Environmental Pollution 331 (2023) 121885

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Origin of non-exhaust PM in cities by individual analysis of particles collected by honey bees (*Apis mellifera*)☆

Marco Pellecchia [a,1], Giulia Papa [b,c,1], Mario Barbato [d,e], Giancarlo Capitani [f], Ilaria Negri [b,*]

[a] KOINE'- Consulenze Ambientali, Via Parmigianino 13, Montechiarugolo, Parma, Italy
[b] Dipartimento di Scienze delle Produzioni Vegetali Sostenibili, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, Piacenza, Italy
[c] Istituto per la Protezione Sostenibile Delle Piante, Consiglio Nazionale Delle Ricerche, IPSP-CNR, Strada delle Cacce 73, 10135, Torino, Italy
[d] Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, Piacenza, Italy
[e] Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Viale Palatucci snc, Messina, Italy
[f] Dipartimento di Scienze Geologiche e Geotecnologie, Università di Milano-Bicocca, Piazza della Scienza 4, Milan, Italy

ARTICLE INFO

Keywords:
Honey bee
Particulate matter
Pollutant
SEM-EDX
Non-exhaust emissions

ABSTRACT

Urban areas present multiple challenges to scientists interested in unraveling the source, transport, and fate of airborne particulate matter (PM). Airborne PM consists of a heterogeneous mixture of particles with different sizes, morphologies, and chemical compositions. However, standard air quality stations only detect the mass concentration of PM mixtures with aerodynamic diameters ≤10 μm (PM_{10}) and/or ≤ 2.5 μm ($PM_{2.5}$). During honey bee foraging flights, airborne PM up to 10 μm in size attaches to their bodies, making them suitable for collecting spatiotemporal data on airborne PM. The individual particulate chemistry of this PM can be assessed using scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy on a sub-micrometer scale, allowing accurate identification and classification of the particles.

Herein, we analyzed the PM fractions of 10–2.5 μm, 2.5–1 μm, and below 1 μm in average geometric diameter collected by bees from hives located in the city of Milan, Italy. Bees showed contamination by natural dust, originating from soil erosion and rock outcropping in the foraging area, and particles with recurrent heavy metal content, most likely attributed to vehicular braking systems and possibly tires (non-exhaust PM). Notably, approximately 80% of non-exhaust PM was ≤1 μm in size. This study provides a possible alternative strategy to apportion the finer fraction of PM in urban areas and determine citizens' exposure. Our findings may also prompt decision-makers to issue policy addressal for non-exhaust pollution, especially for the ongoing restructuring of European regulations on mobility and the shift toward electric vehicles whose contribution to PM pollution is debated.

1. Introduction

Particulate matter (PM) is a ubiquitous air pollutant responsible for millions of deaths annually (World Health Organization, 2020). Airborne PM is a heterogeneous mixture of particles of different sizes, morphologies, and chemical compositions and is commonly classified according to their aerodynamic diameters. Particles less than 10 μm in aerodynamic diameter (PM_{10}) can penetrate the respiratory tract below the larynx, whereas those less than 2.5 μm ($PM_{2.5}$) can reach the gas-exchange interface of the lungs (Brown et al., 2013). The sub-micrometer fraction (PM_1) is of much concern, because of its ability to enter the blood circulation and distribution to most organs, including the brain (Maher et al., 2016).

Current PM monitoring systems only assess PM_{10} and/or $PM_{2.5}$ mass concentrations (Directive, 2008/50/EC of the European Parliament and of the Council of May 21, 2008 on ambient air quality and cleaner air for Europe), and fail to assess the contribution of the sub-micrometer fraction. Moreover, trace metal analyses in PM, if available, usually lack a physical-chemical characterization of single particles, whereas it is acknowledged that particle size, shape, and chemical composition are responsible for adverse biological effects (Valavanidis et al., 2008; Brown et al., 2013; OECD, 2020; Plutino et al., 2022; Papa et al., 2021a).

☆ This paper has been recommended for acceptance by Admir Créso Targino.

* Corresponding author.
E-mail address: ilaria.negri@unicatt.it (I. Negri).

[1] These authors contributed equally to the work

https://doi.org/10.1016/j.envpol.2023.121885
Received 23 August 2022; Received in revised form 22 May 2023; Accepted 23 May 2023
Available online 24 May 2023

In urban areas, road traffic is one of the major sources of PM. Airborne particles may be emitted from both exhaust (fossil fuel combustion) and non-exhaust sources (abrasion of vehicle components, e.g., braking system, tires, and friction with road surfaces). However, while stringent regulations have substantially mitigated exhaust-derived PM emissions, regulations addressing non-exhaust emissions are lacking (OECD, 2020; Piscitello et al., 2021). Further, information on the contribution of non-exhaust particulate matter in daily life, with special regard to the sub-micrometer fraction is lacking (Panko et al., 2018; Baensch-Baltruschat et al., 2020; OECD, 2020; Rausch et al., 2022). Moreover, the actual impact on human and environmental health is debated as less data are available on the size, morphology, and chemical composition of airborne particles, especially for those below 1 μm (Baensch-Baltruschat et al., 2020; Järlskog et al., 2022; Plutino et al., 2022; Rausch et al., 2022; Papa et al., 2023).

