



## Review

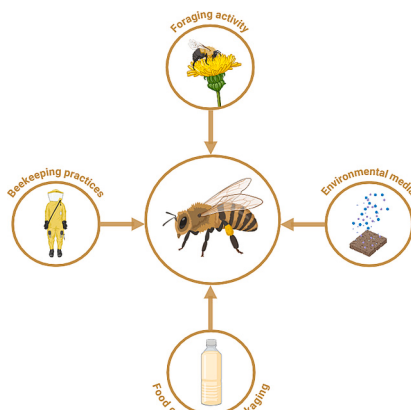
## Honeybees as active bioindicators of plastic pollution: Environmental exposure, analytical strategies, and monitoring perspectives

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## HIGHLIGHTS

- Honeybees act as proactive bio-indicators of plastic pollution.
- Microplastics can accumulate in honeybee tissues and hive-derived products.
- Environmental and human factors explain geographic variation in contamination.
- Analytical strategies involve microscopy, spectroscopy, and chromatography.
- Standardized and green analytical methods are urgently needed in this field.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Honeybee (*Apis mellifera*) has emerged as a valuable bioindicator for environmental pollution due to its morphological and behavioural traits, wide distribution, short life cycle, high reproductive rate, and ease of domestication. Through their extensive foraging activity, bees interact with multiple environmental compartments. They are increasingly exposed to a wide range of contaminants, including plastic-related compounds. In this work, we examine the potential of honeybees as active samplers of plastic pollution and identify the main exposure pathways. A brief overview of current sampling approaches, sample preparation techniques, and analytical strategies for detecting plastic contaminants is also presented. This study offers practical insights for beekeepers and policymakers to promote more sustainable hive management practices that reduce plastic exposure. Finally, we emphasize the need for harmonized methodologies, expanded geographic monitoring, and

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interdisciplinary collaboration, alongside the adoption of greener and standardized analytical techniques to improve detection accuracy and mitigate environmental impact.

## Abbreviations

|          |                                                |
|----------|------------------------------------------------|
| EDX      | energy-dispersive X-ray spectroscopy           |
| GC–MS    | gas chromatography–mass spectrometry           |
| LC-MS/MS | liquid chromatography-tandem mass spectrometry |
| MPs      | microfibers                                    |
| MPs      | microplastics                                  |
| NPs      | nanoplastics                                   |
| Py-GC–MS | pyrolysis-gas chromatography–mass spectrometry |
| SEM      | scanning electron microscopy                   |
| SML      | specific migration limits                      |
| TEM      | transmission electron microscopy               |
| WAC      | white analytical chemistry                     |
| μFTIR    | micro-Fourier transform infrared spectroscopy  |
| μRaman   | micro-Raman spectroscopy                       |

## 1. Introduction

Plastic pollution has emerged as one of the most pressing environmental challenges of the 21st century. While the initial concern centered on visible plastic waste accumulating in oceans and landscapes, attention has increasingly shifted toward the microscopic dimension of this problem (Alma et al., 2023). Microplastics (MPs), typically defined as plastic particles ranging from 1 μm to 5 mm in size, and nanoplastics (NPs), with dimensions below 1 μm, have been detected in air, water, soil, and biota, food and human samples (Basaran et al., 2024). These particles originate either as primary MPs, which are intentionally manufactured for use in commercial applications, or as secondary MPs, which result from the fragmentation and degradation of larger plastic debris through environmental processes (Edo et al., 2021). Their small size, mobility, and persistence in the environment raise significant concerns about their potential to infiltrate biological systems and enter the food chain (Martín-Gómez et al., 2024a). For many years, plastic particles were considered too large to penetrate the physical barriers of intact plant tissues. However, recent findings have challenged this assumption, revealing that both MPs and NPs have the potential to contaminate edible plants, including vegetables commonly consumed by humans (Li et al., 2020). In that study, the authors investigated the uptake of different types of MPs by crop plants such as wheat and lettuce, using treated wastewater under various growing conditions, including hydroponic systems, sand matrices, and sandy soils. Such evidence highlights a critical pathway through which MPs could infiltrate the food chain, the honeybees and its products (Naggar et al., 2021). This widespread distribution has led them to be called as “contaminants of emerging concern” and “everywhere chemicals” (Liu et al., 2021; Vighi et al., 2023). They possess considerable toxicological potential for ecosystems and living organisms, raising serious concerns about their long-term impacts.

Despite the growing awareness, current methods for environmental monitoring of MPs and related plastic additives (such as phthalates and bisphenols) remain limited in scope and effectiveness. Traditional sampling strategies often involve stationary or passive collection systems that fail to capture the dynamic and spatially heterogeneous nature of airborne and terrestrial MPs. In this context, the use of bioindicators has gained increasing attention. Organisms that interact closely with their environment can serve as integrative samplers of pollution, reflecting both the presence and intensity of contaminant exposure (Oliveira et al., 2019). Among potential candidates, the Western honeybee (*Apis mellifera*) has emerged as a particularly promising bioindicator (Martín-Gómez et al., 2024b).

Honeybees play a crucial role as pollinators worldwide, supporting

the reproduction of numerous flowering plants, including many key agricultural crops essential for human consumption. Features such as pollen baskets on their hind legs and branched body hairs allow them to efficiently collect and transfer pollen between flowers. As a result, honeybees contribute significantly to the pollination of a wide range of crops, including fruits, vegetables, nuts and oilseed crops. Honeybees have long been recognized as effective biological monitors for a variety of environmental pollutants, including heavy metals or pesticides (Fuente-Ballesteros et al., 2024b) due to their high sensitivity to stressors, broad foraging range, and continuous interaction with multiple environmental compartments. In previous monitoring studies, researchers have collected and analyzed honeybee bodies to detect the external deposition and ingestion of contaminants (Gómez-Ramos et al., 2019), examined hive products as indirect indicators of environmental exposure (Glinski et al., 2024), and implemented non-invasive in-hive passive samplers that trap pollutants (Murcia-Morales et al., 2020). These complementary approaches demonstrate the versatility of honeybees as bioindicators in environmental monitoring.

These characteristics also position them as promising sentinels for emerging forms of pollution, such as MPs and other plastic-related contaminants. However, despite this potential, only a few studies to date have directly examined whether exposure to plastic pollutants affects colony vitality, leaving a critical gap in our understanding. This is particularly relevant given that previous research has already raised concerns about the harmful effects of other emerging pollutants - such as environmental contaminants, metals, metal nanoparticles, and nanotechnology-based pesticides - on honeybee health (Hooven et al., 2019).

