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Long-term dynamics of microplastic accumulation in the intestinal tract of terrestrial insects on the example of *Vespula vulgaris* (Linnaeus, 1758) (Hymenoptera: Vespidae)

Многолетняя динамика накопления микропластика в кишечном тракте наземных насекомых на примере осы обыкновенной *Vespula vulgaris* (Linnaeus, 1758) (Hymenoptera: Vespidae)

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КЛЮЧЕВЫЕ СЛОВА: микропластик, *Vespula vulgaris,* многолетняя динамика, биоаккумуляция, волокна, частицы.

ABSTRACT. The dynamics of microplastic (MP) accumulation in the intestinal tract of terrestrial insects was studied based on the data obtained for the common wasp *Vespula vulgaris* (Linnaeus, 1758) (Hymenoptera: Vespidae) for over 10 years. Wasps were collected in the vicinity of the village of Kireevsk (Tomsk region, Western Siberia, Russia) during the period of 2012 to 2021. A total of 167 individuals were examined. MPs were detected in wasps during all years of the study; fragments and fibers of various colors and sizes were recorded. The MP content did not show statistically significant changes over the past decade. Since 2019, the number of MP particles per individual increased. The ratio of multi-colored fragments and fibers over the years did not show statistically significant changes. In recent years, the proportion of larger fibers and fine particles has grown. The increased number of fibers may be due to an enhanced production and consumption of synthetic textiles; an increased proportion of fragments <0.05 mm may indicate a gradual MP fragmentation.

РЕЗЮМЕ. Проведено изучение динамики накопления микропластика (МП) в кишечном тракте наземных насекомых на примере осы обыкновенной *Vespula vulgaris* (Linnaeus, 1758) (Hymenoptera: Vespidae) за 10 лет. Осы собраны в окрестностях с. Киреевск (Томская область, Западная Сибирь, Россия) в период с 2012 по 2021 гг. Всего на наличие МП исследовано 167 особей. МП в осах обнаружен во все годы исследования, зарегистрированы фрагменты и волокна разного цвета и размера. Статистически значимых изменений в содержании МП в осах за последнее десятилетие не обнаружено. Наблюдается тенденция к увеличению количества частиц, приходящихся на одну особь, начиная с 2019 г. Не выявлено статистически значимых изменений в соотношении разноцветных фрагментов и волокон по годам. В последние годы возрастает доля волокон большего размера и мелких частиц. Повышение количества волокон, возможно, связано с увеличением производства и потребления синтетического текстиля, а возрастание доли фрагментов <0,05 мм может свидетельствовать о постепенной фрагментации МП.

Introduction

Microplastic (MP) includes synthetic polymer particles sized less than 5 mm along the longest axis [Thompson *et al*., 2004]. Reliable and durable materials made from artificial polymers are extensively used during the last 70 years. The largest market share includes low-cost thermoplastic polymers, i.e., polyethylene terephthalate (PET), high-density and low-density polyethylene (PE), linear low-density polyethylene, as well as polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), etc. [Chamas *et al*., 2020].

MP pollution caused by intensive production and consumption of plastic products is a widespread concern. Microparticles of artificial polymers can be found

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in different environments, such as marine and freshwater ecosystems, the atmosphere, agroecosystems, natural soils, food, and drinking water [Nizzetto *et al*., 2016; Kumar *et al*., 2020]. MP ingestion by living organisms is not associated with direct fatal effects, but it causes chronic disorders, for example, reduced food intake or even starvation, developmental disorders and behavioral changes, and can induce long-term toxic effects [He *et al*., 2020; Weber *et al*., 2020; Bartkova *et al*., 2021; Cappello *et al*., 2021; Frank *et al*., 2023]. In addition, MPs can act as a substrate for malignant microorganisms (for example, opportunistic or antibiotic-resistant bacteria) [Bartkova *et al*., 2021]. This feature raises concerns since it could potentially have an adverse effect on biodiversity and functioning of the ecosystems [Reid *et al*., 2019; Rillig *et al*., 2020].