Here, we used an innovative methodology to characterize and assess the relative abundance of airborne PM, distinguishing between particles with an average geometric diameter ranging 10–2.5 μm, 2.5–1 μm, and below 1 μm. We employed honey bees (*Apis mellifera*) as PM collectors in a metropolitan city in north Italy and analyzed PM using scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX). Honey bees are eusocial insects, mainly known for their role in pollination, a fundamental ecosystem service for maintaining plant biodiversity and, ultimately, for human and planetary health and wellbeing (Papa et al., 2022). Honey bees are also important biomonitors of pollutants (Devillers and Pham-Delegue, 2002; Satta et al., 2012; Zarić et al., 2018) and particularly airborne PM (Negri et al., 2015; Pellecchia and Negri, 2018; Capitani et al., 2021;Edo et al., 2021; Papa et al., 2021b). During their flight, the abundant pubescence on the body of the bees is charged with frictional electricity, allowing for electrostatic pollination and facilitating the attraction of PM (Vaknin et al., 2000; Bonmatin et al., 2015). Airborne PM attached to the bee's body - especially the forewings - can be analyzed using SEM/EDX, providing individual particle chemistry on a sub-micrometer scale (Negri et al., 2015; Pellecchia and Negri, 2018; Capitani et al., 2021; Papa et al., 2021b).

The use of bees as PM biosensors offers several advantages over other pollution monitoring systems with respect to sampling and analysis (Negri et al., 2015; Pellecchia and Negri, 2018; Capitani et al., 2021; Papa et al., 2021b), in particular: (i) each forager bee acts as an autonomous, efficient, and cheap sampling unit, as the insects thoroughly interact with all environmental domains (air, soil, water, and vegetation); (ii) the bee acts as a size-selective sampling unit with a cut-off of 10 μm in size, as PM >10 μm are only occasionally found on the bee forewings; (iii) as bees fly several hundred meters around a hive and visit up to 1000 flowers per day, they can collect representative samples of PM - both suspended in air and deposited on surfaces - emitted from different sources, and (iv) PM collected by bees can be analyzed down to the ultrafine fraction using SEM/EDX with no sample processing, eventually providing morpho-chemical information on the particles and information about their emission sources.

2. Materials and methods

2.1. Honey bee samples

Three beehives were installed in March 2021 in Parco Segantini, a public park in the center of Milan (Italy; lat. 45°26′50.7156″N; long. 9°10′12.7596″E) surrounded by the major city road networks, covering approximately 9 ha (Fig. 1). The area falls inside a limited traffic zone (known as "area B″), with no access and circulation allowed for the most polluting vehicles from Monday to Friday from 7:30 a.m. to 7:30 p.m. Despite this, the area always suffers from congested traffic, and more than 630.000 transits of vehicles per day in 2021 have been recorded (www.comune.milano.it).

Ten worker bees at the end of their life were collected monthly from June to September 2021, yielding a total of 40 bees, and euthanized. For each bee sampled, both forewings were analyzed using a Tescan VEGA TS5136XM (Brno - Kohoutovice, Czech Republic) and a Zeiss Gemini 500 (Oberkochen, Germany) SEMs, both equipped with an EDX system, as previously described (Negri et al., 2015). Briefly, measurements were conducted on particles present in four easily identifiable areas, 450 × 450 μm wide, on the insect forewings. Forewings are affected by the leading edge vortex phenomenon that enhances the accumulation and deposition of the particles (Pellecchia and Negri, 2018). Back-scattered electron (BSE) images, whose contrast is dominated by the average atomic number of the particle, were obtained to locate PM on the wings; EDX point analyses were performed to determine particle composition. Secondary electron (SE) images, sensitive to sample topography, were acquired to determine particle sizes and morphology.

A total of 4860 particles were counted (114 particles/bee) and grouped based on their compositions and sizes. The results were compared with those of negative control bees consisting of adult individuals that emerged from a brood frame placed in a growth chamber at 36 °C and 60% relative humidity (Capitani et al., 2021; Papa et al., 2021b).

2.2. Soil analyses

Topsoil/exposed sediment samples were collected from areas surrounding the hive (Fig. 1). The sites selected for sample collection were soils with and without grass (located 5 and 60 m from the hives,

Fig. 1. Maps of the sampling area generated using QGIS V. 3.28.0. (A) Location of the hive in Milan. (B) Topsoil/exposed sediment samples were collected approximately 5 m (a) and 40 m (b) away from the hives (soil with and without grass, respectively) and 60 m (c) from the hives (artificial path for pedestrians and bicycles).

respectively) and pedestrian-cycle paths (located approximately 40 m from the hives). From each site, three to five samples were collected. Briefly, shallow pits were excavated (maximum 5-cm deep) and up to 0.5 kg of material was recovered per pit. The samples were air-dried and approximately 1 g of material was mixed with equal aliquots of samples from the same site to obtain representative samples. These mixtures were sieved and the fraction <45 μm was investigated using X-ray powder diffraction (XRPD), and energy-dispersive X-ray fluorescence (EDXRF) analyses.

