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Detection of microplastics in honey of stingless bee (*Heterotrigona itama*) and honey bee (*Apis mellifera*) from Malaysia

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Abstract. *Ibrahim YS, Rosazan MN, Mamat MII, Anuar ST, Azmi WA. 2025. Detection of microplastics in honey of stingless bee (*Heterotrigona itama*) and honey bee (*Apis mellifera*) from Malaysia. Biodiversitas 26: 1271-1278.* The demand for stingless bee honey and European bee honey has increased rapidly due to its medicinal benefits. Honey of the Indo-Malaya stingless bee, *Heterotrigona itama,* and European honey bee, *Apis mellifera,* are among the most popular bee products that Malaysians commonly consume. It has been reported that the contamination of honey with microplastics (MPs) can occur at various stages of production, from bees collecting the contaminated floral sources to the harvesting and packaging processes. With the emerging plastics pollution in the environment and concerns about potential health risks, this study aims to investigate the presence of MPs in honey samples from stingless bees, *H. itama*, and honey bees, *A. mellifera*, from Malaysia. Microplastic particles were extracted from 10 g of honey and characterized under a stereomicroscope to determine their color, size, and type. Polymer types were further identified using FTIR analysis. All honey samples from both species were found to be 100% contaminated with microplastics. *H. itama* honey contained a higher concentration of microplastics (8.18 ± 2.57 MPs/g) compared to *A. mellifera*'s honey (5.52 ± 1.13 MPs/g). The MPs found in honey from both species were predominantly fibers and fragments, mostly transparent in color, with sizes ranging from 0.7 to 1.8 mm. The findings of this preliminary study are intended to provide an awareness of MPs in honey, especially in the food safety aspect, which needs a better understanding of good practices of beekeeping and processing procedures to minimize the contamination of honey.

Keywords: Food safety, honey bee, microplastics, South East Asia, stingless bee

INTRODUCTION

Humans globally produce over 400 million tonnes of plastic waste each year, a staggering figure that has a devastating impact on our environment (Zhang et al. 2019). Shockingly, only 9% of this waste is recycled, 12% is incinerated, and a massive 79% is carelessly released into the environment (Chen et al. 2021). The majority of this waste ends up in our oceans and on our land, causing untold harm to our ecosystems and the creatures that inhabit them (Ouyang et al. 2022).

Microplastics (MPs) are not just the result of macroplastic degradation, which can occur through microbial degradation or natural weathering processes (Dees et al. 2020; Chen et al. 2021). They can also originate from a wide range of sources, including agricultural plastics, textiles, cosmetics, personal care products, paints and coatings, industrial abrasives, and more (Zhang et al. 2019; Diaz-Basantes et al. 2020; Jin et al. 2021; Ziani et al. 2023). These tiny pollutants, measuring less than 5 mm, are a growing concern globally. They are pervasive, infiltrating various ecosystems, from soil and surface waters to coastal sediments, beach sands, freshwater, and even deep-sea environments (Ziani et al. 2023). Recent research has even found microplastics in rain and snow, as well as in our food supplies (Wang et al. 2021).

Several studies have reported that there is contamination of MPs in human daily intake, including fish, salt, drinking water, beverages, packaged food, and other food (Jin et al. 2021; Ziani et al. 2023). For instance, Lee et al. (2019) reported the presence of microplastics (MPs) in salts from Taiwan, while Vidyasakar et al. (2021) identified MPs of various sizes in salts from Gujarat and Tamil Nadu, India. Similarly, a study by Oßmann et al. (2018) detected small MPs and pigmented particles in 32 bottled mineral water samples in Germany. Notably, Li et al. (2020) discovered that sterilizing polypropylene infant feeding bottles and exposing them to high-temperature water significantly increased microplastic release, highlighting the urgent need to assess whether this exposure poses a risk to infant health. This underscores the importance of protecting vulnerable populations from the potential health risks associated with MPs. Additionally, multiple studies have confirmed the presence of MPs in various commercial seafood, such as mussels, oysters, crabs, shrimp, and fish (Lee et al. 2019). These findings raise concerns about the potential risks to food safety and human health, particularly for infants and other vulnerable groups.

