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Microplastic contamination in *Heterotrigona itama* bee products (Honey, Pollen, Propolis) from Meratus Geopark, South Kalimantan

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ABSTRACT

Microplastic pollution has become a significant environmental concern, with recent studies detecting microplastics in various ecosystems and natural products, including those produced by bees. This study investigates the presence, morphology, and distribution of microplastics in products of the stingless bee Heterotrigona itama—specifically honey, pollen, and propolis—collected from six meliponiculture sites within the Meratus Geopark, South Kalimantan, Indonesia. Samples were analyzed using stereomicroscopy to identify microplastic types, quantify particles, and measure sizes. The findings reveal that fibers and fragments are the predominant microplastic forms, with urban locations exhibiting higher contamination levels (approximately 309 to 318 particles per 100 ml) compared to rural areas. Notably, one rural site recorded the highest contamination level (approximately 362 particles per 100 ml), suggesting that factors beyond urbanization, such as agricultural practices and atmospheric deposition, contribute to microplastic pollution. The study underscores the role of bees as natural bioindicators for environmental monitoring and highlights the potential risks of microplastic contamination to bee health, food safety, and ecosystem sustainability. These insights are aligned with Sustainable Development Goal 12, which advocates for responsible consumption and production.

Conflict of interest: The authors declare that they have no conflicts of interest.

Introduction

Microplastics have emerged as a significant environmental pollutant in recent decades[1]. Their existence has been identified across several habitats, encompassing both aquatic and terrestrial environments, as well as in natural products such as honey, pollen, and propolis produced by bees[2]. This issue stems from the proliferation of microplastics, which arise from the decomposition of larger plastics or are directly produced by industrial processes, resulting in widespread pollution. A study by Widianarko and Hantoro demonstrated that microplastics can accumulate in honey, pollen, and propolis, indicating the extent of environmental contamination in the vicinity[3]. Nonetheless, additional research is required to comprehend the effects of microplastics on bee health, including their accumulation within the bee body, their influence on the colony, and their interactions with other pollutants, including heavy metals and pesticides[4]. The disparity in microplastic dispersion between urban and rural areas highlights the complex nature of pollution sources, influenced by several factors. In urban areas, elevated population density, industrial operations, vehicular usage, and the existence of wastewater treatment facilities substantially contribute to microplastic contamination. Conversely, rural areas encounter difficulties stemming from agricultural methods, atmospheric deposition, inadequate waste management, and the conveyance of microplastics through rivers and streams. Common sources in both consist of single-use plastics, synthetic fabrics, and personal care products. Comprehending the intricacies of microplastic pollution sources in urban and rural areas is crucial for formulating effective measures to alleviate their effects on ecosystems and human health[5].

Prior research has discovered many microplastic types, including fibers, fragments, filaments, and foams, in bee products[2]. Nonetheless, a deficiency persists in comprehending the precise effects of microplastics on bee health, their accumulation processes, and their interactions with other contaminants. Moreover, the absence of standardized methodologies for the detection and analysis of impediments hinders the comparability of data across various areas[6].

The study employs a comprehensive paradigm[7] that incorporates microplastic morphological characterization, particle quantification, and spatial distribution mapping. The study posits that bees serve as natural bioindicators for monitoring microplastic contamination in the environment due to their active resource collection from their surroundings. The rationale for employing this hypothesis is grounded in the observation that microplastics found in stingless bee products indicate the degree of environmental pollution[8] hence serving as a potential early warning mechanism for pinpointing sources and pathways of pollution.

