Agraria La Molina (UNALM) (12°04'44"S; 76°56'49"W), situated in the district of La Molina (central zone); and (3) La Arena Apiary (12°13'30"S; 76°50'60"W) in the district of Pachacamac (southern zone) **(Figure 1)**. The collection of *A. mellifera* was conducted based on a convenience sampling method between October and December 2023.

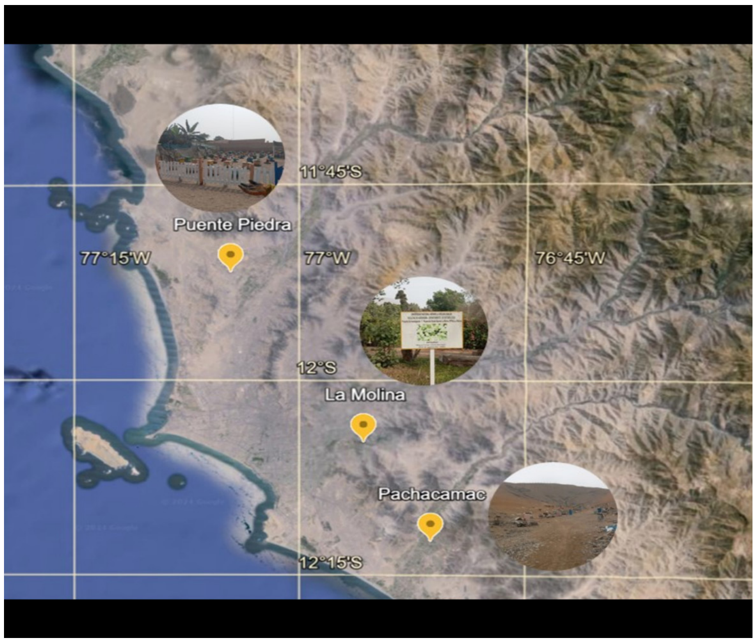

Figure 1. Cartographic map of the three *Apis mellifera* collection zones in Metropolitan Lima, Peru.

Biological Material

Worker bee specimens (*A. mellifera*) were collected from the three apiaries (zones). Two hives were selected from each location. The bees were collected directly by blocking the hive entrance (a small opening at the base of the hive that facilitates the bees' entry and exit at any time) [30,31]. A total of 90 individuals were obtained from each zone. Each evaluation zone included three replicates per hive, resulting in six replicates per zone, grouped in sets of 15 individuals. These groups were weighed collectively using a four-decimal precision analytical balance before undergoing the digestion process. In total, 270 worker honey bees were analyzed.

Procedure for detecting microplastics in *A. mellifera*

Microplastics were extracted in groups of 15 bees per sample, with six samples analyzed per hive. MPs were evaluated on the bees' body surfaces and cuticles, as well as in their digestive tracts. The process was performed in three stages: washing, digestion, and filtration.

Bees were washed for 15 minutes using ethanol and ultrapure water to remove external particles, including MPs. The solution was filtered through a 47-mm diameter nitrocellulose membrane with a 6-µm pore size using a vacuum pump (Rocker 400®) [32,33,34]. The samples were digested using 30% hydrogen peroxide for 24 hours at 60°C in an incubator (Biobase, China). The process continued until no visible tissue remained. The supernatant was then filtered using the vacuum pump [35]. MPs were analyzed from external, internal, and total sources on the bees. The number of particles was expressed as MPs per 90 bees.

Classification of MPs: Microplastics were categorized by shape into four groups: fragments, fibers, filaments, and pellets. Fragments were defined as hard, irregular plastic particles with a length no more than four times their width. Fibers and filaments were elongated particles with a length exceeding four times their width; fibers were straight, while filaments were flattened and rounded. Pellets were hard, rounded plastic particles. MPs were further classified by color, including blue, fuchsia, red, black, light blue, yellow, and white. Each particle was measured using a calibrated micrometric ocular lens (Nikon**®)** and a standard calibration slide [36].

Quality control measures were implemented to minimize external contamination. The individual collecting the bees wore non-synthetic clothing and nitrile gloves. In the laboratory, all glassware and stainless-steel tools were thoroughly cleaned with ethanol and ultrapure water, wrapped in aluminum foil, and baked at 250°C for 6 hours. All solvents used were filtered through 0.45-µm filters at least twice. Sample processing was conducted within a laminar flow hood. During the procedure, laboratory coats (100% white cotton) and nitrile gloves were used. Controls were prepared daily to assess background contamination from solvents or laboratory equipment. Results were corrected by subtracting MPs identified in the controls, where 1–2 particles were typically observed.