Honey has long been appreciated for its numerous health benefits and therapeutic properties (Katsara et al. 2022). Recent research has unveiled the contamination of MPs in honey, which is thought to originate to be derived from the exposure of bees to MPs contamination either from floral sources (i.e., nectar, pollen, resin), water, air, or probably due to beekeeping practice or contamination of the beekeeping materials (Edo et al. 2021). It is believed that bees may be exposed to MPs through various environmental pathways, including floral sources such as nectar, pollen, and resin, which may accumulate MPs from air pollution or contaminated soil. Water sources like rivers, ponds, and dew, which often contain MPs from industrial, agricultural, or domestic waste, may also contribute to contamination. Additionally, airborne MPs from dust or particulate matter can settle on bees or enter the hive. Beekeeping practices such as plastic-based materials used in hive construction, feeders, or storage containers, along with improper handling during honey extraction, may introduce MPs. This contamination highlights the potential risks to honey quality, pollinator health, and human consumption, emphasizing the urgent need for further research and mitigation strategies (Liebezeit and Liebezeit 2013; Rainieri and Barranco 2019).

For instance, MPs have been found in 12% of the honey, beer, milk, and refreshment samples collected in Ecuador (Diaz-Basantes et al. 2020). A study by Al Naggar et al. (2021) found that MPs have been detected in honey bees collected from 19 different apiaries in Copenhagen, Denmark. Liebezeit and Liebezeit (2013) discovered colored fibers and fragments in honey samples from Germany, France, Italy, Spain, and Mexico. These particles are provisionally identified as environmental, indicating they may have been transported by bees into the hive, introduced during honey processing, or both. Another study revealed that MPs contaminated 19 samples of honey from five countries, which are already present in bees' feed and transported from flowers to the hive by the bees (Jin et al. 2021). These findings suggest that MPs are widely distributed in the apiaries, but the potential induced effects on bees remain unknown. The presence of MPs in honey also raises serious concerns that it may have potential toxicity or threat to human health (Rainieri and Barranco 2019).

Despite growing concerns about MP contamination in bee-related products, limited studies have examined how different bee species, with varying foraging distances, behaviors, and beekeeping practices, contribute to MP contamination. In addition, most of the studies are conducted outside of Southeast Asia, and the presence of MPs in honey has not been investigated yet in Malaysia. In this study, honey samples of the two most domesticated bee species in Malaysia were analyzed for the detection of MPs. We hypothesize that variations in bee species, environmental conditions, and management practices across different areas of Malaysia may influence the level of MP contamination in honey. Addressing this gap will provide valuable insights into the potential impact of environmental pollution on bee-derived products. Thus, the present study aims to bridge this knowledge gap by determining the presence of MPs in the honey of the Indo-Malaya stingless bee, *Heterotrigona itama*, and European honey bee, *Apis mellifera*.

MATERIALS AND METHODS

Study collection

Twelve honey samples from stingless bees, *H. itama* (6) and honey bee, *A. mellifera* (6), were used in this study. Six honey samples were collected from farms and another six samples were obtained from local stores in different areas of Malaysia. The honey samples were collected from various ecosystems and geographical regions, including lowland forests, agricultural farmlands, coastal areas, and wetlands. Additionally, the selection of honey samples from farms and local stores considered several factors, such as floral sources, packaging types, and harvesting or processing methods, to ensure a representative analysis. However, the information on the locations of sample collection could not be revealed due to confidentiality and to protect the privacy of the beekeepers and the companies. Honey samples were placed in universal glass bottles and stored in a chiller at 4°C before the MP's examination. The analysis was conducted at the Laboratory of Microplastics Research Interest Group (MRIG), Universiti Malaysia Terengganu (UMT).

Samples digestion

Honey sample digestion was conducted using methods described by Liebezeit and Liebezeit (2015) and Diaz-Basantes et al. (2020) with some modifications. 10 g of honey was diluted with 10 mL of filtered deionized water with a ratio of 1:1. The solutions were then mixed with 30% hydrogen peroxide to allow digestion. Next, to ensure accuracy and consistency, each sample was analyzed in triplicate. The samples were stirred for 6 hours using a hot plate. To determine the microparticle size, the digested solutions were filtered through a 1.2 µm pore size GF/C glass filter membrane using vacuum filtration (Rocker 300, #167300-22, Taiwan) at 55°C. The filter papers were dried in a desiccator for at least 24 hours.

Determination of amount, size, color, and composition of microparticles

The MPs contained on each filter paper were carefully observed to determine the amount and size of the microparticles, whether they were fragments, fibers, granules, flakes and pellets (Diaz-Basantes et al. 2020) under a stereomicroscope (Carl Zeiss Stemi 508, Germany) that attached to a digital camera (Carl Zeiss Axiocam 208 color, Germany). The size of MPs was categorized into four categories: <0.3 mm, 0.3-0.5 mm, 0.5-1.0 mm, and 1.0-5.0 mm, based on Anuar et al. (2023). The methods used to determine the color of MPs (transparent, black, red, blue, purple, brown, and yellow) followed a widely used protocol suggested by Hidalgo-Ruz et al. (2012).

Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analyses

Scanning Electron Microscope (SEM, TESCAN/VEGA LMH, USA) at an accelerating voltage of 5 to 15 keV was used to further morphologically visualize and characterize the MPs. Prior to the analysis, microplastic particles were mounted on SEM stubs and coated with a thin layer of gold coating. Additionally, Fourier Transform Infrared spectroscopy

(FTIR) (Invenio-S Bruker, Germany) was used to screen for the polymer identification. The analysis was performed in the wavenumber range of 4000-500 cm^{-1} with 4 cm^{-1} resolutions and at 36 scans/min. All FTIR spectra were visualized as absorption spectra (plots of absorbance vs. wavenumber) to illustrate the characteristic peaks of the detected polymers. The spectra were analyzed using OPUS software (Bruker Inc.) and compared to the polymer library (OPUS Bruker ATR-FTIR Complete Library) with a>70% matching index. Identified functional groups were annotated and referenced against EFSA (2016) and Jung et al. (2018) to confirm polymer classification. Representative spectra with peak assignments were included in the results to support data interpretation.

Contamination control

All filtration units and glassware were covered throughout the workup procedure to prevent airborne contamination. All the equipment was rinsed with filtered deionized water, and all solutions used in the study were filtered using GF/C filter paper (filtered with 1.2 μm GF/C glass filter membrane). The procedure for controlling airborne contamination involved placing a new blank filter paper as a procedural control to prevent contamination of airborne MPs before and during analysis. Additionally, the analysis of sorting and measurement was carried out in a closed-contamination room equipped with a portable air filtration system to avoid excessive contamination from airborne.

Statistical analysis

Shapiro-Wilk test was used to test the normality of the data due to the small sample sizes (n<50 samples). Data were log (x+1) transformed to improve normality and homogeneity of variance. T-test was used to evaluate differences in the honey samples between *H. itama* and *A. mellifera*. All statistical analyses and visualization were performed in OriginPro® version 2022 software (OriginPro 2022).

RESULTS AND DISCUSSION

A total of 822 MPs were collected from 12 samples of *H. itama* and *A. mellifera* honey. Interestingly, the honey of stingless bees (*H. itama)* contained a significantly higher abundance of MPs (8.18±2.57 MPs/g) compared to that of honey bee (*A. mellifera*) honey (5.52±1.13 MPs/g) (Table 1). As shown in Figure 1, an independent t-test revealed a statistically significant difference ($p<0.05$) of MPs/g in honey between both species. Honey sample F from *H. itama* recorded the highest count of MPs with 12.1 MPs/g, while sample C had the lowest at 5.1 MPs/g. For *A. mellifera*'s honey sample B contained the highest abundance of MPs with 6.5 MPs/g, and the lowest was found in sample F with 3.5 MPs/g (Table 2).

In the current study, only two types of microplastic particles were found, including fragments and fibers. Figure 2 presents two representative microscopy images of the detected MPs. Fibers were the most prevalent type in both honey samples, constituting 94.3% (463 MPs) of *H. itama's* honey and 95.15% (314 MPs) of *A. mellifera*'s honey. Conversely, fragments were less abundant, accounting for 5.7% (28 MPs) of *H. itama's* honey and 4.85% (16 MPs) of *A. mellifera's* honey (Figure 3.A). These findings align with a recent study by Rani-Borges et al. (2024), which also reported high levels of fiber contamination in honey from both stingless bees and honey bees. The abundance of fibers found in honey samples from both species may be attributed to the presence of textile-derived fibers, which are commonly introduced into the environment through routine activities such as physical wear, washing, and laundry processes (Periyasamy and Tehrani-Bagha 2022). These synthetic fibers, often used in clothing and household textiles, are shed during every activity and cleaning routine, releasing microfibres that can enter terrestrial ecosystems and aquatic systems (Gavigan et al. 2020; Le et al. 2022; Ali et al. 2023). Bees may unintentionally collect these fibers indirectly by coming into contact with contaminated pollen, nectar, or water sources. For example, synthetic fibers can shed into the environment from textiles and accumulate on vegetation, in the air, or in water sources that bees use for foraging. These fibers can then be incorporated into the nectar or pollen that bees collect. Additionally, fibers could be carried by wind or deposited onto flowers and plants, which bees might then visit and incorporate into their hive, resulting in honey contamination (Alma et al. 2023).