The selection of *Heterotrigona itama* as the subject of this research is imperative due to its considerable ecological and economic significance, alongside existing scientific knowledge deficiencies and its prospective role as a biomonitoring agent for plastic pollution. *Heterotrigona itama* is among the most extensively farmed stingless bee species in Southeast Asia, particularly in Indonesia[9]. It's distinguished by its superior honey production relative to other *Trigona* bees[10], serving as a significant source of income for local communities through the selling of honey and other hive products[11]. In addition to its economic significance, *H. itama* serves as an essential pollinator for both commercial crops and indigenous plants[12]; highlighting its critical ecological function in sustaining biodiversity and agricultural output. Secondly, although stingless bees constitute the bulk of eusocial bees worldwide, scientific understanding of their biology, morphology, and nesting attributes remains inadequate, including those of *H. itama*[13]. Recent research has emphasized unresolved discrepancies in *H. itama*'s morphology, nest structure, and environmental adaptations[14]. Studies demonstrate that meliponiculture procedures and the environment substantially affect the nest features and morphology of these bees[15].

This paper examines the morphological examination and distribution of microplastics in the products of *Heterotrigona itama*, together with their consequences for bee health and food safety[16]. Examining microplastics in this species addresses a significant deficiency in comprehending the impact of environmental contamination on under-researched pollinators. The widespread prevalence of plastic pollution renders *H. itama* an ideal choice for biomonitoring. While foraging for nectar and pollen, bees intensively interact with their environment, potentially acquiring microplastic particles. Analyzing their products allows us to discern the extent and types of microplastic contamination in many ecosystems, utilizing bees as natural indicators of environmental health. This work aims to clarify the pattern of microplastic contamination to support efforts to reduce plastic pollution and promote more sustainable consumption and environmental conservation practices[17].

This study seeks to examine the morphology, distribution, and effects of microplastics in stingless bee products, particularly *Heterotrigona itama*, as indicators for environmental biomonitoring. The stingless bee, *Heterotrigona itama,* is a crucial and efficient insect pollinator, inhabiting tropical rainforests and human settlements[18]. Stingless bees serve as essential pollinators and natural bioindicators, significantly contributing to the ecosystem[19]. Thus, the contamination of their products with microplastics jeopardizes bee health, as well as food security and environmental sustainability. This study addresses this gap by providing an examination of the morphology, distribution, and size of microplastics in bee products, while also offering a more effective biomonitoring method.

Method

Sampling Methodologies and Criteria for Selection

The research employed a purposive sample method to identify six particular meliponiculture locations, concentrating on locations recognized for *Heterotrigona itama* production. This non-probability sampling method facilitated a focused examination of microplastic contamination in their products.

The sampling locations were selected due to their varied environmental conditions and degrees of urbanization, pertinent to plastic pollution: Urban-yard locations (Cempaka Madu Kelulut, adjacent to SMP 3 Banjarbaru and Budidaya Kelulut Kalimantan in the Sungai Tiung Subdistrict), Semi-urban yard locations (Bumdes Ozi Kelulut in Lihung Village and Kelulut Dua Puteri in Kiram Village), and Rural-garden/plantation locations (Budidaya Kelulut Rafasya in Padang Panjang Village and Kelulut AsSyifa in Beruntung Jaya Village).

The distinction among n urban, semi-urban, and rural locations, as well as between home gardens and plantations, was essential for evaluating microplastic concentrations across different levels of human activity and population density. The designated meliponiculture locations are illustrated in Fig. 1.

Fig. 1 | Research sampling location

Data Collection

At each of the six designated meliponiculture locations, triplicate samples of honey, pollen, and propolis were obtained from *Heterotrigona itama* nests. This repetition was essential for guaranteeing the consistency and trustworthiness of the data acquired from the microplastic examination.

Measurement and Analysis of Microplastics

All microplastic analyses were performed in the Anatomy and Physiology Laboratory at the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University (FMIPA ULM). The gathered samples of honey, pollen, and propolis were examined with a stereo microscope to detect and describe the existing microplastics. The microscopic examination focused on several key aspects. First, the form or morphology of the microplastic particles was observed, revealing four main types: fiber, filament, fragment, and foam. Each morphology was identified based on its distinct shape under microscopic analysis. Next, the quantity of microplastic particles was determined by counting the number of particles present in every 100 milliliters of the sample, providing an estimate of the level of microplastic contamination. In addition, the size of the particles was measured, specifically focusing on their diameter, which was recorded in micrometers (µm). This measurement aimed to assess the distribution of particle sizes within the sample.