In contrast to well-studied marine and freshwater ecosystems, MP occurrence in terrestrial ecosystems remained virtually unmonitored. Preliminary estimates show that the amount of MPs in the terrestrial environment may be several times larger than that in the oceans [Horton *et al*., 2017]. Insects are effectively used to study MP contamination in terrestrial ecosystems. These animals are of great ecological significance to most aquatic and terrestrial systems. Few other groups of organisms can compete with insects in biomass productivity and species diversity, which makes them preferred targets for analysis of the accumulation of toxic substances [Oliveira *et al*., 2019]. At present, there are no reported data on the long-term dynamics of MP bioaccumulation in terrestrial insects. Moreover, few studies address this issue on a worldwide scale, and the territory of Western Siberia remains completely unexplored in this respect.

The common wasp *Vespula vulgaris* (Linnaeus, 1758) (Hymenoptera: Vespidae) was used as a model object to monitor MP accumulation in insects in Western Siberia. This species is ubiquitous and plays an essential role in forest and agro-ecosystems. The common wasp inhabits almost all continents, reproduces rapidly, exhibits high biomass productivity, and its diet is extremely wide, which makes it a preferred object of the present study.

The aim of this work is an analysis of the dynamics of MP accumulation in the intestinal tract of terrestrial insects on the example of *V. vulgaris*.

Materials and methods

Field methods. During the period of 2012 to 2021, wasps were collected in the vicinity of the village of Kireevsk (56°21′50″ N 84°05′28″ E), Tomsk region, Western Siberia, Russia. Insect collecting was carried out as follows: sweep netting was performed on dry grass, in sunny weather, along a 100 m survey path; the path was laid to include all biotopes or ecotones available in the area; after every 10 sweeps, insects were removed from the net into a separate killing jar to facilitate recording (all killing jars were immediately labeled); netting in the air; Moericke traps (plastic rectangular yellow pan traps 25 cm long, 19 cm wide and not less than 3 cm deep) were placed on the ground in series; the series consisted of 10 pan traps 1 meter spaced in one line through the selected biotope; the pan traps were filled with soapy water; the traps were inspected once a day [Bagirov et al., 2011]. The captured wasps were stored in 96% ethanol. A total of 167 individuals were examined for MPs.

Laboratory methods. Prior to homogenization, each individual was thoroughly washed in distilled water to prevent particles on the insect surface from entering the sample. The abdomen contents were separated from the exoskeleton, and after that, the entire abdomen contents were homogenized. MPs could be found only in the intestine, since the detected particles (0.01–2 mm in size) were unable to penetrate through the peritrophic membrane of the insect. For further microscopic analysis and quantitative determination of MPs, wasps were homogenized in pools of 13–25 individuals each annually; 35% H_2O_2 with 0.05 M $FeSO_4$ was added as a catalyst in a ratio of 3/1 (v/v) until complete dissolution of organic tissues [Claessens *et al*., 2013; Karami *et al*., 2017; Lusher *et al*., 2020; Simakova *et al*., 2022]. The filters were stored in glass Petri dishes to prevent external plastic contamination. For the control, filters used to process blank samples were simultaneously examined to monitor possible contamination from the atmospheric air and/or reagents. During calculation, MP morphological features were also analyzed to group the detected polymer particles. The analyzed features included MP shape (irregularly shaped fibers/fragments), size (large MP for fibers/fragments >1 mm, small MP for fibers <1 mm), and color (red, blue, black, transparent, orange and 'other colors') [Hidalgo-Ruz *et al*., 2012]. Microphotographs were taken using an Axiocam ERc5s camera (Zeiss, Germany).

Statistical analysis. Data were analyzed using R statistical package v4.0.5 [R Core Team, 2021]. The dynamics of MP accumulation in wasps was analyzed via Spearman's rank correlation analysis and Fisher's exact test.

Results

The study of wasps from natural populations collected in the vicinity of the village of Kireevsk during the period of 2012 to 2021 revealed MPs in their intestines. On average, 0.92 ± 0.19 (±standard error) MP units per individual were detected over the entire study period. The number of particles varied significantly in different years, from 0.40 (2012) to 2.40 (2020). Spearman's rank correlation analysis did not reveal statistically significant changes in the MP content in wasps over the studied period ($r_s = 0.55$, p-value 0.104); however, the number of particles per individual tends to increase starting from 2019 (Fig. 1).