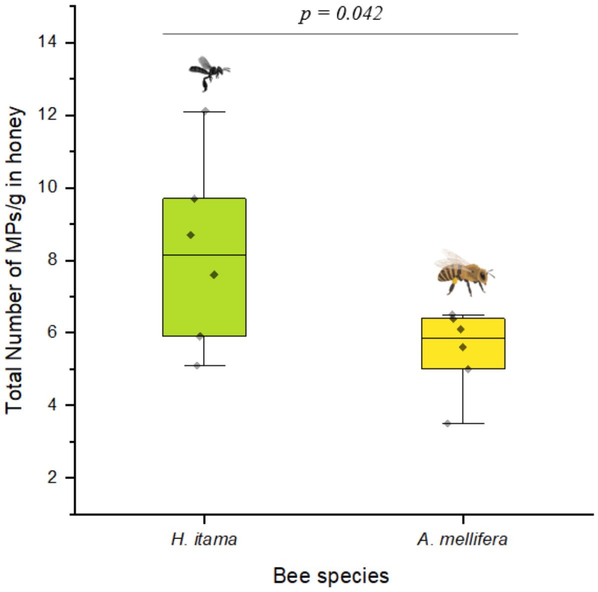

Figure 1. Box plots depict the amounts of MPs/g in honey produced by two different types of domesticated bees, which are stingless (*H. itama*) and honey bees (*A. mellifera*). Each box plot represents the total number of MPs of a specific sample (A-F). Black horizontal lines within the box plots represent the mean value. Box shows the interquartile range; the whiskers represent the maximum and minimum values of MPs/g. Swarm data points (diamond dots) are shown within the box plot, which denotes each value of MPs/g in each honey sample

Table 1. Summary of mean ± SD of MPs/g in honey samples produced by *H. itama* and *A. mellifera*

Source of honey	**Bee species**	**Mean ± SD of MPs/g of honey**
Stingless bee (n=6)	*Hetetrigona itama*	8.18 ± 2.57
Honey bee (n=6)	*Apis mellifera*	5.52 ± 1.13

Table 2. Microplastics MPs/g in honey samples of two different types of domesticated bees: stingless bee (*Heterotrigona itama*) and honey bee (*Apis mellifera*)

Honey samples code	**Total number of MPs/g of honey**	
	Bee species	
	Heterotrigona itama	***Apis mellifera***
A	9.7	6.1
B	5.9	6.5
C	5.1	5.6
D	7.6	5
E	8.7	6.4
F	12.1	3.5

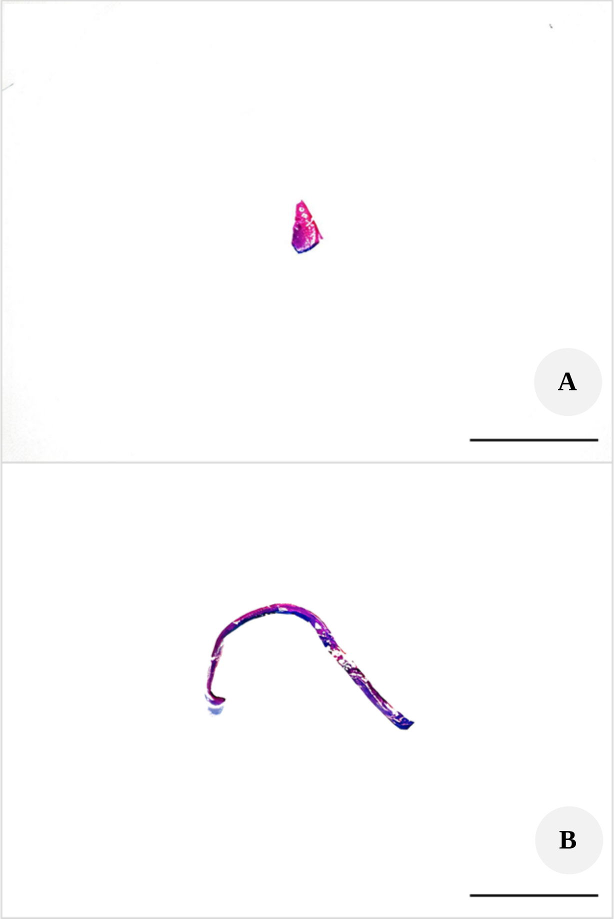

Figure 2. Microscopy images of microplastic particles found in honey samples of stingless bees (*H. itama*) and honey bees (*A. mellifera*). A: Red fragment; B: Purple fiber. Scale bar: 0.5 mm