This work presents an up-to-date and integrative perspective on the role of honeybees as proactive samplers of plastic pollution. It explores their potential to detect plastic-related compounds, the mechanisms of exposure, analytical strategies for detection, current evidence, and the implications for environmental monitoring. We aim to underscore the importance and urgency of advancing research in this field, as a means to preserve honeybee populations, strengthen environmental surveillance systems, and contribute to the broader framework of planetary health.

## 2. Honeybees as proactive samplers of plastic pollution

The concept of a proactive bioindicator refers to an organism that not only accumulates contaminants passively from its surroundings but actively engages with the environment in a manner that enhances its potential as a sampler. In the case of honeybees, their biology and behavior provide multiple pathways for the acquisition and transport of pollutants. Unlike other biomonitors such as mosses, lichens or fishes, honeybees possess excellent characteristics and actively forage over several kilometers, interacting with all environmental domains (air, water, soil, and vegetation) (Martín-Gómez et al., 2024b) and anthropogenic sources (Papa et al., 2024) (see Table 1). This extensive spatial coverage allows them to act as natural vectors of environmental contamination (see Fig. 1). Other organisms, including bivalves, earthworms, and seabirds, have been used as bioindicators of pollution, particularly in aquatic and soil systems. However, honeybees offer distinct advantages, and they are classified as good biological indicators of environmental contamination (Schiano et al., 2024). Their wide flying capacity, great mobility, and social structure ensure that collected pollutants are brought back to the hive where they can accumulate and be detected in hive products (Alma et al., 2023). Additionally, honeybee colonies are widely distributed across urban, agricultural, and natural environments, facilitating comparative and large-scale monitoring.

**Table 1**  
Comparison of honeybees and other widely used bioindicators of environmental contamination.

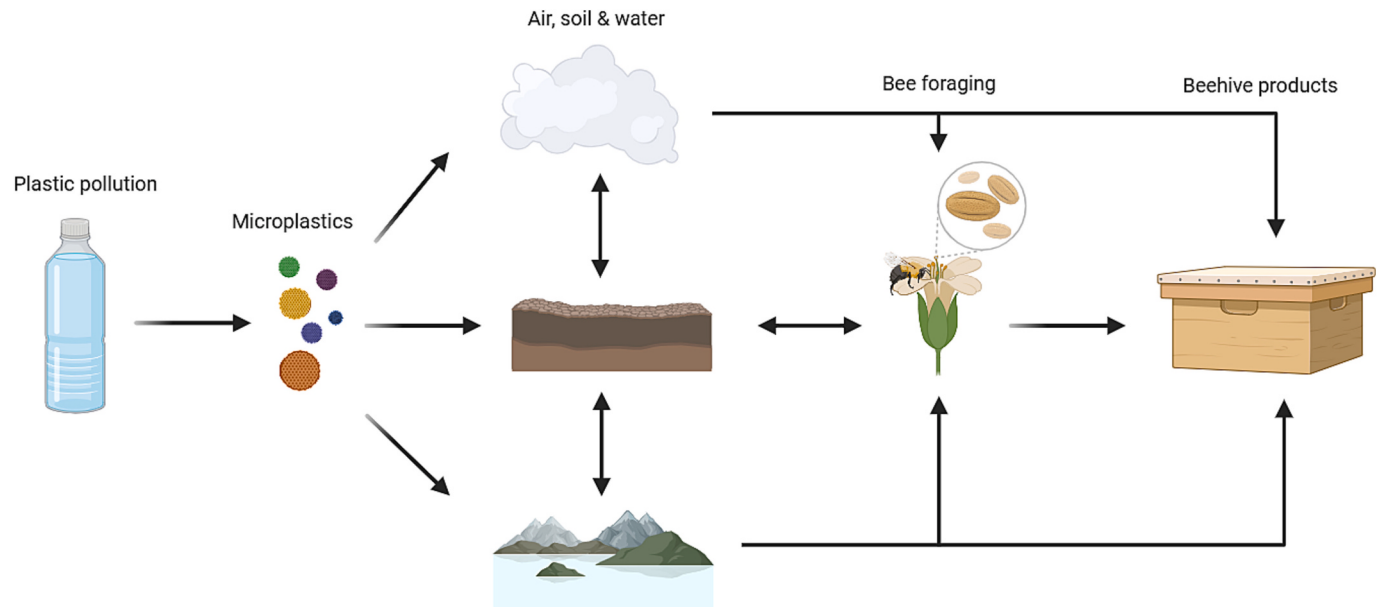
| Bioindicator | Advantages                                                                                                                                                                                                    | Disadvantages                                                                                                                                                 |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Honeybees    | Wide foraging range (up to several km); integrate pollutants from multiple compartments (air, water, soil, vegetation); easy sampling via bees, hive products, or passive samplers; citizen science potential | Sensitive to multiple stressors (pesticides, pathogens, climate), which may confound results; seasonal activity; ethical considerations in colony management  |
| Mosses       | No root system, pollutants mainly absorbed from atmosphere; accumulate trace metals and airborne particles; cost-effective and easy to transplant; tolerant to harsh environments                             | Provide only local information (few cm <sup>2</sup> surface); slow growth; limited to airborne contamination, no insight into soil/water matrices             |
| Lichens      | Long lifespan; high capacity to accumulate metals, persistent organic pollutants, and airborne particles; widely distributed; strong indicator of air quality                                                 | Sensitive to environmental stress (e.g., drought, UV); difficult taxonomic identification; reflect mainly long-term exposure, less suitable for rapid changes |
| Fishes       | Reflect waterborne contamination (organic pollutants, metals, microplastics); integrate exposure over time; relevant for food safety and human health risk                                                    | Ethical and legal restrictions in sampling; mobility makes spatial attribution difficult; bioaccumulation depends on age, diet, and species                   |

Honeybees possess several traits that make them particularly suited to monitoring plastic-related compounds through multiple proposed exposure pathways (Alma et al., 2023) (see Table 2).