Data analysis

The results for MP characterization were expressed as mean ± standard deviation (SD). The normal distribution of the data was assessed using the Shapiro-Wilk test, and Levene's test was applied to evaluate the homogeneity of variances. A Chi-square test (X^2) was used to associate the total number of MPs and their shapes with the hive zones (Puente Piedra, La Molina, and Pachacamac). ANOVA was performed to determine whether MP sizes differed across the evaluated zones. Pearson's correlation coefficient was used to assess the degree of association between the weights of groups of 15 bees and the total, external, and internal MPs recorded. All analyses were conducted with a 95% confidence level using SPSS statistical software, version 25.

Ethical considerations

All Peruvian and international institutional regulations were followed, including obtaining authorization from hive managers and adhering to biosafety and waste disposal protocols for chemical and biological substances. This study was approved by the Ethics Committee of the Faculty of Natural Sciences and Mathematics at the Universidad Nacional Federico Villarreal (Lima, Peru), under resolution 003-2023-Ethics Committee.

RESULTS

The quantity and percentage of MPs categorized by shape, hive location, and the external and internal parts of *A. mellifera* are detailed in **(Table 1)**. Similar amounts of MPs were observed in hives located in Pachacamac (41 external particles and 10 internal) and La Molina (38 external particles and 12 internal), with a lower amount recorded in Puente Piedra (19 external particles and 15 internal) (X^2 = 6.62, p = 0.03) **(Table 1)**. Filamentous MPs were the most abundant type across all three hives, followed by fragments and fibers. Notably, fibers were found exclusively in the hive located in La Molina (central zone). Overall, the body surface (72.6%) exhibited a higher quantity of MPs compared to the digestive tract (27.4%) in *A. mellifera*.

When filamentous MPs were associated with external and internal parts of the bees from each of the three hives, significant differences were observed (X^2 = 10.37, p = 0.005). The hive in Puente Piedra showed distinct differences in filamentous MPs compared to the other two locations. However, when fragment-type MPs were associated with the parts of the bees and the hive locations, no significant differences were found (X^2 = 2.63, p = 0.26).

Table 1. Quantity (MP particles/90 bees) and percentage of microplastics by shape, hive location, and body region of *Apis mellifera* in Metropolitan Lima, Peru.

Type	Pachacamac			La Molina			Puente Piedra			Total			
	E	I	%	E	I	%	E	I	%	E	I	T	%
Filament	40	6	90.2	27	4	62	19	13	94	86	23	109	**80.7**
Fragment	1	4	9.8	10	7	34	0	2	5.9	11	13	24	**17.8**
Fiber	0	0	0	1	1	4	0	0	0	1	1	2	**1.5**
Total	**41**	**10**		**38**	**12**		**19**	**15**		**98**	**37**	**135**	
%	**80.4**	**19.6**		**76**	**24**		**55.9**	**44.1**		**72.6**	**27.4**		

Body surface (E = external) and digestive tract (I = internal), (T= total)

Table 2. Maximum (Max), minimum (Min), and mean values in micrometers (µm) of microplastic sizes by shape and hive location of *Apis mellifera* in Metropolitan Lima, Peru.

Type	Pachacamac			La Molina			Puente Piedra		
	Max	Min	Mean + SD	Max	Min	Mean + SD	Max	Min	Mean + SD
Filament	4.398	41	745 ± 1.007	3.755	147	1.015 ± 944	2.929	185	725 ± 664
Fragment	344	116	190 ± 89	419	41	490 ± 102	738	325	488 ± 292
Fiber	0	0	0	741	496	610 ± 181	0	0	0

SD = standard deviation.

The minimum, maximum, and mean sizes recorded during the characterization of MPs by shape and hive location in *Apis mellifera* are presented **(Table 2)**. Filaments exhibited the highest maximum and mean values in bees from all three hives. No differences were observed in the sizes of total MPs (F = 0.81, p = 0.46), external MPs (F = 3.09, $p = 0.07$), or internal MPs (F = 0.76, $p = 0.48$) in *A. mellifera*. However, fragment sizes were, on average, smaller in Pachacamac compared to the other two hives ($p < 0.05$) **(Table 2)**. Fibers were exclusively found in the hive located in La Molina. The dominant colors observed across all evaluated sites were blue > black > red > white **(Figures 2, 3, and 4)**. Regarding size, MPs were most frequently recorded in the 40–585 μm range, followed by the 586–1131 μm range **(Figure 5)**. No differences were observed in the group weights of bees evaluated across the three hives (F = 1.62, p = 0.23), and no correlation was found between this parameter and the total MPs (r = -0.11, $p = 0.63$), external MPs (r = -0.02, $p = 0.90$), or internal MPs (r = -0.21, $p = 0.38$).