A significant difference was observed in the total abundance of fibers and fragments between the two honey types (t=2.3454; df=10; $p<0.05$). Generally, the largest dimension sizes of MPs are between the range of 1.0 mm to 5.0 mm (Figure 3.B). The average size of MPs size in *H. itama*'s honey was 1.100 ± 0.653 mm, while *A. mellifera*'s honey was 0.883 ± 0.232 mm. A higher abundance of MPs ranging from 1.0-5.0 mm (28.63%) was found in *H. itama*'s honey, followed by MPs with sizes ranging from 0.5-1.0 mm (28.63%), 0.3-0.5 mm (27.84%) and <0.3 mm (21.26%). Similarly, a higher of MPs in the 1.0-5.0 mm size range (33.87%) was observed in *A. mellifera*'s honey, followed by MPs ranging from 0.5-1.0 mm (24.28%), 0.3-0.5 mm (21.73%) and <0.3 mm (20.13%). It is important to classify MPs based on size, as different sizes of MPs can potentially lead to varying toxicity potential towards the honey's composition or humans that consumed the contaminated honey. As reported previously in aquatic organisms (Lyu et al. 2022), the toxicity towards the medium can vary due to the size of MPs. However, further and continuous detailed study needs to be done focusing on how the size of MPs can affect the honey physically and chemically.

Various colors of MPs were detected in both types of honey. Transparent was the dominant color observed in both types of honey, followed by black, red, blue, purple, brown, and yellow (Figure 3.C). The observed MPs were selected randomly for surface imaging using SEM analysis and chemical analysis using FTIR spectroscopy. Based on the morphological observation, the surface of MPs showed the level of chemical weathering and mechanical erosion experienced by the MPs (Cai et al. 2017). Few degradation patterns were detected, such as numerous pick-holes and cracks on the rough surface, as well as heterogenous fragmentation and the presence of adhering particles (Figures 4.A-D), suggesting that the particles were environmentally originated (Furfaro et al. 2022). The surface morphology of MPs is an important factor that allows MPs to be an ideal substrate for further settlement of foreign particles and microorganisms.

The purpose of FTIR analysis is to confirm the polymer associated with MP particles based on the main functional groups recorded in the infrared region after preliminary examination using a hot needle test procedure. Due to size limitation, only six particles could be identified by the FTIR, and all MPs showed the same type of polymer, which was polyethylene (PE) (Figure 5). The spectrum shows strong absorption at 2914-2847 cm^{-1} for CH_2 stretching, followed by bending at 1470-1377 cm^{-1} and non-splitting CH_2 rocking at 720 cm^{-1} (Jung 2018), that potentially attributed to low-density polyethylene (LDPE) polymer. This is probably due to honey packaging in plastic bottles or beekeeping materials, which are mostly from PE, and this type of plastic is commonly used in Malaysia. The detachment of the plastic-packaging materials and beekeeping equipment may have contributed to the presence of MPs in the honey, as recorded by Liebezeit and Liebezeit (2013), Mason et al. (2018), and Koelmans et al. (2019). However, certain specific analytical methods can significantly affect the outcomes. For example, micro-Raman spectroscopy may provide more accurate estimates of MPs than traditional FTIR due to the size limitation of FTIR (>300 µm). Moreover, as discussed by Cabernard et al. (2018), ASPEx-µ-Raman could identify two times higher

MP numbers compared to FTIR imaging. Thus, it shows that the lack or the absence of one of these techniques might result in potential biases in our results, which can lead to underestimations of other materials of MP particles. It is recommended to combine both spectroscopic methods (micro-Raman and FTIR) or the use of a combination of chromatographic and spectroscopic methods (e.g., pyrolysis-GC/MS), which is necessary for a complete and reliable characterization of the chemical composition, particularly in identifying MPs from food samples like honey.