Firstly, the passive mode is considered. Hairy bee bodies generate electrostatic charges during flight, enhancing the adhesion of airborne microfibers (MFs) and particles (Edo et al., 2021). These contaminants can attach passively to the bee's exoskeleton, especially on the thorax and legs (Negri et al., 2015). MP fibers can easily remain in the bee's cuticle, digestive tract or even transfer to larvae, honey and wax (Alma et al., 2023; Buteler et al., 2022). The presence of MF and microfragments on the wings and the proboscis was also observed (see Fig. 2) (Schiano et al., 2024). Edo et al. reported that polyethylene was one of

**Table 2**  
Main characteristics of honeybees as proactive bioindicators of plastic pollution.

| Feature                                     | Description                                               | Relevance to Plastic Monitoring                                                               |
|---------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Foraging range                              | 2–10 km radius around the hive                            | Enables wide spatial sampling across ecosystems (urban, rural, natural)                       |
| Interaction with environmental compartments | Air, water, soil, vegetation, artificial surfaces         | Increases likelihood of contact with diverse pollution sources                                |
| Flight behavior                             | Active and electrostatically charged during flight        | Enhances passive adhesion of airborne microfibers and particles                               |
| Water collection behavior                   | Bees collect water for hive thermoregulation and humidity | Potential ingestion or transport of MPs and plastic additives from contaminated water sources |
| Pollen and nectar collection                | Daily contact with flowers and plant surfaces             | Risk of misidentification and collection of microplastics mimicking pollen grains             |
| Hive centralization                         | All collected materials are brought back to the hive      | Facilitates accumulation and detection in products such as wax, honey, propolis, and pollen   |
| Social structure                            | Tens of thousands of individuals per colony               | Enables large-scale exposure and representative sampling                                      |
| Year-round activity (in temperate climates) | Active foraging over several seasons                      | Allows temporal monitoring and assessment of seasonal variations in contamination             |
| High metabolic sensitivity                  | Physiologically responsive to environmental stressors     | Sublethal effects of MPs (e.g., immune, gut, neurological) can be detected                    |
| Widely distributed colonies                 | Beekeeping present in most global regions                 | Enables global comparisons and large-scale monitoring networks                                |
| Use in previous biomonitoring studies       | Proven bioaccumulator of pesticides, heavy metals, PAHs   | Demonstrates analytical feasibility and historical precedent                                  |
| Human relevance                             | Bee products are consumed by humans (honey, pollen, wax)  | Contamination may reflect human exposure risk as well                                         |
| Ecological role                             | Key pollinators in agroecosystems                         | Links environmental monitoring to food production and ecosystem health                        |



**Fig. 1.** Environmental microplastic mass flow and its potential transfer to honeybees and hive-derived products. Reproduced from Naggar et al. (2021).

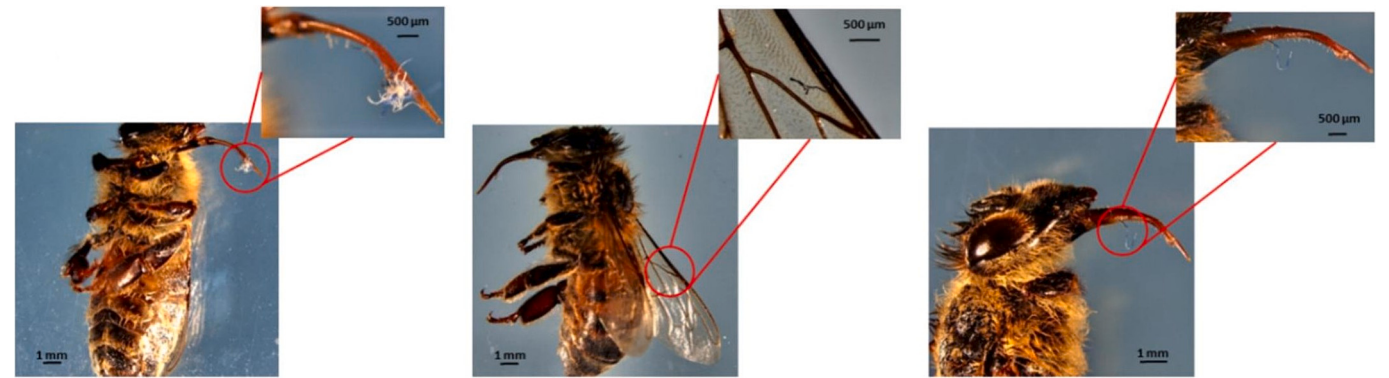


Fig. 2. Optical micrographs showing microfibers and microfragments deposited on the body of honeybees. Reprinted with permission from Schiano et al. (2024) Copyright©Elsevier.

the common polymers found in the bodies of honeybees (Edo et al., 2021). Similarly, Schiano et al. hypothesised that MFs adhering to their bodies originate from textile fibers released into the air, which are subsequently captured by the bees during flight (Schiano et al., 2024). Furthermore, bees may ingest microparticles during foraging, mistaking them for pollen due to similar size, shape, or coloration. Brightly colored MPs may mimic pollen grains, leading to unintentional collection and transport back to the hive. Bees also gather water from puddles, streams, and artificial sources for thermoregulation and hive humidity control. If these water or other natural sources are contaminated with MPs or plastic additives, bees may become vectors for their distribution (Martín-Gómez et al., 2024c).

In addition to these pathways, there is evidence that bees, particularly solitary species, may use plastic fragments as construction material (MacIvor and Moore, 2013). Bees also collect plant resins to produce propolis, a key substance used for hive maintenance and antimicrobial defense. If the plant surfaces or exudates are contaminated, plastic particles may inadvertently be incorporated into the propolis. Moreover, production processes that involve direct contact with unsuitable plastic cups, containers/materials, honeycombs extractors, or uncappers can contribute to contamination (Fuente-Ballesteros et al., 2024a). Since MPs ubiquitously exist in the atmosphere it is difficult to assess the possible sources of plastics although some potential sources were described (Basaran et al., 2024) (see Table 3). For example, Mühlischlegel et al. reported low levels of MP contamination in commercial honey samples, which they attributed to textile fibers originating from beekeepers' clothing (Mühlischlegel et al., 2017). Katsara et al. noted that plastic packaging materials such as polyethylene terephthalate is used to store honey in small, flexible packages, which also increases the risk of MP migration (Katsara et al., 2024). Schiano et al. identified polycaprolactone MPs in honey samples confirming that biodegradable materials could be further pollutants in the environment (Schiano et al., 2024). Another understudied source of contamination is the use of MF sheets to trap the hive beetle (*Aethina tumida*). Buteler et al. investigated the effects of placing non-woven MF wipes within beehives as a management method (Buteler et al., 2023). Once introduced, bees chew on these sheets, causing them to fray and become fuzzy, which effectively traps the beetles in the tangled fibers. After three months the researchers found that both the honey and the bees - specifically their gut and cuticle - were contaminated with MFs. These findings raise significant concerns about the unintentional introduction of MP pollutants into the ecosystem and the food chain through commonly used beekeeping practices (Shavali Gilani et al., 2025).