The acquired data regarding microplastic presence, morphology, quantity, and size were further analyzed statistically. Specifically, t-test and ANOVA were employed to assess contamination levels across various meliponiculture locations (urban, semi-urban, rural) and among different types of bee products (honey, pollen, propolis). This meticulous laboratory investigation and statistical approach provided accurate identification and quantification of microplastics, yielding substantial proof of environmental pollution.

Results and Discussion

Form (Microplastic Morphologies)

The research indicates the existence of microplastics in goods derived from stingless bees, including honey, pollen, and propolis. It encompasses the identification of four principal morphologies: Fiber, Filament, Fragment, and Foam (Fig. 2). Fiber and Fragment were the predominant categories, with urban locations exhibiting elevated contamination levels (±309 to ±318 particles/100 ml) relative to the majority of rural locations (Bumdes Ozi Kelulut, Budidaya Kelulut Rafasya). The rural location Kelulut Dua Puteri exhibited the greatest contamination level (±362 particles/100 ml), indicating that microplastic pollution extends beyond urban areas. This discovery corroborates prior studies that have consistently found fibers and fragments as the predominant kinds of microplastic in environmental samples, frequently originating from synthetic textiles, packaging, and industrial processes. Increased pollution in urban areas is anticipated due to heightened human activities. The heightened concentrations in Kelulut Dua Puteri underscore the potential for rural areas to serve as notable contributors to microplastic pollution, likely attributable to agricultural activities.

In comparison to the experiment conducted, the microplastic concentrations in this research are significantly elevated[20]. Research on microplastics in honey and bee products has generally indicated reduced particle counts, frequently ranging from 10 to 20 particles/100 ml. The markedly elevated amounts recorded in this study (±309 to ±362 particles/100 ml) may indicate variations in sample locations, environmental factors, or analytical methods. The prevalence of fibers and fragments aligns with global patterns, as these morphologies are commonly detected in air, water, and soil samples. The unexpectedly high contamination in the rural meliponiculture location (Kelulut Dua Puteri) contradicts the idea that urban areas are the principal contributors of microplastic pollution. This underscores the necessity for more investigation into the origins and routes of microplastics in rural environments, along with their possible effects on pollinators and human health. Overall, the study highlights the widespread nature of microplastic pollution and underscores the urgent need for effective mitigation strategies to protect ecosystems and ensure food safety.

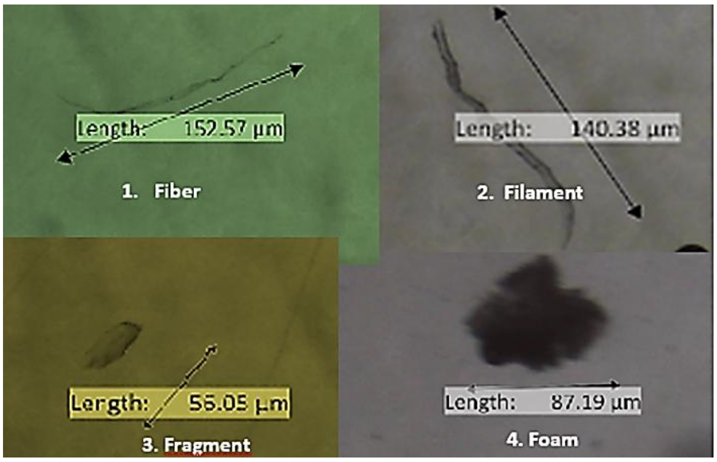

Fig. 2 | Examples of microplastic morphology (fibers, filaments, fragments, and foam) and sizes found in the stingless bee *Heterotrigona itama* products

Quantity (Count of Microplastics)