The analyzed MPs were categorized by shapes, sizes and colors. In terms of shape, MPs were divided into shapeless fragments and fibers. The detected particles were less than 0.05 mm in size, and the fibers were divided into groups of <1 mm and >1 mm. The analyzed distribution of particles of different shapes and sizes in wasps over the studied decade revealed changes in their ratio over the years (Fisher test, p-value = 0.018). In 2012–2015, fibers <1 mm predominated significantly (76.9–88.9%); in 2016, the proportion of larger fibers and small fragments increased (Table 1). Among MP fibers, transparent and black particles were distinguished; blue ones were less common (Table 2). MP fragments were typically red and orange, and blue fragments could also be detected (Table 2). The ratio of multi-colored particles and fibers has not changed over the years. Among fibers, transparent (39.5% of the total MP number) and

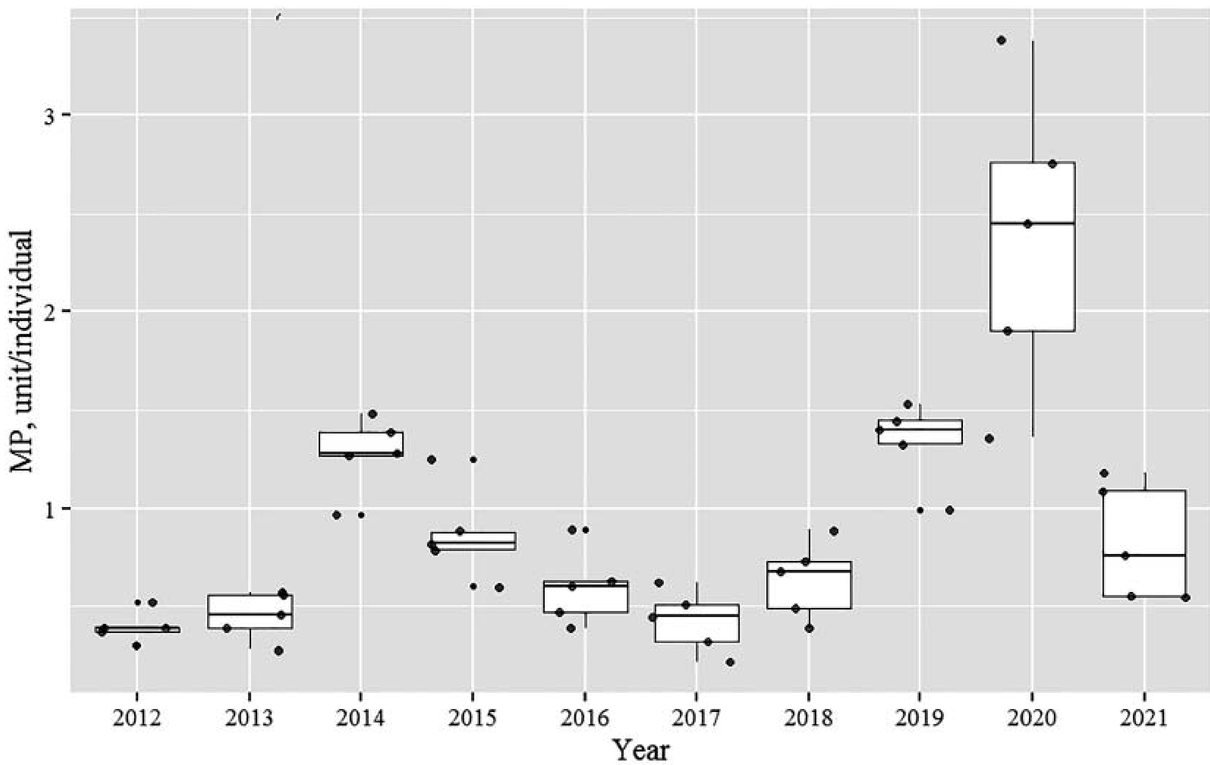

Fig. 1. Box and whisker plot showing average content of MP particles inside wasps from natural populations sampled in the vicinity of the village of Kireevsk (five samples/year; Tomsk region, Russia).
Рис. 1. Диаграмма, демонстрирующая среднее содержание частиц МП в осах из природных популяций (5 выборок/год) в окрестностях с. Киреевск (Томская область, Россия).

black (36.2%) particles were most frequently observed, blue ones (5.9%) were less common, and fibers of other colors were infrequent (Fig. 2).