XRPD investigations were performed with a Bragg-Brentano PANalytical (Malvern, UK) X'PertPro PW3060 system. Data were collected in the 5–80° 2θ range (CuKα radiation), with steps of 0.02°/s, at room temperature, with operating conditions of 40 mA and 40 kV. Mineral phase abundances were determined using the Rietveld method (Rietveld, 1969) using the PANalytical X'Pert High Score Plus software.

EDXRF chemical analyses were performed using a PANalytical Epsilon 3X instrument. Sample briquettes were prepared by pressing a mixture of 15 g of powdered sample, 5 g of boric acid, and a few drops of polyvinyl alcohol for 1 min at approximately 1.5 GPa, and left to dry for 2–3 h at 60 °C. The Omnian-standardless method was used for quantitative analyses. Aliquots of the same sample powder were used for the determination of both volatile components (H_2O and CO_2) through weight loss on ignition (LOI) and the Fe^{3+}/Fe^{2+} ratio through $KMnO_4$ redox titration.

2.3. Disc, brake pad, and tire samples

Particles were directly collected from used discs, brake pads, and tires removed from light vehicles by pressing SEM stubs carrying carbon adhesive pads against them. Samples were analyzed using SEM/EDX under the same operating conditions used for honey bee wings. Analyses were also performed on wear debris generated from traditional brake pads using a full-scale dynamometer adapted to measure and collect wear particles, as described by Matějka et al. (2020). Additionally, fragments of new brake disks, pads, and used tires were cut, mounted onto SEM stubs, and analyzed. Six replicates were prepared for each sample.

3. Results and discussion

The SEM/EDX analyses revealed the presence of particles up to 10 μm in size (2D geometric diameter) on the honey bee wings from Milan (Fig. 2A and B), while the negative control did not display PM contamination.

The mineral particles found on honey bees living in the urban park were typical of both the local natural and urban environments (i.e., quartz, calcite, clays, alkali feldspars, plagioclase, and dolomite). Up to 78, 50, and 22% of such particles were in the size ranges 10–2.5, 2.5–1, and <1 μm, respectively.

This mineral association is consistent with the XRPD analysis of the soil and ground surrounding the beehive (Table 1) and is typical of soils and alluvial deposits in the Po Valley, the area harboring the city of Milan. Dolomite is also the main constituent of "*calcestre*", a finely crushed material commonly used in pedestrian-cycle paths in this region. During their flight and foraging activity, the bees may have therefore been exposed to soil dust and wear of the pedestrian-cycle paths (dolomite) lifted by wind, bicycles, and pedestrians, as well as to inert minerals (e.g., quartz and feldspar) from the asphalt of the city road.

In addition to common crust minerals, EDX analyses on the bee wings revealed the presence of heavy metal particles in the following recurrent combinations: Fe + Mn, Fe + Cu, Fe + Cr + Mn ± Ni, and Fe + Zn plus minor amounts of S, Si, and Ca (Figs. 3 and 4). Importantly, C and O are always present in the spectra since both are major constituents of the honey bee wings (chitin, proteins, wax, hemolymph, etc.) and can be struck by the electron beam, especially if the particles are smaller than 3–5 μm (Capitani et al., 2021). Moreover, C is present in the coating used to make the sample conductive, and both C and O are strongly absorbed within the particle, due to their low-energy kα radiation. Therefore, the relative heights of the C and O peaks may depend not only on the particle composition, but also on these other factors. However, in most cases, their exact quantification is not required to identify a compound, but a qualitative estimation is sufficient.

Other compounds on the bee wings were Ba sulfate (commonly known as barite) and Fe oxides/hydroxides (Figs. 3 and 4). Barite and Fe compounds (alloys, oxides, hydroxides) are not typically abundant in local soils, as they usually occur in different geological settings (lateritic soils, mine areas); however, both are widely present in areas of heavy traffic (Capitani et al., 2021; Papa et al., 2021b; Papa et al., 2021b).

The metropolitan area of Milan suffers from congested traffic; hence, we analyzed vehicle components (brake discs, brake pads, and tires) and their worn parts. EDX spectra acquired on both types of material overlapped qualitatively with those acquired on particles contaminating the insect wings (Figs. 3 and 4, S1, and S2).

EDX spectra acquired on brake pads and discs displayed significant levels of Fe + Mn, Fe + Cu (spectra in Fig. 3B, D acquired on pads), and Fe, Fe + Mn + Cr (spectra in Fig. 4B, D acquired on discs), often associated with minor amounts of other elements such as Si, S, Ca, and Al on bee wings (Figs. 3 and 4). Elemental mapping performed on wear debris from brake pads showed Fe, Cr, and Al in great abundance, while Cu and S were primarily concentrated in a few particles (Fig. 5).