Fig. 3 illustrates substantial microplastic contamination in honey, pollen, and propolis, with rural-yard honey (Bumdes Ozi Kelulut) exhibiting the highest concentration at 60 particles/100 ml, followed by urban-yard pollen (Budidaya Kelulut Kalimantan) at 37 particles/100 ml, and rural-yard propolis (Kelulut Dua Puteri) at 49 particles/100 ml. The prevalence of microplastic fibers (80.4%) in all samples indicates that textiles and synthetic materials are the principal sources of contamination. This corresponds with prior research, which has repeatedly recognized fibers as a significant contributor to microplastic pollution across several environmental matrices, including air, water, and soil. The size distribution of microplastics, with foam particles at 124.8 µm and fibers at 120.5 µm, aligns with earlier studies that frequently describe particles within the 100–150 µm range. These highlight the ubiquitous presence of microplastic pollution, even in natural products such as honey and bee-derived materials.

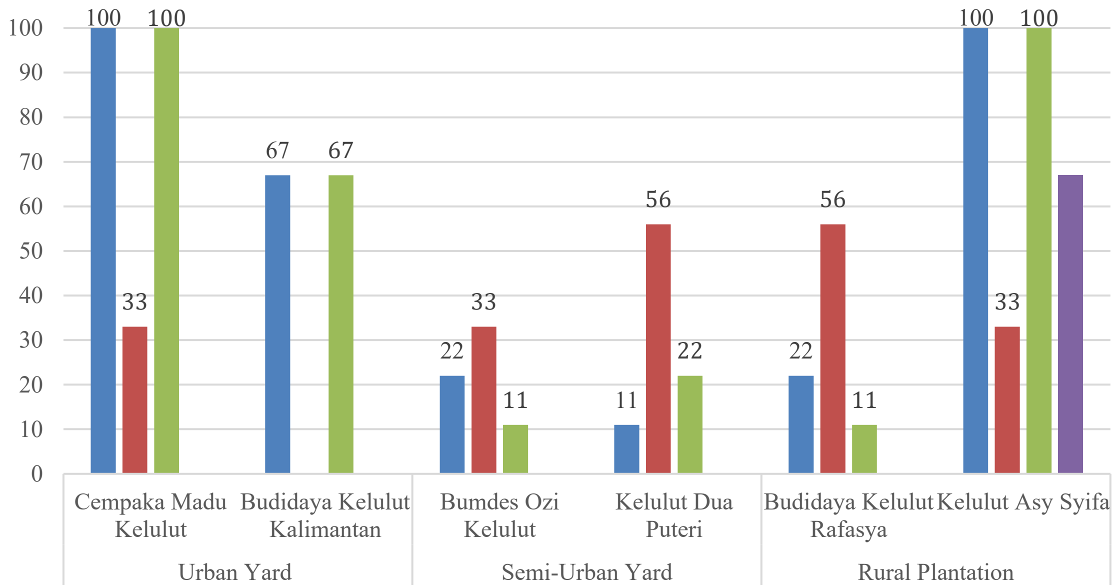

Fig. 3 | Microplastic forms in samples (percentage)

In comparison to previous investigations, the microplastic concentrations identified in this research are significantly elevated. Lower concentrations, ranging from 10 to 20 particles per 100 ml, have been reported in honey[21]. The elevated contamination in rural-yard samples (Bumdes Ozi Kelulut and Kelulut Dua Puteri) contradicts the prevailing idea that urban areas, owing to increased human activity, would demonstrate higher pollution levels. This indicates that rural areas may also serve as considerable sources of microplastic pollution, maybe attributable to agricultural methods or atmospheric deposition. The results underscore the necessity for additional research into the origins and routes of microplastic pollution in both rural and urban environments, along with the possible effects on bees and human health. This study highlights the necessity of mitigating microplastic pollution to safeguard ecosystems and guarantee food safety.