However, more recent studies have demonstrated that both air and water are widely contaminated with MPs, suggesting that honeybees may predominantly incorporate these environmental MPs into the hive (Beaurepaire et al., 2021). Regardless of the source of contamination, several potential solutions have been proposed in the literature (Basaran

Table 3  
Potential sources of plastic-related compounds detected by honeybees.

| Polymer / Additive                 | Common Use / Source                          | Example of Bee Exposure Route                                           |
|------------------------------------|----------------------------------------------|-------------------------------------------------------------------------|
| Polyethylene                       | Plastic bags, packaging films                | Carried by wind to flowers or soil; contact during foraging             |
| Polypropylene                      | Food containers, bottle caps, fibers         | Found in dust particles, agricultural residues                          |
| Polyethylene terephthalate         | Beverage bottles, synthetic textiles         | Fiber release from clotheslines, urban aerosols                         |
| Polystyrene                        | Disposable cutlery, insulation foam          | Degraded foam near urban waste, transport via air                       |
| Polyvinyl chloride                 | Pipes, flooring, banners                     | Weathered materials in urban/rural settings; dust inhalation or contact |
| Polyamide                          | Fishing nets, clothing, ropes                | Fiber contamination near water sources or vegetation                    |
| Styrene-Butadiene Rubber           | Vehicle tires                                | Tire wear particles in road dust; urban and roadside foraging areas     |
| Phthalates                         | Plasticizers in flexible plastics            | Contaminated water or contact with treated surfaces (e.g., greenhouses) |
| Bisphenol A                        | Food can linings, epoxy resins, receipts     | Ingestion via pollen or plant resin from contaminated sources           |
| Brominated flame retardants        | Electronics, upholstery, building materials  | Atmospheric deposition; contact with surfaces near human settlements    |
| Polyfluoroalkyl substances         | Stain-resistant coatings, firefighting foams | Contaminated water used by bees for cooling the hive                    |
| Acrylic polymers                   | Paints, adhesives, textiles                  | Fragments on plant surfaces or aerosols in urban areas                  |
| Cellulose acetate                  | Cigarette filters, optical films             | Fragmentation of discarded filters in foraging zones                    |
| Microbeads                         | Personal care products (toothpastes, scrubs) | Washed into water bodies and accessible to bees collecting water        |
| Latex and natural rubber           | Gloves, balloons, tires                      | Found in residues on vegetation or near human activity zones            |
| Additives in agricultural plastics | UV stabilizers, antioxidants                 | Migration from mulch films or greenhouse materials to nearby plants     |

et al., 2024) including: (a) using materials such as wood or metal instead of plastic equipment; (b) conducting beekeeping activities in areas far from urban and industrial settlements; (c) ensuring proper recycling and management of plastic waste; (d) providing training to beekeepers on this issue to help reduce the accumulation of MPs in honey; and (e) continuing research on the contamination of bee ecosystems by plastic-related compounds.

Significantly, the potential for bees to interact with plastic-related compounds is not limited to MPs and NPs. Additives such as phthalates, flame retardants, and bisphenols may also be present in the

environment and co-transported by bees. These families of compounds are of high relevance today and have been defined by the European Chemicals Agency as hot topics in research studies due to their ubiquitous presence in the environment and their considerable toxicological potential for ecosystems and living organisms. However, while these analytes have been extensively studied in food matrices and water, their occurrence in pollinators and hive products remains understudied (Martín-Gómez et al., 2024a) (see Table 4). Nonetheless, honeybees have already been shown to bioaccumulate a wide range of pollutants, including pesticides, antibiotics, and heavy metals, demonstrating their capacity to reflect complex environmental exposure (Fuente-Ballesteros et al., 2023b, 2023a).

### 3. Environmental exposure pathways in honeybees

The geographic variability in plastic burden among apiaries suggests

**Table 4**  
Representative studies on the occurrence of plastic-related compounds on honeybees and bee-related matrices.

| Matrix                       | Analytes                   | Location                          | Detection              | Ref.                               |
|------------------------------|----------------------------|-----------------------------------|------------------------|------------------------------------|
| Honeybees                    | Microplastics              | Denmark                           | Microscopy, $\mu$ FTIR | (Edo et al., 2021)                 |
| Honeybees, honey, and pollen | Microplastics, microfibers | Italy                             | Microscopy, $\mu$ FTIR | (Schiano et al., 2024)             |
| Honeybees, honey             | Microplastics              | Hawaii                            | Raman                  | (Buteler et al., 2023)             |
| Bee pollen                   | Plasticizers               | Spain                             | GC-MS                  | (Martín-Gómez et al., 2024b)       |
| Bee pollen                   | Bisphenols                 | Spain                             | UHPLC-MS/MS            | (Ares et al., 2024)                |
| Bee pollen                   | Bisphenols                 | China                             | HPLC-DAD               | (Zhang et al., 2021)               |
| Honey                        | Microplastics              | Kosovo                            | Microscopy, FTIR       | (Özçifçi et al., 2025)             |
| Honey                        | Microplastics              | Turkey                            | Microscopy, FTIR       | (Basaran et al., 2024)             |
| Honey                        | Microplastics              | Ecuador                           | Microscopy, FTIR       | (Díaz-Basantes et al., 2020)       |
| Honey                        | Bisphenols                 | China                             | UHPLC-MS/MS            | (Zhou et al., 2019)                |
| Honey                        | Bisphenols                 | Spain, Helsinki, Finland, Estonia | UHPLC-MS/MS            | (Martín-Gómez et al., 2024c)       |
| Honey                        | Plasticizers               | Brazil, Spain, Finland, Estonia   | GC-MS                  | (Fuente-Ballesteros et al., 2024a) |
| Honey                        | Bisphenols                 | Iran                              | GC-MS                  | (Khani et al., 2021)               |
| Honey                        | Bisphenols, Plasticizers   | NS                                | GC-MS                  | (Peñalver et al., 2021)            |
| Honey                        | Bisphenols, Plasticizers   | Italy                             | GC-IT/MS               | (Notardonato et al., 2020a, 2020b) |
| Honey, royal jelly           | Plasticizers               | China                             | GC-MS                  | (Zhou et al., 2014)                |
| Royal jelly                  | Bisphenols                 | China                             | LC-FLD                 | (Tu et al., 2019)                  |
| Royal jelly                  | Plasticizers               | China                             | GC-MS/MS               | (Xu et al., 2025)                  |
| Propolis                     | Plasticizers               | China                             | GC-MS/MS               | (Raka et al., 2025)                |

FTIR, Fourier transform infrared spectroscopy; GC-IT/MS, gas chromatography-ion trap/mass spectrometry; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; HPLC-DAD, high-performance liquid chromatography with diode array detection; LC-FLD, liquid chromatography with fluorescence detection;  $\mu$ FTIR, micro-Fourier transform infrared spectroscopy; UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry.

that bees can be used as mobile samplers to generate spatial maps of contamination. By placing colonies in different environments - from city centers to agricultural fields and remote forested areas - it is possible to assess the distribution of MP pollution and identify hotspots. Such data can contribute to pollution mapping initiatives and environmental risk assessments. Building upon this idea, different ecological and anthropogenic factors may explain the observed geographical variation in MP contamination in honeybees (Bashir et al., 2024).