Our results are consistent with previous findings based on SEM/EDX of single particle analyses of traffic-related non-exhaust PM collected by passive sampler devices (Sommer et al., 2018; Weinbruch et al., 2014; Rausch et al., 2022). Brake discs are primarily made of Fe alloys and brake pads can contain Ba sulfate, commonly used as a filler (Ingo et al., 2004; Kukutschová et al., 2011; Pant and Harrison, 2013; Carrero et al., 2014; Grigoratos and Martini, 2015; Adamiec et al., 2016; Venkatesh and Murugapoopathiraja, 2019). In addition, Fe oxides/hydroxides are abundant in brake pads owing to long-term exposure to air and humidity (Österle et al., 2001; Ingo et al., 2004).

EDX spectra acquired on used tires and tire wear showed significant peaks of Zn, Fe, Si, S, and Ca (Fig. 4F, S1, and S2). S and ZnO are used as vulcanization agents in tires, and SiO_2 and $CaCO_3$ as rubber fillers (Panko et al., 2018; Sommer et al., 2018). Therefore, EDX spectra acquired on new tires are characterized by significant peaks of Zn, S, Si, and Ca in a C-rich matrix (rubber). Conversely, used tires and tire wear debris are usually contaminated by other vehicle-derived components, especially mineral particles originating from the road surface and fine dust stemming from brakes (Baensch-Baltruschat et al., 2020; Rausch et al., 2022; Järlskog et al., 2022). In our study, EDX spectra acquired from used tires and tire wear displayed a significant amount of Fe, i.e., the dominant element of brake discs (Fig. 4F and S1). Hence, if Zn alone is barely considered a specific tracer of tire wear because it may be present in particles from other common anthropogenic sources (e.g., zinced surfaces, road markings, brake wear, industrial emissions, etc.; Baensch-Baltruschat et al., 2020), its co-occurrence with S, Si, O, Ca, and Fe can be used as a distinctive fingerprint of tire wear PM analyzed with EDX, consistent with previous findings (Järlskog et al., 2022; Rausch et al., 2022). The presence of Fe, Zn, Si, Al, and S in the EDX spectra of some PM acquired from bees (Fig. 4E), prompts us to speculate that such particles might be derived from tire wear. However, further analyses are necessary to confirm whether such particles are actually tire wear particles contaminated by fragments from discs or metallic fragments of the braking systems, especially considering that the C-rich matrix characterizing the groundmass (rubbers) of tires cannot be easily detected in bee samples because carbon is a major constituent of the honey bee cuticle.

SEM observations confirm that brake particles have irregular morphology and sharp edges (Fig. 2A–C), while no specific morphologies have been detected for tire wear PM other than being multigrain aggregates (Fig. 2 H and S2). In the relevant literature, the morphology