Dimensions (Diameter of Microplastics)

Fig. 4 illustrates the variance in microplastic diameter (in micrometers/µm) present in stingless bee honey. The analysis results reveal that the predominant microplastic category consisted of fragments, with the maximum diameter measuring 154.1 µm at Kelulut Asy Syifa (Farm - Rural) and 141.3 µm at Budidaya Kelulut Kalimantan (Urban). Foam-type microplastics were exclusively detected in Semi-Urban and Rural areas, with diameters measuring 23.3 µm at Bumdes Ozi Kelulut and 78.4 µm at Kelulut Asy Syifa. Fibers and filaments displayed diverse sizes, predominantly within a moderate range of 25.8–120.5 µm.

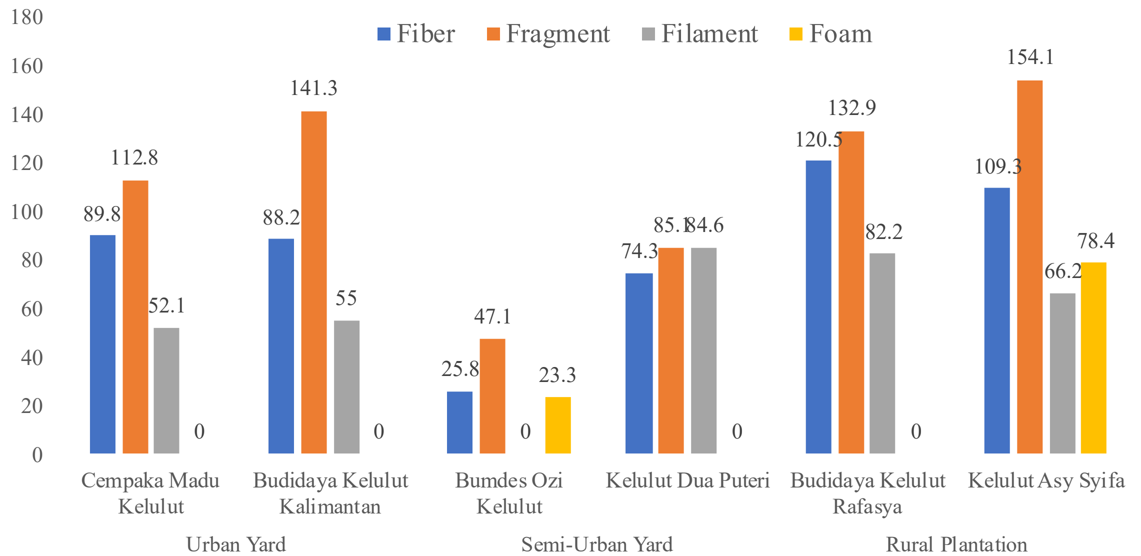

Fig. 4 | Comparison of microplastic diameters in honey (µm) – analysis results from the Anatomy and Physiology Laboratory, FMIPA ULM.

The microplastic diameters in this study exhibited variability but were confined to a shorter range than those reported by Diaz-Basantes et al., who documented a wider diameter range (13.45–6,742.48 µm for fibers and 2.48–247.54 µm for fragments)[22]. In addition to polluting honey, microplastics have been detected in other food products ingested by humans. The study's results indicate that fiber-type microplastics had a greater diameter (84.7 µm) compared to those reported in previous research, which measured an average diameter of 64 ± 39 µm[2]. Although fragment diameters were markedly smaller (112.2 µm compared to 234±156 µm). Nevertheless, for the filament (56.7 µm) and foam (17.0 µm) categories in this investigation, no comparison data were accessible.

The findings of this investigation affirm that, despite urban areas exhibiting significant contamination potential, bigger microplastics are also present in rural locations. This signifies a substantial cross-regional dispersion of microplastic pollution, which must be taken into account regarding the safety of the stingless bee product. The data indicate that geographical variations and human activities around meliponiculture sites affect the degree of microplastic contamination in honey. Differences in location, analysis methods, or environmental characteristics—such as settlement density and the airborne properties of microplastics—are believed to be significant determinants influencing microplastic size and distribution[2].