#### 3.1. Environmental conditions

Environmental conditions might help explain where and how honeybees are exposed to MP pollution. Conditions like wind, rain, and temperature shape how plastic particles move through the environment and end up in places where bees might encounter them. For example, wind can carry tiny plastic fragments over long distances, meaning even seemingly untouched natural areas can become contaminated. In areas with frequent or heavy rainfall, surface runoff can wash MPs from urban or agricultural land into nearby rivers, lakes, or coastal zones (Wei et al., 2022). Temperature also plays a major role. In sunnier, warmer regions, high ultraviolet light exposure speeds up the breakdown of larger plastic debris into smaller pieces through photodegradation (Sutkar et al., 2023). These smaller particles can then settle onto flowers, leaves, or soil, making it easy for bees to pick them up while collecting nectar or pollen. Additionally, airborne MPs can be carried by the wind across great distances, spreading far beyond their original sources. As a result, honeybees may end up collecting these particles while foraging, even in areas that appear to be clean and unpolluted. This means that bees can encounter plastic contaminants not only near obvious pollution hotspots, but also in remote or rural environments where wind-blown debris has silently settled (Rezaei et al., 2019).

#### 3.2. Beekeeping practices

Beekeeping methods differ widely depending on the region, and these differences can influence how much MP contamination honeybees are exposed to. It seems evident that hives located in urban environments are often closer to pollution sources, increasing the likelihood that bees come into contact with airborne or surface-bound plastic particles. In that context, a recent study performed by Edo et al. detected MPs in honeybees collected from 19 apiaries across Copenhagen, including urban, suburban, and rural areas (Edo et al., 2021). The highest concentrations were found in urban apiaries. However, a notable and unexpected finding was the presence of comparable levels of MPs in suburban and rural hives. This may be attributed to the proximity of urban settlements within the foraging range of worker bees, as well as the high dispersal capacity of small MPs through wind transport. Similar results were reported in Gualaceo (Ecuador), where bees located farther from the urban centre ended up exhibiting lower levels of MP contamination, highlighting the potential impact of urban proximity on MP accumulation in bees (Arévalo et al., 2024). Authors reported that 54.06 % of the detected MPs in dead worker bees were fibers and 45.94 % were fragments. The identified synthetic polymers included polyamides, polyethylene, ethylene propylene diene monomer, polytetrafluoroethylene, polycarbonate, and polyethylene terephthalate. According to Lithner et al. the toxicity risk scores (hazard levels) for polyamide, polyethylene, polypropylene, polyethylene terephthalate, and polycarbonate were determined as 50 (II), 9 (II), 1 (I), 4 (II), and 1177 (IV), respectively (Lithner et al., 2011). The same study reported that inhalation of polyamide is harmful and can cause eye and skin irritation; polycarbonate causes eye and skin irritation and can be fatal if inhaled; and polyethylene can cause drowsiness or dizziness. The data reported by them highlight significant differences in the hazard profiles of commonly used polymers, which may have implications for honeybee health when such materials are used in beekeeping equipment or hive components. The exceptionally high toxicity score of polycarbonate,

combined with its potential to cause fatal effects upon inhalation, raises concerns about chronic or acute exposure of bees to volatile or leachable compounds in enclosed hive environments. Polyamide, with a moderate hazard score, and polyethylene and polypropylene, with comparatively lower scores, may still pose risks if their reported acute effects translate into disorientation, reduced foraging efficiency, or impaired navigation in bees. These findings suggest that material selection for apiculture should not be based solely on durability or cost, but should also account for toxicological properties, potential exposure pathways within the hive, and the long-term impacts on colony health and productivity (Lithner et al., 2011).

As mentioned before, the plastic materials used in apiculture, such as plastic hive frames, synthetic foundation sheets, and even the protective clothing of beekeepers, can serve as unintended sources of MP contamination inside the hive (Buteler et al., 2023; Diaz-Basantes et al., 2020). Additionally, regarding anthropogenic sources, tire wear, fragmented plastics dispersed through littering, and the application of biosolids such as sewage sludge and manure in agriculture represent significant human-driven contributors to environmental MP pollution (Naggar et al., 2021). A recent study on honey from Kosovo reported that the high MP contamination detected in some samples could be associated with increasing industrial development, high population density, and the absence of waste disposal sites in the areas where the honey samples were collected (Özçifçi et al., 2025).

### 3.3. Foraging behavior

Honeybees typically forage within a 2–5 km radius from their hive. In some cases, they may travel even farther in search of food. This wide-ranging activity means that bees interact with a diverse environment ranging from urban parks and roadside vegetation to agricultural fields and natural woodlands, which can vary significantly in their levels and types of MP contamination. The specific plants bees visit, the surfaces they land on, and the environmental conditions during foraging all influence the likelihood of MP pickup. For example, flowers located near roadsides or industrial zones may be more likely to have MPs or plastic particles deposited on their petals and leaves due to air currents and vehicle emissions. Furthermore, bees use water sources for cooling the hive and feeding larvae, and these sources - especially in urban or agricultural landscapes - may also contain suspended MPs. Additionally, differences in foraging preferences among bee colonies, which can be influenced by seasonal blooms, plant diversity, or colony health, might also lead to variation in MP exposure even within the same geographic area. Over time, repeated exposure through foraging can lead to bioaccumulation of MPs not only in the bees themselves but also in hive products such as honey, wax, and pollen (Edo et al., 2021).

## 4. Analytical strategies for detecting plastic-related compounds

The detection of plastic-related compounds in bees and their products requires sensitive and reproducible analytical methods. These methods must accommodate the complexity of bee-related matrices, which include both biological tissues and organic substances such as honey, wax, and propolis. In practice, its analysis involves three main stages: sampling, sample preparation and analytical detection. The choice of which technique to use at each stage strongly depends on the specific compounds and the matrix being analyzed (Martín-Gómez et al., 2024a).