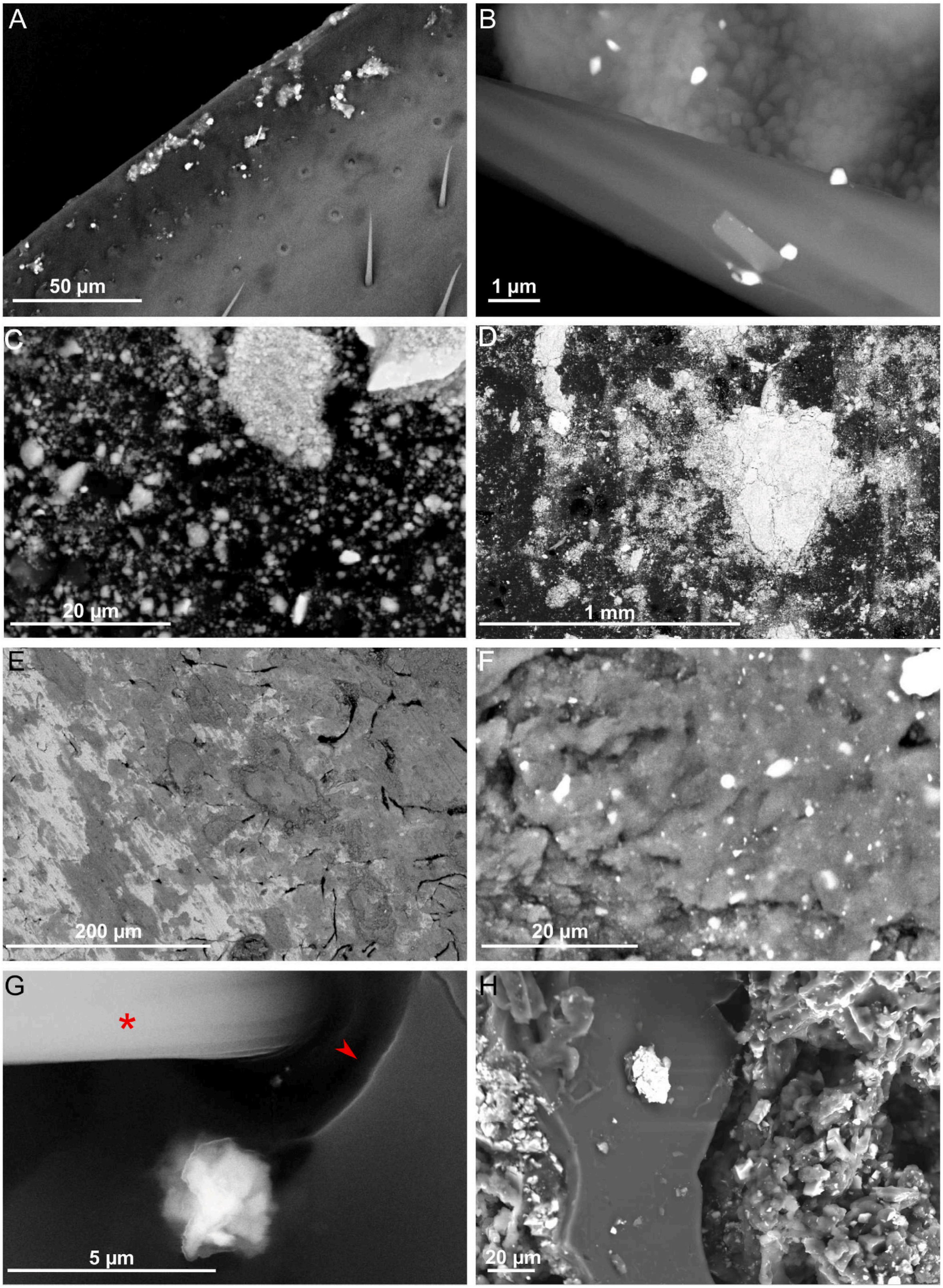

Fig. 2. (A): Back-scattered electron (BSE) micrograph of a bee wing with particulate matter (PM; bright spots) mostly from disc and tire wear. (B): A wing with fine and ultrafine PM (bright spots) from brake pads. (C): Particles collected from the surface of a used brake pad displaying a highly heterogeneous population of particle in term of size, including PM < 1 μm, with sharp edges and irregular morphology (D): A new pad. Heavy metals are concentrated in brighter areas. (E) A new disc. Heavy metals are concentrated in brighter areas (F): A tire displaying bright particle material, primarily containing Fe and Zn. (G) A hypothetical tire wear particle on the bee wing, containing Zn, S, Si, O, Ca, and Fe (asterisk, hair; arrowhead, hair socket). (H) Tire wear particles. Brighter particles contain heavy metals, mainly Zn and Fe. Fe-rich fragments may originate from brake discs (i.e., the bright particle on the gray substrate possibly made of tire rubbers).

Table 1
Percentages of minerals obtained using X-ray powder diffraction (XRPD) on topsoil/exposed sediment samples collected approximately 5 m (a) and 40 m (b) from the hives (soil with and without grass, respectively) and 60 m (c) from the hives (pedestrian-cycle artificial path).

Topsoil samples	Quartz	Plagioclase	K-feldspar	Mica	Calcite	Dolomite	Clinochlore
a	48%	16%	4%	20%	–	4%	4%
b	52%	22%	<1%	24%	–	–	2%
c	6%	–	–	1%	2%	91%	<1%