Prevalence of Plastic Waste

The quantity and variety of plastic waste in an area can signify the degree of urbanization or human activity adjacent to meliponiculture sites. Increased urbanization results in heightened plastic waste, which can contaminate the environment and even infiltrate beehives, thereby impacting pollen and honey through microplastics. Table 1 depicts the incidence of plastic waste across different meliponiculture areas. The research indicates a distinct association between urbanization and the prevalence of plastic waste dominance in meliponiculture areas, with urban-yard locations (Bumdes Ozi Kelulut, Kelulut Dua Puteri, Cempaka Madu Kelulut) demonstrating the highest concentrations of plastic waste (36.67% to 46.67%). This indicates that human activities and urbanization significantly contribute to plastic pollution, which can permeate beehives and taint bee products like honey and pollen. Rural-yard areas, such as Budidaya Kelulut Kalimantan, demonstrate a significant prevalence of plastic waste (38.75%), suggesting that even less urbanized regions are susceptible to plastic pollution. Conversely, rural-garden locations (Budidaya Kelulut Rafasya and Kelulut Asy Syifa) demonstrate markedly reduced plastic waste prevalence (10.92% to 12.00%), presumably attributable to their expansive land areas and diminished human activity levels. The Morisita Distribution Index corroborates these findings, indicating that plastic waste is more concentrated in urban and rural-yard areas (approaching 1.00). Conversely, rural-garden locations demonstrate a more scattered distribution. This clustering effect underscores the localized influence of human activity on plastic pollution, potentially jeopardizing the health of stingless bees and the quality of their products.

Table 1 | Calculation of the dominance value of plastic waste in the meliponiculture area (%)

Parameter	urban-yard		rural-yard		rural-garden	
	Bumdes Ozi Kelulut	**Kelulut Dua Puteri**	**Cempaka Madu Kelulut**	**Budidaya Kelulut Kalimantan**	**Budidaya Kelulut Rafasya**	**Kelulut Asy Syifa**
Atotal (m^2)	507	300	240	480	19000	12679
Agrid (m^2)	1	1	1	1	100	100
Awaste (m^2)	224	110	112	186	2300	1400
Dominance (%)	**44.18**	**36.67**	**46.67**	**38.75**	**12.00**	**10.92**
Morisita Distribution Index	0.98	0.97	0.95	1.00	1.69	1.21
Waste Distribution Status	clustered				dispersed	

Atotal=Total area of meliponiculture (m^2), Agrid=Area of each measurement grid (m^2), and nWaste=Number of grids on the measurement map containing plastic (n)

These findings are consistent with previous research identifying urban areas as hotspots for plastic pollution due to higher population density and consumption levels[23]. Nonetheless, the significant prevalence of plastic waste in rural-yard areas (e.g., Budidaya Kelulut Kalimantan) contradicts the prevailing belief that rural areas are less impacted by plastic pollution. This discovery indicates that areas with moderate human activity can amass considerable plastic waste, either attributable to insufficient waste management or the utilization of plastic materials in agricultural activities. The reduced prevalence of plastic waste in rural-garden locations (Budidaya Kelulut Rafasya and Kelulut Asy Syifa) aligns with research indicating that larger, less disturbed areas generally exhibit lower pollution levels. This study highlights the widespread prevalence of plastic pollution and its possible consequences on meliponiculture, emphasizing the necessity for improved waste management methods and additional research into the influence of microplastics on bee health and ecosystem sustainability.

The impact of plastic prevalence and microplastic existence

The prevalence of plastic waste across various meliponiculture sites indicates the degree of urbanization and human activity, affecting the occurrence of microplastics in stingless bee products. Table 1 reveals a significant association between the prevalence of plastic waste and urbanization, with urban-yard areas (Bumdes Ozi Kelulut, Kelulut Dua Puteri, Cempaka Madu Kelulut) displaying the highest dominance percentages (36.67%–46.67%). This indicates that heavily populated and highly active areas substantially contribute to plastic pollution, which can permeate beehives. The Morisita Distribution Index corroborates this observation, revealing a concentrated distribution of plastic waste in both urban and rural-yard areas, suggesting that localized human activity is a principal source of pollution. This shows that human activities not only have an impact on heavy metal pollution[24-26], air pollution[27,28], but also on water pollution. Conversely, rural-garden locations (Budidaya Kelulut Rafasya, Kelulut Asy Syifa) exhibited markedly reduced plastic waste prevalence (10.92%–12.00%), underscoring the influence of land area and diminished human activity pollution mitigation.