Sampling remains overlooked mainly as the vast majority of studies focus on simply collecting honeybees from apiaries or analyzing hive products - mostly honey and pollen (see Table 4) to monitor the presence or absence of plastic-related compounds. However, when working with airborne MPs, accurate sample collection is a crucial step. To date, the predominant methods for collecting airborne MPs include passive atmospheric deposition samplers and active pump samplers (Cortés-Corrales et al., 2024). Atmospheric deposition is widely used to study how

MPs settle from the air onto surfaces, making it well-suited for continuous, long-term sampling, especially in remote areas without electricity. Despite it, weather conditions such as rain, snow, and wind can significantly affect its accuracy. In contrast, active pumped samplers are commonly used for rapid sampling and provide quantitative data. However, they are expensive and require electricity, limiting their use in remote settings. Both methods face difficulties in capturing very small MPs due to filter pore size and the challenges of trapping fine airborne particles. To overcome these issues, new samplers have been developed. Cortés-Corrales et al. designed a non-invasive in-hive passive sampler named the APITrap to assess environmental contamination by MPs. This sampler, made of polyethylene plastic with a polyvinyl acetate adhesive, offers several advantages: (i) it captures MPs through indirect adsorption from the air circulating within the hive; (ii) operates over prolonged periods; (iii) functions independently of electricity; (iv) is resistant to adverse weather conditions; (v) demonstrates superior reproducibility, with no adverse effects on bee health; and (vi) can be easily implemented in successive samplings (Cortés-Corrales et al., 2024). As mentioned before, beyond airborne pathways, honeybees may also act as vectors of pollutants originating from different natural compartments. In these cases, the sampling of soil, water, and vegetation is usually performed directly by collecting the material to be analyzed. Standard approaches include grab sampling for water, core sampling for soil, and direct cutting or swabbing for vegetation. When applying these methods, it is important to consider precautions such as (i) the potential degradation or transformation of contaminants between collection and laboratory analysis, which requires appropriate preservation and (ii) the strict avoidance of cross-contamination during sampling and transport, as detailed below.

Sample preparation is crucial for isolating target particles while minimizing contamination and degradation. Importantly, plastic analysis involves unique challenges, including broad particle size heterogeneity, variable polymer structures, strong matrix effects, and high susceptibility to contamination to cross-contamination (Jeffries et al., 2025). Given the critical role of sample preparation in plastic analysis, improper or insufficient protocols can result in inaccurate quantification, polymer misidentification, or false positives. Since MPs are airborne and ubiquitous, preventing sample contamination is essential, and various strategies have been implemented to address this issue. Some of the most effective measures include (Cortés-Corrales et al., 2024; Fuente-Ballesteros et al., 2024a) (i) using only glass or metal containers and tools, as plastic labware may introduce background signals; (ii) filtering all reagents to eliminate synthetic particles; (iii) running procedural blanks with each batch to monitor potential contamination; (iv) working under laminar flow hoods or cleanroom conditions, and wearing cotton lab coats to minimize synthetic fiber shedding; and (v) calcining glass materials and covering them with aluminum foil until analysis. Samples are typically digested using alkaline (e.g., potassium hydroxide), oxidative (e.g., hydrogen peroxide), or enzymatic protocols to eliminate organic matter without altering the integrity of plastic polymers followed by different filtration/washing steps (Mallek and Barcelo, 2025). In some cases, a staining step using dyes (e.g., Nile red) may be included to improve the efficiency and accuracy of MP identification and quantification, due to its solvatochromic properties (Arévalo et al., 2024). Moreover, a saturated sodium chloride solution has proven effective for extracting MPs from honeybee samples, as it facilitates the separation of particles and low-density polymers from bee tissues. This option is cost-effective, widely accessible, and environmentally friendly, making it a practical choice for routine analysis (Cortés-Corrales et al., 2024).

The subsequent plastic detection steps can accomplish three groups of advanced analytical techniques: (a) microscopy (visual identification); (b) spectroscopy; and (c) chromatography (Ivleva, 2021) (see Fig. 3). The presence, amount and measurement of the microparticles, whether fibers or are typically first determined through visual examination in a microscope (Diaz-Basantes et al., 2020). Microscopy methods

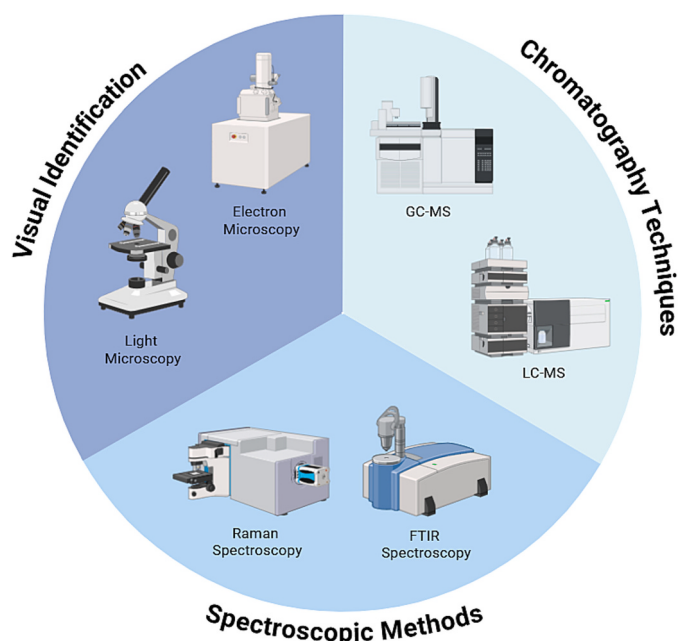


Fig. 3. Main analytical methods for identification and quantification of microplastics.

such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide high-resolution images of particles and surface morphology. Papa et al. used a SEM coupled with energy-dispersive X-ray spectroscopy (EDX) to characterize airborne particles attached to the bees in terms of their morphology, size, and chemical composition (Papa et al., 2024). Arévalo et al. used fluorescence microscopy for a highly sensitive detection of MPs in honeybees (Arévalo et al., 2024).

Spectroscopic tools, such as micro-Fourier transform infrared ( $\mu$ FTIR) and micro-Raman spectroscopy ( $\mu$ Raman), are ideal for polymer identification and can analyze the structure and functional groups of MPs and NPs.  $\mu$ FTIR is a widely used technique for MP identification, as it measures the infrared absorption patterns of molecular bonds characteristic of different polymers. However, its spatial resolution and sensitivity limitations may hinder the accurate detection of MPs and especially NPs present in fine particulate (Zhou et al., 2025). Additionally,  $\mu$ FTIR-based techniques are often costly and time-consuming, and their detection efficiency can be affected by several factors, including the wavelength of the radiation used, baseline drifts resulting from the irregular shapes and rough surfaces of certain particles, and spectral noise that may obscure or distort peak clarity (Arévalo et al., 2024; Mallek and Barcelo, 2025). These limitations highlight the importance of using complementary techniques to obtain a more accurate and reliable assessment of the presence and characteristics of MPs in the samples. A FTIR-based approach was employed to characterize MPs in Turkish honey samples. A total of 568 particles per 50 g of honey were detected across 32 samples, with 97 % of these particles successfully identified (92 % plastics, 7 % cellulose, and 1 % chitosan) (Basaran et al., 2024). Similarly, the analysis of Italian honey samples revealed its contamination by polyethylene, polyester and polyvinyl stearate MFs as well as by rayon and cellulosic fibers. In this study, Erminia Schiano et al. also reported a FTIR spectrum for the microparticles identified in honeybees, revealing that 137 MFs were natural, 93 were synthetic, and 26 were artificial. The analysis of MFs of natural origin allowed to determine that all the MFs were of cellulosic nature, the MFs of artificial origin were identified as rayon and the synthetic MFs included polyester (60 %), polyamide (20 %), polyacrylonitrile (15 %), and polyurethane (5 %) (Schiano et al., 2024).