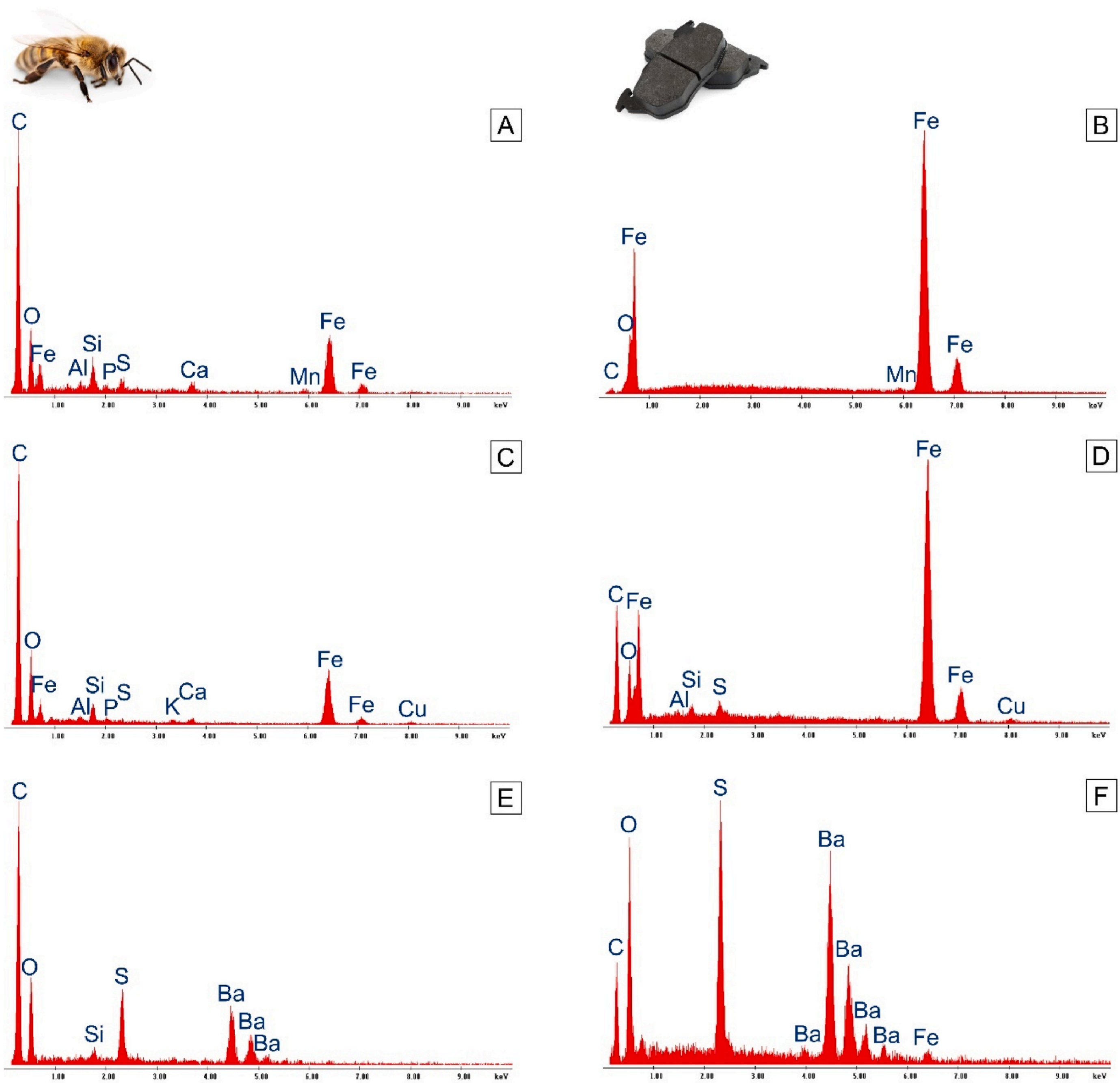

Fig. 3. EDX spectra of honey bee wings and brake pads. (A–F): EDX spectra acquired for particulate matter from bees (left) and brake pads (right), displaying distinctive combinations of Fe + Mn (A, B), Fe + Cu (C, D), and barite (E, F).

of tire wear particles is highly variable, ranging from roundish to kidney-shaped or elongated (Panko et al., 2018; Sommer et al., 2018; Rausch et al., 2022).

The relative abundance of primary PM counts in the size range 10–2.5 μm attributable to vehicular braking systems and tires accounted for approximately 15% of the total analyzed particles on honey bee wings, whereas PM in the size range 2.5–1 μm and <1 μm accounted for 50% and 78%, respectively. These results suggested that honey bees, and therefore humans, are primarily exposed to non-exhaust friction-derived sub-micrometer particles associated with road transport. Such