The statistical trend indicates that proximity to human settlements significantly contributes to plastic waste accumulation, underscoring the necessity for enhanced waste management measures in urban and semi-urban areas engaged in meliponiculture. The occurrence of microplastics in stingless bee products, specifically honey, pollen, and propolis, underscores the pervasive extent of plastic pollution. The research revealed four principal microplastic morphologies—fiber, filament, fragment, and foam—with fibers and fragments being the most prevalent. Urban locations (Cempaka Madu Kelulut, Budidaya Kelulut Kalimantan, Kelulut Asy Syifa) demonstrated elevated microplastic contamination levels (±309 to ±318 particles/100 ml), whilst the rural-yard site Kelulut Dua Puteri recorded the greatest contamination (±362 particles/100 ml). This challenges the presumption that urban areas are the exclusive sources of microplastic pollution, indicating alternate mechanisms, such as agricultural practices or air deposition. The existence of synthetic textile-derived microplastics in rural areas underscores the necessity for additional investigation into the mechanisms of microplastic dissemination beyond direct human activities. Statistical comparisons with prior studies indicate markedly elevated microplastic concentrations in this research, suggesting that environmental conditions, sampling methodologies, and local pollution sources are pivotal in influencing contamination levels.

The quantitative data about microplastic contamination in various bee products offers essential insights into pollution patterns. Rural-yard honey (Bumdes Ozi Kelulut) demonstrated the greatest contamination level at 60 particles/100 ml, followed by urban-yard pollen (Budidaya Kelulut Kalimantan) at 37 particles/100 ml and rural-yard propolis (Kelulut Dua Puteri) at 49 particles/100 ml. The prevalence of microplastic fibers (80.4%) corresponds with current research that designates textiles and synthetic materials as significant sources of pollution. The size distributions of foam (124.8 µm) and fibers (120.5 µm) align with prior studies, which often identify microplastic particles within the 100–150 µm range. Rural-yard samples demonstrated unexpectedly elevated microplastic concentrations, challenging the prevailing belief that urban areas would exhibit the highest levels of contamination. This indicates that microplastic contamination may be affected not only by direct plastic waste accumulation but also by wider environmental influences, including air and water transport processes.

Conclusion

The study's findings validate that microplastic pollution has permeated the products of the stingless bee (*Heterotrigona itama*), such as honey, pollen, and propolis, in both urban and rural meliponiculture locations, with fiber and fragment morphologies being the predominant types identified. This pollution is especially evident in urban-yard areas, underscoring the connection between urbanization and environmental plastic waste. Nevertheless, several rural areas, such as Kelulut Dua Puteri, exhibit unexpectedly elevated contamination levels, indicating potential atmospheric or agricultural sources of microplastics. These findings substantiate the concept that stingless bees can function as excellent bioindicators of environmental pollution, owing to their foraging behavior and exposure to local circumstances. The study's innovation resides in its combination of microplastic morphology analysis and spatial distribution mapping in several environmental contexts, offering a methodological enhancement for biomonitoring microplastic pollution using stingless bee products. This research enhances both theoretical and practical insights into environmental contamination routes, highlighting the necessity for standardized detection methodologies to enable cross-regional comparisons. Future research should further examine the health consequences of microplastic deposition in bee colonies and identify mitigating techniques, especially in meliponiculture practices, to improve ecosystem and food safety. These findings significantly influence environmental education, sustainable agriculture, and the formulation of SDG-aligned policies designed to mitigate plastic pollution.

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by **[Anang Kadarsah]**, **[Aminuddin Prahatama Putra]**, and **[Anni Nurliani]**. The first draft of the manuscript was written by **[Eko Suhartono]**, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.