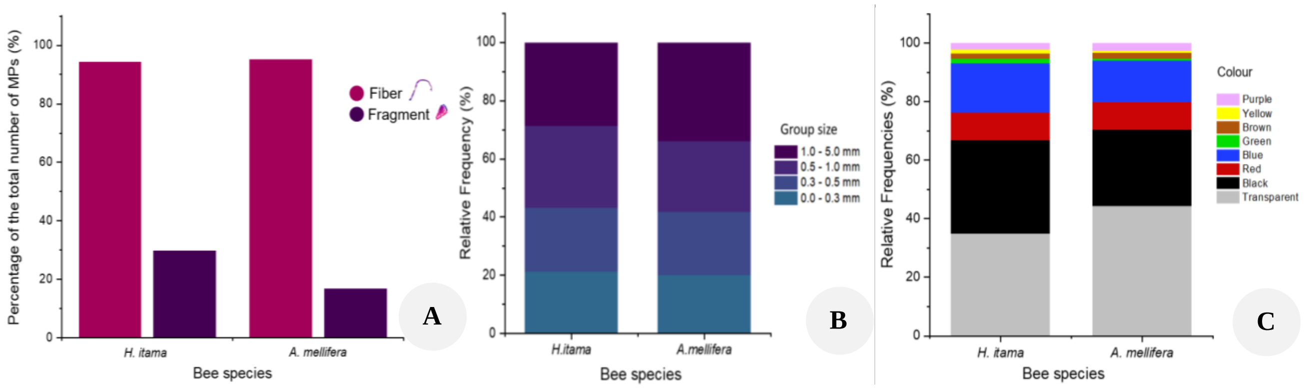

Figure 3. The bar graph visually represents the microplastic contaminants (MPs) found in honey produced by *H. itama* and *A. mellifera*. A. Types of MPs; B. Sizes; C. Colors