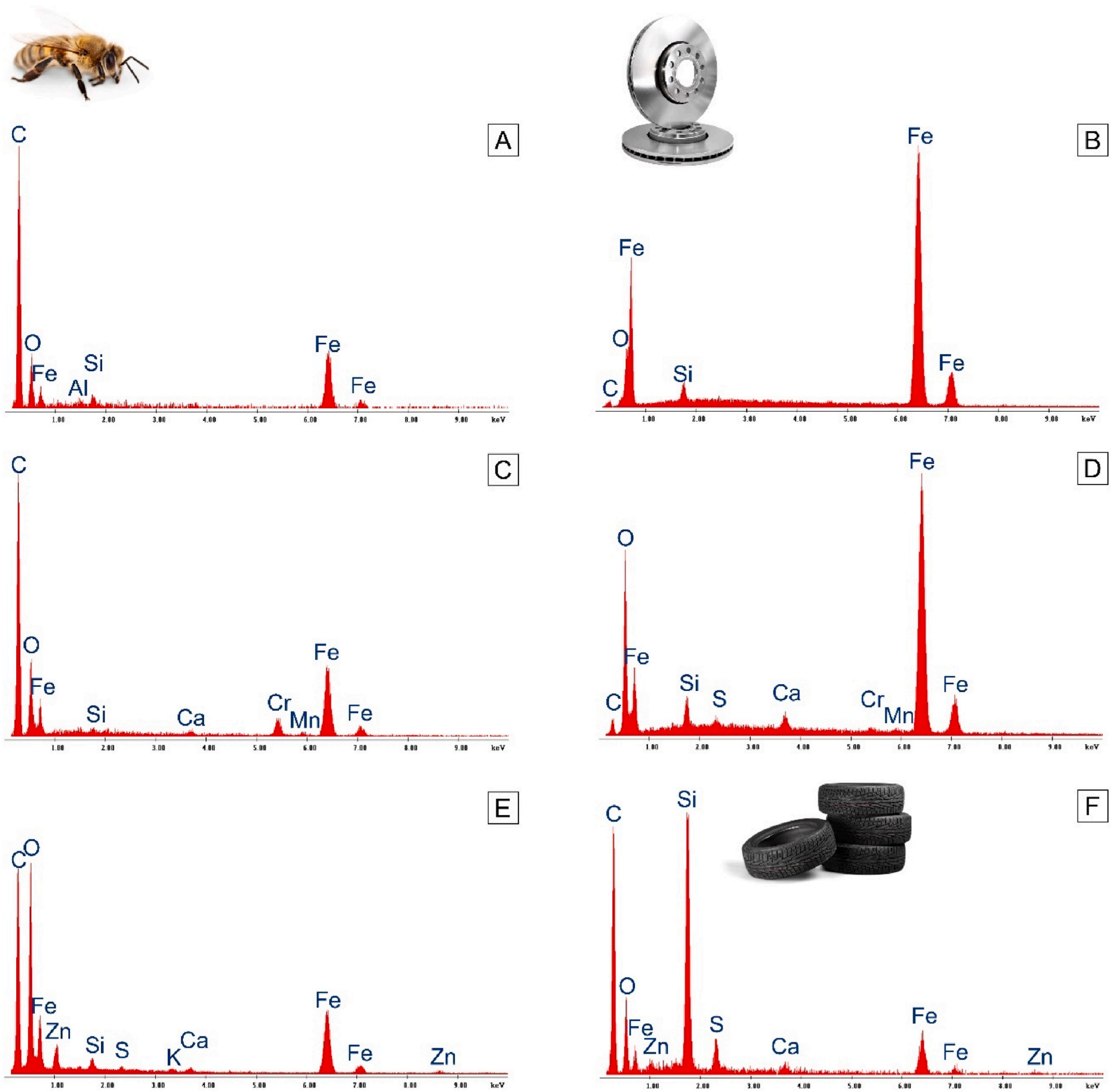

Fig. 4. EDX spectra of honey bee wings, discs, and tires. (A–D) EDX spectra acquired for particulate matter (PM) from bees (left) and on discs (right), displaying the presence of iron oxides/hydroxides, with minor amounts of Si (A, B), and combinations of Fe + Cr + Mn (C, D); (E, F) EDX spectra for PM acquired from bees (left) and on a used tire tread (right), displaying Fe + Zn + Si + Al + S.

exposure may adversely affect public health through inhalation or swallowing, enhanced inflammation, DNA damage via oxidative stress, and neurological disorders (Dashtipour et al., 2015; Soltani et al., 2018; Jan et al., 2020; Maher et al., 2016; Jan et al., 2020). Our assessment does not take into account the contribution of secondary PM and organic compounds which are often not stable under SEM.

Our findings also have key implications for the future of mobility and the design of ecological vehicles. The current trend for sustainable development and ecological transitions in Europe is pushing governments and policymakers toward the adoption of hybrid and electric motor vehicles instead of traditional internal combustion engine vehicles. Electric vehicles emit 11–19% less PM_{10} than gasoline-fueled vehicles (OECD, 2020). However, depending on the additional weight due to the battery pack, the emission of $PM_{2.5}$ may be lowered by 11.2–13.3% in light vehicles but 2.6–7.8% higher in heavier vehicles, and this increase is mostly due to tire wear (OECD, 2020). To date, no data on submicrometric PM emissions from electric vehicles are available (OECD, 2020). Policymakers are therefore called upon to take urgent action for addressing non-exhaust particulate emissions, which are currently not included as potential pollutants in the existing environmental legislation.

XRF bulk analyses of the soils surrounding the hives revealed traces of the same heavy metals detected on bees as in brake systems and tires, including Cr, Cu, Zn, and Ba (Table 2). Certainly, these elements may be