Discussion

Published works on the interaction between MPs and insects primarily address the effect of MPs on viability and food selectivity, growth, development, and physiology of terrestrial insects, as well as on the insect ability to degrade a wide range of synthetic polymers. The effect of MPs on biological functions has been thoroughly investigated in model experiments on some species of terrestrial insects, such as: flies *Drosophila melanogaster* Meigen, 1830 and *Hermetia illucens* (Linnaeus, 1758); the honey bee *Apis mellifera* Linnaeus, 1758, and the cricket *Gryllodes sigillatus* (Walker, 1869) [Boots *et al*., 2019; Oliveira *et al*., 2019; He *et al*., 2020; Kumar *et al*., 2020; Weber *et al*., 2020; Baho *et al*., 2021; Cappello *et al*., 2021]. A number of experiments was carried out on *A. mellifera* to reveal the effect of the diet supplemented with microfibers. No adverse effects on the biological functions of bees were found, yet the authors do not exclude such effects under a longer exposure to MPs [Buteler *et al*., 2022]. In addition, the effect of MPs on the survival rate was studied on the example of the larvae of the black soldier fly *H. illucens*. The survival rate of individuals grown on medium supplemented with PS was 5% lower compared to the control [Cho *et al*., 2020]. Different types of MPs were found to have different effects on insect viability. A certain type of MPs added to the diet of the cricket *G. sigillatus* during its development resulted in a significantly lower body weight

Table 1. Percentage of MPs of different sizes inside wasps from the vicinity of the village of Kireevsk (Tomsk region, 2012–2021).
Таблица 1. Процентное соотношение размерных частиц МП в осах из окрестностей с. Киреевск (Томская область, 2012–2021 гг.).

Year	MP shape and size		
	Fragments <0.05 mm	Fibers <1 mm	Fibers >1 mm
2012	11.1	88.9	0.0
2013	0.0	80.0	20.0
2014	12.0	80.0	8.0
2015	11.5	77.0	11.5
2016	0.0	63.6	36.4
2017	20.0	60.0	20.0
2018	12.5	50.0	37.5
2019	20.0	50.0	30.0
2020	35.3	50.0	14.7
2021	42.9	35.7	21.4