Raman spectroscopy uses a monochromatic laser (500–800 nm) to interact with the vibrational, rotational, and low-frequency modes of

molecules, producing Raman shifts ( $\text{cm}^{-1}$ ) that reflect the force constants and symmetry of molecular bonds. Each polymer's unique spectral signature, determined by its bond structure and crystallinity, enables MP identification. Coupled with microscopy,  $\mu$ Raman can simultaneously determine particle morphology, polymer type, and color, and detect MPs as small as  $\sim 2 \mu\text{m}$  on filtration membranes.  $\mu$ Raman offers high-resolution identification of MPs with Raman mapping, eliminating the need for organic solvents or dyes. Laser direct infrared imaging also provides high-resolution spectral data, though automated analysis methods require improvement, and standardized protocols for MP detection in foods are still needed (Liu et al., 2023). However, among the studies conducted on honeybees and honey products, only the work of Buteler et al. employed Raman spectroscopy (see Table 4).

Pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) is the leading analytical technique for polymer identification and fingerprinting (Santos et al., 2023). In Py-GC–MS, MPs are thermally degraded into smaller molecular fragments through pyrolysis, which are subsequently separated and identified based on their characteristic pyrolysates using gas chromatography–mass spectrometry (GC–MS) (Zhou et al., 2025). As a thermoanalytical technique, Py-GC–MS provides a robust approach for quantifying both nano- and MPs, with the advantage of having no lower size detection limit. However, its application to aerosol MPs remains controversial, particularly concerning the optimization of the thermal program, the selection of appropriate quantitative markers, and the standardization of calibrant preparation (Seeley and Lynch, 2023). A major limitation of Py-GC–MS lies in the clearance of interferences, since many natural or anthropogenic compounds present in complex matrices produce the same pyrolysis products as plastics, potentially leading to false positives and overestimation (Rauert et al., 2022). This challenge is especially relevant in the context of bees and hive products, where waxes, lipids, and other organic components may generate pyrolysates indistinguishable from plastic-derived markers. Overcoming these interferences requires rigorous clean-up steps and careful marker selection to ensure accurate identification. Similarly, although chromatography is less commonly employed for MPs analysis than spectroscopy, it can help detect halogenated compounds bisphenols, as well as degradation products like oligomers and plastic additives in biological and apicultural matrices (Martín-Gómez et al., 2024a). Liquid chromatography–tandem mass spectrometry (LC-MS/MS), with solvent extraction was employed for bisphenols (Martín-Gómez et al., 2024c), while GC–MS combined with a double solvent extraction followed by clean-up was predominantly used for phthalate acid esters analysis (Fuente-Ballesteros et al., 2024a).

## 5. Biological and health effects of microplastic contamination in honeybees

Beyond the spatial and environmental factors influencing plastic contamination exposure, it is crucial to consider the biological consequences of such contamination on honeybees themselves. Although recent studies suggest that MP ingestion has a limited impact on mortality, it can induce a range of sublethal effects (Bashir et al., 2024; Ferrante et al., 2024; Shavali Gilani et al., 2025). These include impaired growth, altered expression of genes related to immunity and detoxification, disruptions in foraging and oviposition behavior, enhanced toxicity of co-occurring environmental contaminants, reduced gut microbiota diversity, morphological abnormalities, and genotoxic effects (Alma et al., 2023). The impact of MPs on insects depends on the dose and the specific polymer involved. This variability is expected, considering that plastics contain different types of additives, and that factors such as particle shape and size can significantly influence their toxicity. Consequently, further research is essential to deepen our understanding of how MPs affect living organisms. Insects, due to their ecological relevance and physiological diversity, offer a valuable model for investigating the potential toxicological effects of MP/NPs on biological systems (Muhammad et al., 2021).

From a One Health perspective, honeybee contamination by plastic-related compounds links environmental degradation to ecosystem and human health (Papa et al., 2024). Given the high-fat content of bee matrices, there is a higher likelihood for these compounds to accumulate, increasing the potential exposure for consumers (Ares et al., 2024). In particular, bisphenols and phthalate esters have been classified as endocrine-disrupting chemicals, with severe health effects on women (Martín-Gómez et al., 2024a). Given its importance, the European Commission defined specific migration limits (SML) on plastic materials and articles intended to come into contact with food (Fuente-Ballesteros et al., 2024a). However, it is important to note that the legislation applies these SMLs uniformly across all types of food without differentiation and information about bee products is quite limited. Additionally, the threshold of  $60 \text{ mg kg}^{-1}$  for those compounds without a defined SML requires careful consideration (Official Journal of the European Union, 2023). For the general well-being of consumers, the residues found in bee products are typically below the SMLs. Risk assessments have shown that the analyzed samples do not pose an apparent risk to human health, although further research in this area remains a priority (Ares et al., 2024; Fuente-Ballesteros et al., 2024a). Importantly, however, the use of bees for biomonitoring should never compromise their conservation. To achieve this, researchers are increasingly prioritizing non-lethal or minimally invasive strategies. For example, hive products such as honey, pollen, and wax can be analyzed as indirect indicators of contamination without harming the insects. In addition, passive in-hive samplers have been successfully developed to environmental contaminant sampling through honeybees colonies, offering continuous and reproducible monitoring while preserving colony integrity (Murcia-Morales et al., 2020).

The determination of plastic-related compounds in bee products may be necessary not only to study the food safety for consumers, but also to evaluate the contamination of the hive environment (Martín-Gómez et al., 2024b). Integrating honeybees into environmental surveillance frameworks aligns with the growing call for biomonitoring systems that capture complex exposures and their biological consequences. Their dual role as pollinators and bioindicators makes them ideal agents to bridge ecological monitoring and public health initiatives, particularly in light of escalating plastic contamination across terrestrial environments.