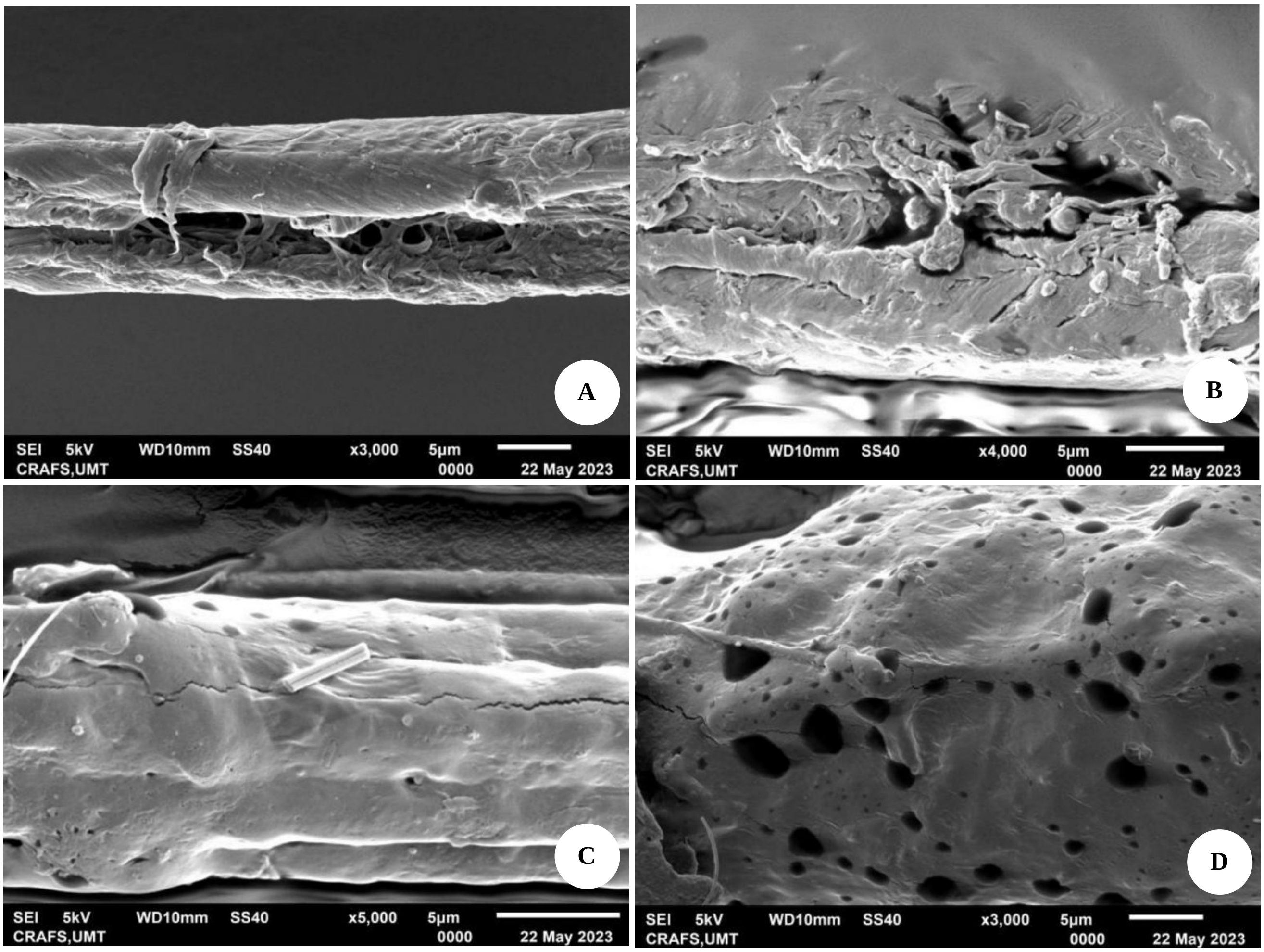

Figure 4. Scanning Electron Microscopy images of microplastics (MPs) found in samples of honey of stingless bee, *Heterotrigona itama*, and honey bee, *Apis mellifera.* A. Crack and grooves; B. Rough surface and noticeable heterogeneity of fragmentation; C. Adhering particle, D. Pick-hole

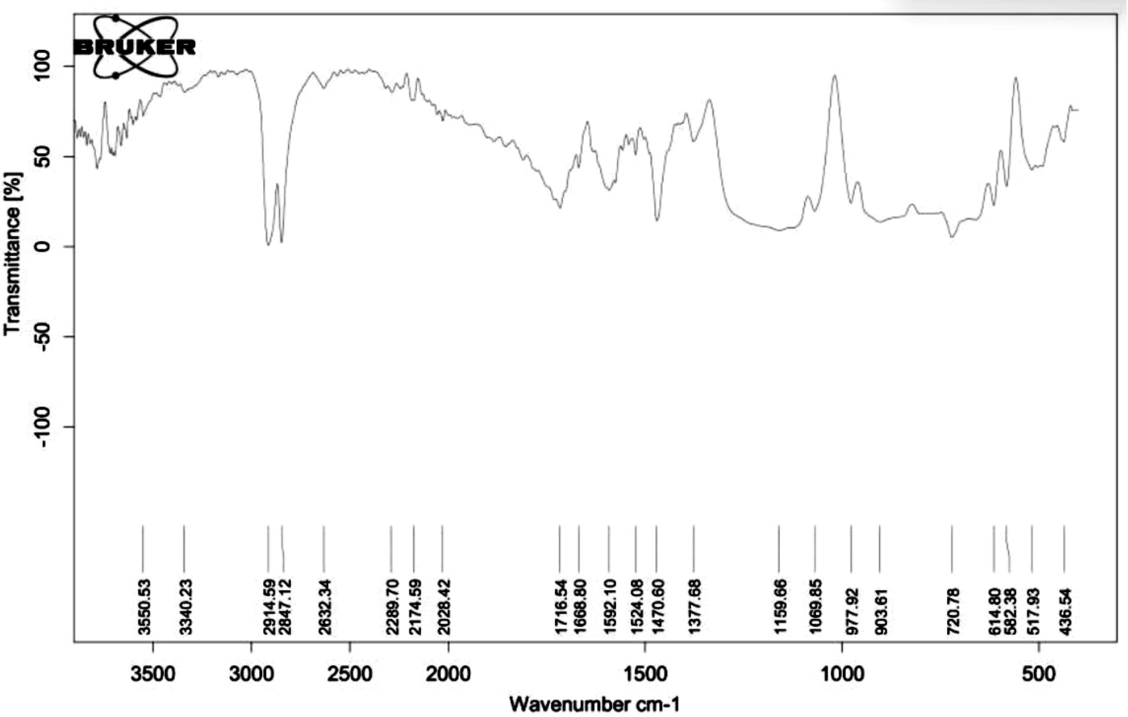

Figure 5. Fourier Transform Infrared spectroscopy of microplastics (MPs) found in samples honey of stingless bee, *Heterotrigona itama*

There are several factors of contamination with microparticles in honey. MPs are ubiquitous in various environmental media, and these microparticles can be carried by air, water, and soil or natural environments (Ouyang et al. 2022). Both bee species in this study are efficient pollinators where they forage for nectar, pollen, and resin from flowers either in the forest or agricultural ecosystems to produce honey (Azmi et al. 2019; Mamat et al. 2023). While foraging, the bees may inadvertently collect the microplastics that are present on the flowers or surrounding vegetation, which can eventually be introduced into the beehives and honey. Stingless bees, *H. itama*, generally have a smaller foraging radius that typically extends up from 500 m to 800 m from the hives (Benedick et al. 2021). In comparison, the honey bees, *A. mellifera* can travel up to 3-5 km from their hives in search of food sources, with the ability to extend their foraging distance up to 7.5 km under certain conditions (Abou-Shaara 2014; Baez-Gonzalez 2024). These foraging differences in both stingless bees and honey bees may reflect the results of MP contamination, which can also be related to factors such as proximity to pollution sources and beekeeping practices. In this study, honey from stingless bees appears more susceptible to MPs contamination due to their foraging behavior, which often involves collecting resources in nearby areas that may be more prone to pollution. In contrast, honey from European honey bees is less likely to be contaminated by MPs as these bees forage over longer distances. Additionally, the use of PE, such as honey suction pumps, during the harvesting process can introduce MPs into the honey.