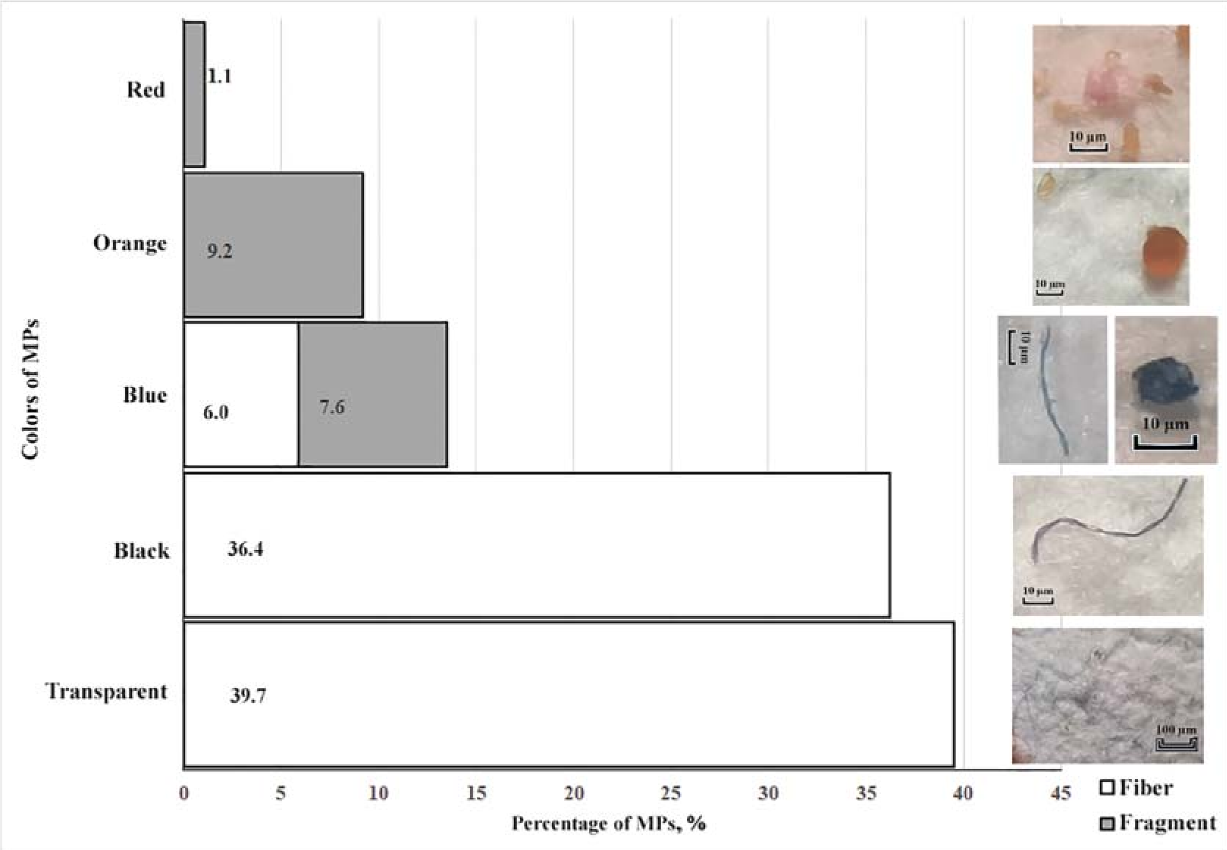

Fig. 2. Occurrence of different types of MPs inside wasps, %.
Рис. 2. Встречаемость разнотипных частиц МП в осах, %.

Table 2. Percentage of MPs of different colors among fibers and fragments inside wasps.
Таблица 2. Процентное соотношение частиц МП разной окраски среди волокон и фрагментов в осах.

Year	Red, %		Orange, %		Transparent, %		Blue, %		Black, %	
	Fiber	Fragment	Fiber	Fragment	Fiber	Fragment	Fiber	Fragment	Fiber	Fragment
2012	0.0	0.0	0.0	0.0	33.3	0.0	22.2	11.2	33.3	0.0
2013	0.0	0.0	0.0	0.0	40.0	0.0	10.0	0.0	50.0	0.0
2014	0.0	0.0	0.0	12.0	56.0	0.0	4.0	0.0	28.0	0.0
2015	0.0	4.0	0.0	0.0	56.0	0.0	0.0	8.0	32.0	0.0
2016	0.0	0.0	0.0	0.0	54.5	0.0	27.3	0.0	18.2	0.0
2017	0.0	0.0	0.0	0.0	30.0	0.0	20.0	0.0	50.0	0.0
2018	0.0	0.0	0.0	12.5	37.5	0.0	12.5	0.0	37.5	0.0
2019	0.0	0.0	0.0	20.0	26.7	0.0	0.0	0.0	53.3	0.0
2020	0.0	0.0	0.0	8.7	32.4	0.0	0.0	26.5	32.4	0.0
2021	0.0	7.1	0.0	21.4	28.6	0.0	0.0	14.3	28.6	0.0

in the adult stage compared to the control [Fudlosid *et al*., 2022]. Interestingly, some insect species exhibit the ability to degrade artificial polymers. For example, the darkling beetle *Tenebrio molitor* Linnaeus, 1758 is apparently able to chew and swallow fragments of plastic packaging, including PVC, PE and PP [Bowditch, 1997; Yang *et al*., 2015; Peng *et al*., 2019]. Caterpillars of the Indian meal moth *Plodia interpunctella* Hübner, 1813 are able to digest PE with the help of intestinal microflora [Yang *et al*., 2014, 2015].

In addition, the experiments with laboratory cultures of blood-sucking mosquitoes *Aedes aegypti* Linnaeus, 1762, *Anopheles albimanus* Wiedemann, 1820, *Anopheles quadrimaculatus* Say, 1824 and *Culex quinquefasciatus* Say, 1823 revealed that larvae of different mosquito species readily digest MPs [Dadd, 1971; Aly, 1988]. Further studies showed the ontogenetic transfer of MPs from larvae to pupae and adults in the laboratory culture of *Culex pipiens* Linnaeus, 1758 [Al-Jaibachi *et al*., 2018]. In this case, MP bioaccumulation depended on the particle size and concentration: the smaller the particles and the higher the MP concentration were, the more successful the ontogenetic transfer was; however, MP ingestion by mosquitoes did not affect their body mass and mortality [Al-Jaibachi *et al*., 2018, 2019]. MPs with a diameter of 2 μm are easily ingested by *Ae. aegypti* larvae with food, partially pass into non-feeding aquatic pupae, and then into flying adults, which live in the terrestrial environment. The larva-to-pupa transition was followed by significant (and almost complete) loss of MPs, while during the pupa-to-adult transition, this loss was insignificant (on average, $7.30 \cdot 10^6$ particles per larva, 15.8 particles per pupa, 10.9 particles per adult) [Simakova *et al*., 2022]. Thus, larvae of bloodsucking mosquitoes actively accumulate a large number of MPs. There are no published data on the quantitative content of MPs in natural populations of mosquitoes or any terrestrial insects to be compared with our experimental results.