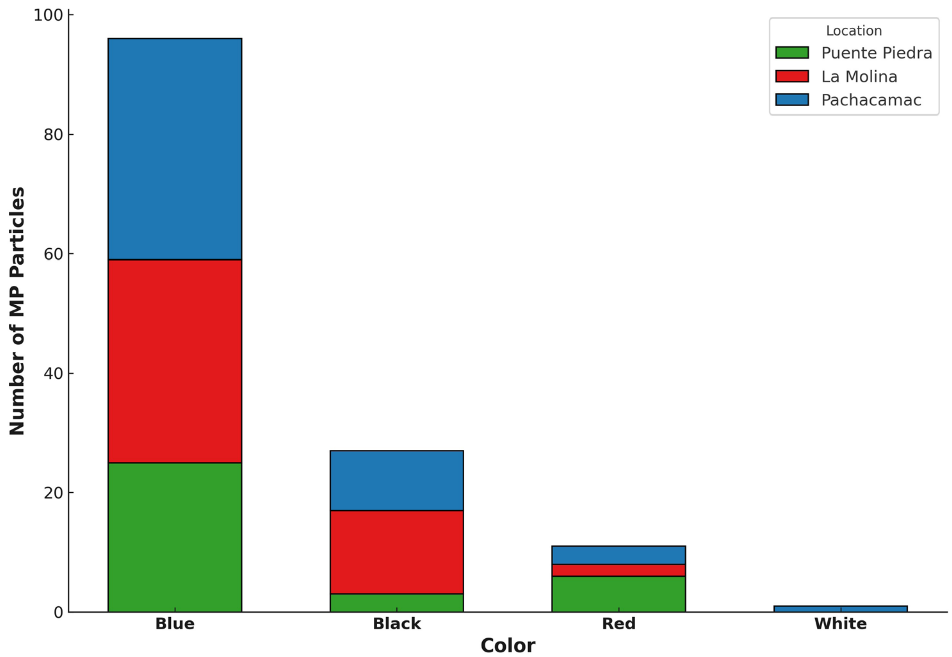

Figure 2. Quantification of microplastics by color in the three hives of *Apis mellifera* in Metropolitan Lima, Peru. Blue = Pachacamac, Red = La Molina, Green = Puente Piedra.

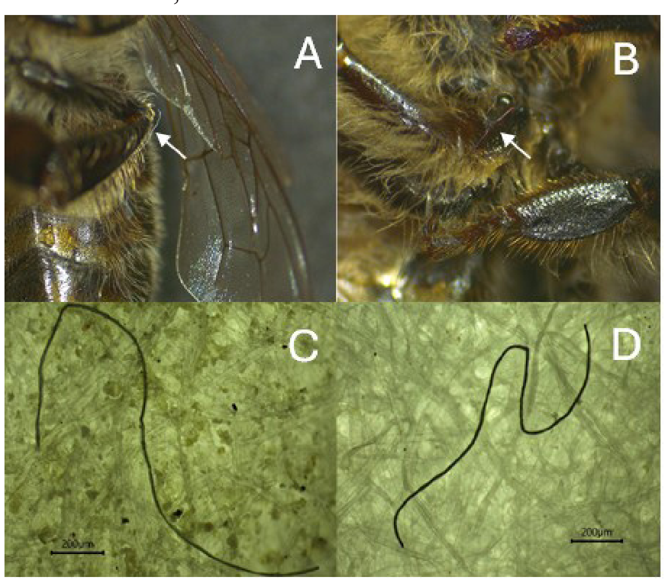

Figure 3. Microplastics on the body surface of two Apis mellifera specimens, located on the leg (A) and mesocoxa (B), respectively. Microplastics found in the digestive tract after the digestion process (C y D).