Both stingless bees and European honey bees play a crucial role in plant pollination. However, according to Liebezeit and Liebezeit (2014), microplastics have been found in the flowers of several plant species. An early study demonstrated that non-living particles, such as latex beads, could be artificially introduced into the transmitting tracts of styles of different plant species and translocated to the ovary (Sanders and Lord 1989). A review study by Oliveira et al. (2019) has revealed that MP beads can mimic the pollen grains, which can disrupt and potentially lead to a decline in plant pollination. Consequently, the MP particles can then be introduced into the honey during its production and processing stages.

Besides, the honey extraction process can further introduce MPs into the honey, as equipment and containers can be a source of MPs in the honey (Al Naggar et al. 2021). In this study, samples of *A. mellifera*'s honey were mainly processed honey. At the same time, samples of *H. itama's* honey were freshly collected from farms, which caused varied rates of exposure to MP contamination. Each sample of honey was collected from different farms or local stores in different areas of Malaysia, which indirectly reflected the results of MPs. Furthermore, the different honey sample is owned by different beekeepers who have different beekeeping practices, procedures, or management. The contamination of MPs probably contributed to the beekeeper suits, honey harvesting equipment, and packaging in plastic bottles or beekeeping materials, which are widely utilized in Malaysia. Studies have shown that microplastics and fibers are present in honey samples, indicating that these contaminants can infiltrate the honey production process (Liebezeit and Liebezeit 2013; Mason et al. 2018; Koelmans et al. 2019). According to a study in Ecuador, MPs are also found in samples of industrial honey and craft honey, comprising 3146 MPs/kg and 4492 MPs/kg (Diaz-

Basantes et al. 2020). Al Naggar et al. (2021) revealed that exposure to MPs can alter the diversity of the bee gut microbiota, followed by changes in gene expression related to oxidative damage, detoxification, and immunity. The effects of the health risks remain uncertain, but it is clear that addressing this issue is vital to safeguard both human and bee health.

In future research, larger sample sizes will be collected to increase statistical power, which would provide more robust results and improve the statistical significance of the findings. Additionally, we will also focus on investigating the environmental sources of microplastics (MPs) to understand better their pathways and accumulation in both stingless bee and honeybee products. Furthermore, exploring the specific factors that may contribute to *H. itama* honey's higher levels of contamination could provide valuable insights into the environmental conditions or biological mechanisms that make certain bee species more susceptible to MP contamination. These directions will help guide future research on the environmental impact of MPs on bees and their products, ultimately leading to a deeper understanding of how microplastic pollution affects ecosystem health.

In conclusion, this is the first report of the presence of MPs in the samples of honey produced by Indo-Malaya stingless bee (*Heterotrigona itama*) and European honey bee (*Apis mellifera*) from Malaysia. The predominant MPs contaminants consist of fibers and fragments, which are primarily transparent in color. These particles are mostly frequently observed in sizes ranging from 1.0 to 5.0 mm. The amounts of MPs in honey ranged varies from 3.5 to 12.1 MPs/g. The FTIR spectroscopy analysis identified the presence of LDPE polymer among the MPs particles. However, due to its limitation in detecting only larger-sized MPs, the presence of other polymer types may have been overlooked. As a result, the specific sources of the MPs could not be conclusively determined. Still, polluted foraging areas of the bees and beekeeping practices (e.g., honey extraction, processing, and packaging) are among the speculations responsible for the MP's contamination in honey. Besides, nectar and pollen collection by the forager bees during pollination and honey processing could be the potential source of the MPs contamination. The presence of these contaminants raises concerns about potential health risks to the bee population, which may be impacted by environmental exposure to MPs, as well as to human consumers, who may unknowingly ingest these particles through honey. Outcomes from this study serve as important information that urgently needs to increase awareness of MPs in honey from a food safety perspective need for further research into best practices in beekeeping and honey processing to reduce MPs contamination risks and ensure product quality, research, and action to mitigate the presence of microplastics in our food supply and food security.

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