Over the studied decade, we did not reveal statistically significant changes in the MP content in the intestinal tract of the common wasp *V. vulgaris*; however, over the last 3 years of study (2019–2021), the MP content was observed to increase. In recent years, the proportion of fibers >1 mm and fragments <0.05 mm has grown up. The increased content of larger fibers accumulated by insects may indirectly indicate an extensive production of synthetic fabrics, and this is further supported by published data. The size range of MPs found in the gastrointestinal tracts of the wasps is determined by their anatomical features. The usual diameter of the pharynx in *V. vulgaris*, according to our measurements, is 13–15 μm, and this explains that detected MP particles did not exceed 10 μm. *V. vulgaris* is an almost omnivorous insect with a very diverse diet. The wasps can ingest insect larvae (caterpillars, bee brood, etc.), adults of other insects, nectar, fruits, meat and other available food. Most likely, the studied wasps consume plastic fragments and fibers along with this diverse food. It is not uncommon to see *V. vulgaris* wasps swarming around fruit and vegetable stalls at outdoor markets. MP particles on the fruit surface ingested by the insects may come from packaging materials and textile clothing. When building a nest, wasps chew cardboard and wood items, and this is another possible way how MPs can enter the wasp's body.

The world production of fibers (synthetic and natural) raised from 57 million metric tons in 2000 to 111 million tons in 2020, and some estimates predict the production of textile fibers of 145 million tons by 2030 [Periyasamy, 2022]. Synthetic textiles are now recognized as the primary source of plastic microfibers in natural environments [Manshoven *et al*., 2022]. Microfibers are shed from synthetic textiles along the entire lifecycle of the fabric, from production and use to waste recycling [Manshoven *et al*., 2022]. The mass of microsized fibers released during washing ranges from 100 to 300 mg per kg of washed fabric [De Falco *et al*., 2019]. According to the European Chemicals Agency [2021], the annual release of secondary MPs (unintended by-product of primary MP decomposition) is 176,000 tons.

The present study revealed higher proportions of plastic microfragments <0.05 mm in wasp intestines over the past 3 years. An increased proportion of small fragments indicates that MP decomposes into smaller particles (up to nanoplastic particles <1 μm), which contributes to its bioavailability. Under mechanical stress caused by wind and water (mechanical degradation), as well as sunlight (ultraviolet degradation), plastic debris degrades into smaller fragments to form secondary MPs, i.e., weathering by-products of larger plastic items released into the environment [Andrady, 2011]. Currently available data on MP degradation in the environment are meager, but circulating microfragments are expected to degrade into smaller particles and eventually turn into nanoplastics [Sait *et al*., 2021]. The amount of secondary MPs resulting from poor waste management has not been estimated yet. However, the rate of its formation attains 1.15–12.7 million tons per year, and the nature of pollution varies in different regions around the world [Manshoven *et al*., 2022]. A global partnership of research organizations, industry and government agencies is therefore required to reduce MP emissions.

Conclusion

MPs were found in wasp samples collected over the years of study (2012–2021). The average number of particles per insect varied from 0.4 in 2012 to 2.4 in 2020. No statistically significant changes in the MP content in wasps were found over the past decade, although the number of particles per insect tended to increase since 2019.

Shapeless fragments <0.05 mm and microfibers of different colors and sizes were found among the detected MPs. Predominantly transparent fibers were recorded (from 27% in 2019 to 56% in 2014–2015). No statistically significant changes in the ratio of multi-colored fragments and fibers could be observed over the years. In recent years, the proportion of fibers >1 mm and small fragments increased. The higher proportion of fragments <0.05 mm can indicate the decay of MPs in the environment. An increased proportion of fibers >1 mm ingested by wasps can be considered as a bioindicator of an extensive use of synthetic fabrics.

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

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