## 6. Research gaps and future directions

Despite significant advances, the study of plastic contamination in bees remains nascent and unevenly distributed. Most existing studies have been conducted in select countries, and there is a conspicuous lack of data from many regions, particularly the Global South. Systematic, large-scale assessments are necessary to establish baseline contamination levels and identify regional patterns. Current methodologies vary widely in terms of sampling, digestion, and detection protocols, making it difficult to compare results across studies. Harmonized procedures and interlaboratory validations are essential to ensure data reliability and reproducibility.

It is evident that honeybees are excellent bioindicators of environmental contaminants, but one may naturally ask: *which part of the bee should be collected for a specific study (legs, wings, thorax, intestinal tract, full body, or perhaps their products)?* Current research offers no definitive consensus, as each matrix provides complementary information. For instance, wings and legs can accumulate particles through external contact, while the intestinal tract may better reflect ingestion and internal exposure. Full-body homogenates maximize detection sensitivity but lack specificity about the route of exposure, whereas hive products such as honey and pollen represent indirect indicators of contamination transferred through foraging activity. For this reason, many researchers analyze not only the bee's body, but also its surrounding environment and the products it produces, in order to obtain a holistic view of the problem. A systematic comparison of these matrices will be essential to

establish standardized recommendations and clarify the relevance of each compartment to environmental monitoring.

Furthermore, future studies must investigate the combined effects of plastic-related compounds and other environmental pollutants, such as pesticides, heavy metals, and antibiotics. Most current research has examined these stressors in isolation; however, bees in natural environments are rarely exposed to a single contaminant. Instead, they face complex mixtures of substances that may interact in ways that amplify or modify their individual toxicity. Exploring these potential synergistic effects is crucial for gaining a more realistic and comprehensive understanding of the risks bees encounter in their habitats. Similarly, another concern is that MPs, due to their lipophilic nature, can adsorb environmental pollutants, acting simultaneously as carriers and reservoirs for these substances. As the surface area of MPs increases their chemical reactivity and capacity to adsorb contaminants also rise. Various environmental factors, including weathering, sunlight exposure, or even pH can significantly influence the adsorption kinetics of contaminants onto MPs.

Multidisciplinary collaboration will be key to future progress. Integrating expertise from scientists, policymakers and industries can enhance the design of comprehensive monitoring systems. The establishment of international networks (e.g., COST Action CA22105 BeSafeBeeHoney), platforms (e.g., the Honey Platform) and initiatives (e.g., INSIGNIA-EU) that actively involve beekeepers in sample collection and preliminary processing, could substantially expand the geographic and temporal reach of monitoring efforts. Beekeepers can reduce the presence of MPs in bee-derived products by opting for wooden equipment instead of plastic alternatives. Establishing apiaries in areas far from urban and industrial zones also offers a practical strategy to minimize the contamination. In addition, adopting proper recycling practices and responsible management of plastic waste can significantly lessen the environmental burden of MPs. Promoting awareness and training among producers on these preventive measures is crucial to limit the accumulation of MPs in honey and other hive products.

On the analytical chemistry side, it is important to note that analytical chemistry methods employed to identify MPs often require the use of toxic reagents and rely on energy-intensive instruments with a significant carbon footprint (Mallek and Barcelo, 2025). In consequence, researchers should prioritize the adaptation of green, efficient, and cost-effective techniques for plastic detection. The emerging paradigm of white analytical chemistry (WAC) (Nowak et al., 2021) - which emphasizes analytical performance (red dimension), environmental sustainability (green dimension), practical applicability (blue dimension), and innovation (violet dimension) - offers a useful framework in that area. However, the application of WAC perspective in MP analysis has received scarce attention. For example, to be greener several aspects require further attention including the development of in-situ sampling techniques, the adoption of direct and non-destructive methods, the miniaturization and automation of procedures, the substitution of hazardous reagents with safer alternatives, and the integration of chemometric tools to optimize experimental design and reduce resource consumption. Moreover, the rapid advancement of artificial intelligence-based software represents a significant potential step for establishing virtual networks for environmental biomonitoring. Despite it, analytical chemistry still faces several critical challenges in this context, such as the complexity of detecting very small plastic particles (especially those below  $20 \mu\text{m}$ ), the risk of cross-contamination during laboratory procedures, the lack of standardized and harmonized protocols across studies, and the extremely low concentrations at which plastic additives and plasticizers (Mallek and Barcelo, 2025). These issues underscore the need for methodological improvements to ensure accurate detection, minimize artefacts, and allow meaningful comparisons across different geographic and environmental settings.

Ultimately, it is also urgent to explore the toxicological implications of plastic exposure for bee health. While some studies have reported changes in immune function, gut microbiota, and behavior following MP

ingestion, the mechanistic pathways remain poorly understood. Longitudinal studies assessing colony survival, reproductive success, and pollination services in relation to plastic load are critical. These findings could inform regulatory policies and environmental health strategies.

## 7. Conclusions

Honeybees possess unique biological and ecological traits that make them effective, proactive bioindicators of plastic pollution. Their extensive foraging activity, interaction with diverse environmental matrices, and ability to transport plastic-related compounds to the hive as well as to transfer them to bee products allow for integrated environmental sampling across landscapes. Multiple studies have demonstrated the presence of MPs and plastic-related compounds in bee bodies and hive products, affirming their role as sentinels of environmental contamination. The implementation of analytical strategies tailored to bee matrices has facilitated the detection and characterization of plastic particles, although significant methodological challenges remain. Expanding global research, standardizing methodologies, and fostering interdisciplinary collaborations are essential to harness the potential of honeybees in environmental monitoring fully. It is evident that honeybees can indeed be considered proactive samplers of plastic compounds and their use as bioindicators not only enriches our understanding of plastic pollution dynamics but also supports broader initiatives in environmental health, food safety, and ecological sustainability. Future efforts should focus on integrating bee-based monitoring into national/international frameworks for environmental surveillance, leveraging their natural behaviors to address one of the most urgent pollution crises of our time. This article aims to offer a fresh perspective on the role of honeybees as active bioindicators of plastic pollution, highlighting their ecological relevance, current analytical methodologies, and future potential within environmental monitoring systems.

## CRedit authorship contribution statement

**Adrián Fuente-Ballesteros:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marco Ciulu:** Writing – review & editing, Writing – original draft, Visualization. **Shaikh Manirul Haque:** Writing – review & editing, Writing – original draft. **Yerkanat Syrgabek:** Writing – review & editing, Visualization. **Burhan Basaran:** Writing – review & editing.

## Declaration of competing interest

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

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## Data availability

Data will be made available on request.

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