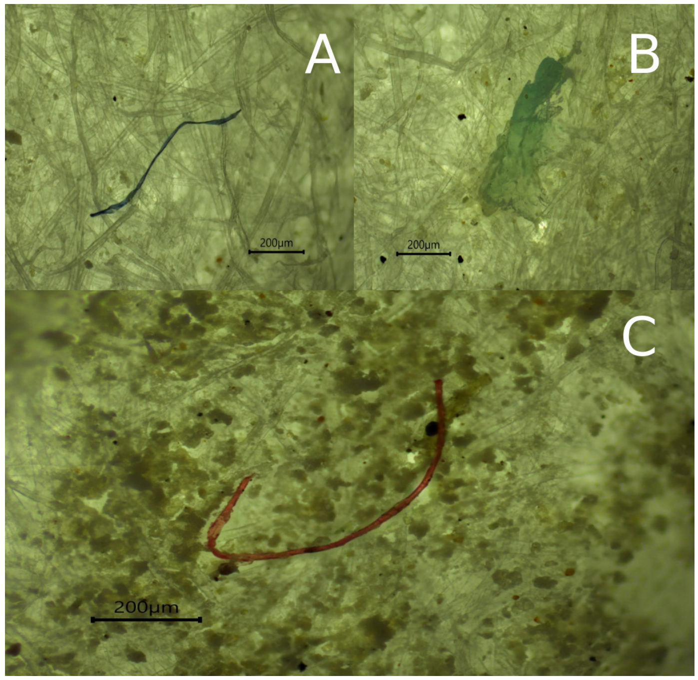

Figure 4. Diversity of colors and shapes of the microplastics found. A: Blue filament; B: Blue fragment; C: Red filament. Measurements in µm.

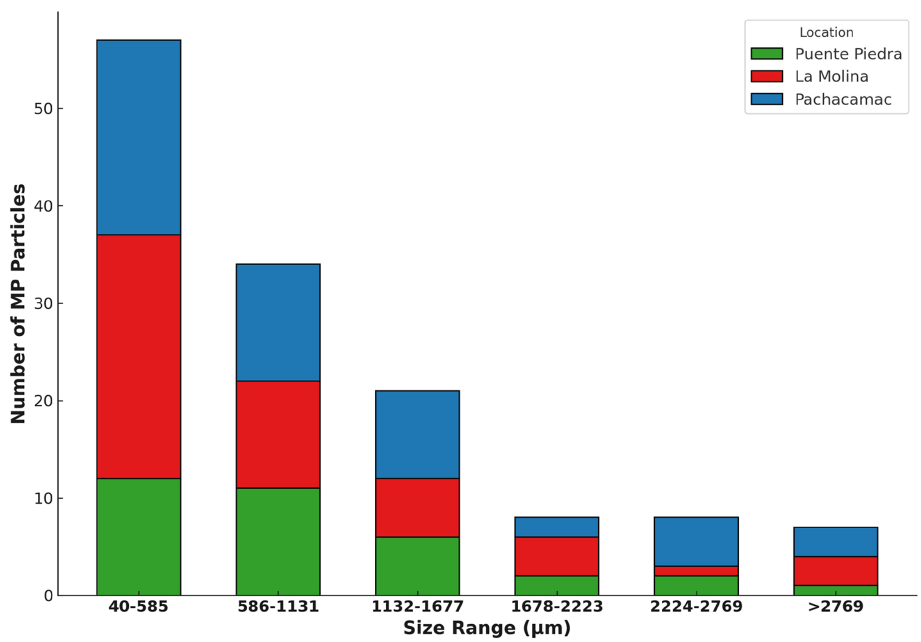

Figure 5. Quantification of microplastics by size range (in micrometers, µm) in the three hives of *Apis mellifera* in Metropolitan Lima, Peru. Blue = Pachacamac, Red = La Molina, Green = Puente Piedra.

DISCUSSION

Recent studies indicate that atmospheric pollution in urban areas has shown that MPs may originate from synthetic textiles, cutting, shredding, or degradation of industrial synthetic macroplastics, tire wear, waste incineration, agricultural activities, and other sources [8]. These findings are consistent with the results from the three sampled areas, where MPs were identified in honey bees in Metropolitan Lima, Peru.

MP contamination in honey bees stems from diverse environmental sources. In this study, comparable quantities of MPs were observed in two hives located in Pachacamac (southern zone) and La Molina (central zone). The findings suggest that the use of plastic feeding chambers in Pachacamac may contribute to higher MP concentrations, acting as a direct and consistent contamination source for hives and bees. Additionally, scientific literature indicates that MPs can originate within the hive itself due to plastic components and management practices, such as the use of disposable microfiber cloths for pest control [37].

In La Molina, exposure to indirect sources, such as extensive anthropogenic activities near the hive, located on the grounds of the National Agrarian University La Molina (UNALM), appears to be a determining factor in the presence of MPs in *A. mellifera* hives [38]. Conversely, the hive in Puente Piedra (northern zone) exhibited a lower quantity of MPs, possibly due to better resource management and hive maintenance practices in relation to its environment [39]. Previous studies have documented that bees become contaminated with MPs primarily through foraging in polluted areas or via airborne particles. Bees ingest MPs from contaminated water, nectar, and pollen during foraging. Additionally, airborne MPs deposit on flowers and plants, which bees collect and bring back to the hive [37].

Studies have demonstrated that atmospheric MP pollution is closely linked to human activities, anthropogenic factors, population density, and industrialization levels [8]. Various mechanisms, including transport, dispersion, and deposition, influence MP movement in the atmosphere, as observed across the three urban zones evaluated in Metropolitan Lima [37].

Regarding MP morphology, filaments were the most abundant type across all three hives, followed by fragments and fibers. Filaments were associated with the bees and hives in all three locations, whereas such an association was not observed for fragments. Previous studies report varied results in *A. mellifera* hives, with some indicating a predominance of fragments varying significantly by location and season, and others of fibers [8, 35]. However, no research highlights filament-type MPs [26,40]. MP characteristics in urban environments vary greatly, as these particles are easily transported over long distances by wind or human activity, such as the clothing or materials people carry in daily life, potentially explaining the results obtained [37,38].

MP morphological characterization is crucial because particles with larger surface areas, such as fragments, provide more adsorption sites for organic compounds, increasing the likelihood of MPs retaining potentially toxic substances [8].

MPs were predominantly found on the body surface of *A. mellifera*, adhering to the cuticle in areas such as hairs, wings, and legs, although they were also identified in the digestive tract [9,37]. These findings align with previous studies suggesting the potential of *A. mellifera* as an environmental bioindicator of MPs in urban settings, showing both external and internal contamination [35]. Given that bees can travel hundreds of meters and visit up to a thousand flowers daily, they serve as excellent environmental indicators of MPs under natural conditions [24].

In this study, blue MPs were the most prevalent (71%), followed by black (20%), red (8%), and white (1%). These results are consistent with findings from research conducted in five hives in Denmark, published in 2024 [8]. The colors of MPs can provide insights into their sources: blue may originate from bottle caps and plastic containers used as feeding chambers, black from tires and automotive products, red from synthetic clothing materials, and white from packaging materials [42]. Moreover, MP color can also suggest associations with chemical additives or infer potential environmental risks [34,41].

Most MPs were observed within the size ranges of 40–585 µm and 586–1131 µm [8]. An analysis of these size ranges revealed that most of fibers measured 100–500 µm, representing 60% of the elements found in bees, followed by fibers over 500 µm (35%). For fragments, 88% were smaller than 100 µm [8]. In *A. mellifera*, fibers ranged from 79–911 µm, and fragments from 31–57 µm [8]. Besides MPs, other microparticles, such as cotton, wax, plant debris, and unidentified particles, were also found on the bees' bodies [35].

Future studies should further investigate the sources of MPs in terrestrial environments [4]. With over 20,000 bee species worldwide, honey bees are not the only pollinators potentially affected by MPs. Wild pollinators, including solitary bees, flies, butterflies, beetles, wasps, moths, birds, bats, and lizards, may also be impacted [9]. In urban environments, it is crucial to assess MP levels and their effects on *A. mellifera* in Peru, as MPs can harm their roles as pollinators and producers of honey and other byproducts, causing ecological and economic losses [14,43,44,45]. Other hive matrices, such as pollen and propolis, could also be analyzed for MP contamination and other potentially toxic compounds. The WHO's One Health Initiative, which aims to balance and optimize the health of people, animals, and the environment, offers a valuable framework for addressing this issue [9].

In this study, the lack of complementary analytical techniques for polymer identification beyond visual characterization constitutes a significant limitation. Future research should include chemical analytical methods, such as micro-Fourier transform infrared spectroscopy (micro-FTIR) [8,35] or Raman spectroscopy [6]. These approaches would provide more precise evaluations of MPs' impacts on bee health and the urban ecosystem. Despite precautions to minimize atmospheric contamination, the possibility of false positives must be acknowledged, as noted in previous studies [35,46].

Finally, based on the results of MP presence in honey bees, it is recommended to evaluate: (i) the potential negative effects of MPs on brood patterns, queen egg-laying, drone vitality, and colony vigor; (ii) MP absorption and accumulation in bee tissues, and whether these are influenced by MP shape, size, and color; (iii) the combined effects of MPs and other environmental contaminants, such as heavy metals, pesticides, nanomaterials, parasites, and pathogens on bee health; and (iv) the potential role of MPs as vectors for honey bee pathogens [9,37].

CONCLUSIONS

This study confirms the significant presence of microplastics (MPs) in honey bees (*Apis mellifera*) from different urban zones in Metropolitan Lima, highlighting variability in MP frequency and morphology among the evaluated areas and their association with anthropogenic activity. The results emphasize the importance of considering *A. mellifera* as an environmental health bioindicator in urban settings, as their foraging behavior exposes them to multiple sources of MPs.

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