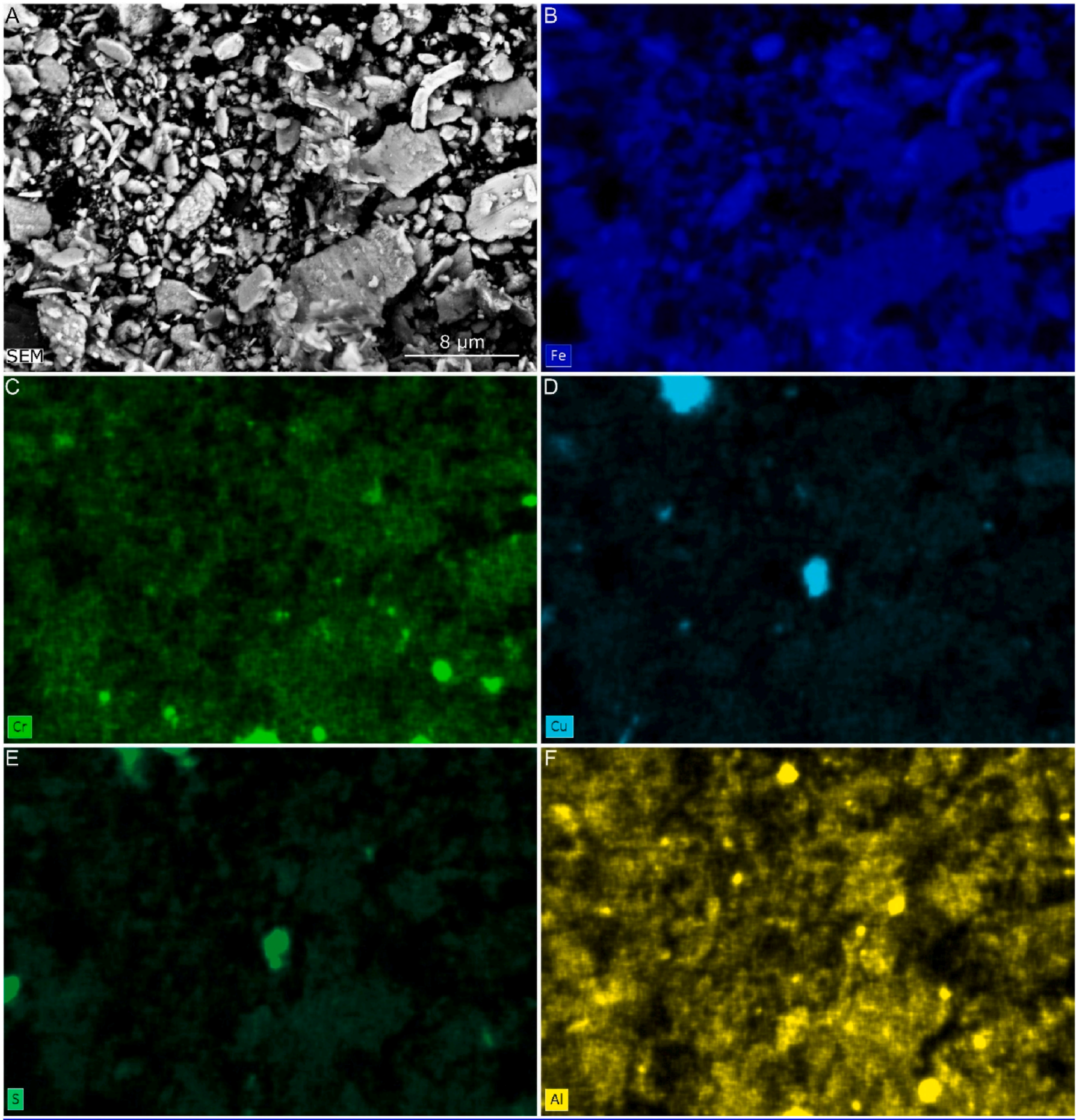

Fig. 5. Elemental mapping of wear debris from brake pads. (A) SEM-BSE image showing particles of different sizes from submicrometric to particles less than 10 µm, with sharp edges and irregular morphology.

naturally present in soils and we recorded concentrations below the legal limit set by Italian regulations (but for Zn and Pb; Decree-Law 3/4/2006, n. 152 "Norme in materia ambientale", Gazzetta Ufficiale n. 88, 14/4/2006 - Supplement n. 96). However, we found concentrations higher than the average concentrations of European and World soils (Alloway, 2013), and higher than those typical of the alluvial deposits of the Po Valley in Italy (Canedoli et al., 2020). Moreover, these elements were not detected in the nearby pedestrian-cycle paths recently constructed, possibly owing to the reduced accumulation time and levels below the detection limit. Overall, our results suggest that heavy metals may be markers of vehicular pollution in urban topsoil.

4. Conclusions

By using honey bees to sample PM and SEM/EDX for single particle analysis, we quantified and established the contribution of non-exhaust emissions from vehicular traffic to urban PM in daily-life situations. Since airborne PM attached to the body of honey bees is typically <10 µm in size, the sub-micrometric and ultrafine fraction of PM can be easily detected and characterized. Moreover, as bees mostly fly at human height, PM collected on their bodies is the same reaching pedestrians. We here show that honeybees are contaminated by PM 10–2.5 µm in size, mostly originating from soil erosion and rock outcropping in the foraging area, and by metallic particles, mostly ≤ 1 µm in size, likely deriving from vehicular braking systems and tires (non-exhaust PM). Policymakers are therefore called upon for urgent action for managing also non-exhaust particulate emissions, which are not included in current environmental legislation as potential pollutants. However, while our approach allows for the determination of particle size, morphology, and chemistry, its quantification efficiency must rely on the development of an automated image processing system. Moreover, the SEM/EDX technique, while sensitive to elements with high atomic number, cannot easily distinguish hydrocarbons from the background (the honey bee body), as they show similar contrast and response to X-ray analysis. Overall, the present study demonstrated that PM monitoring utilizing biological systems, and particularly honey bees,

Table 2
Heavy metal concentrations (mg kg^{-1}) in the studied samples compared to Milan urban soils, Europe soils (median and maximum concentrations), World soils (average), and legal limits according to current regulations in Italy.

Elem.	Segantini[a]			Milan urban soils[b]	Europe soils[c]		World soils[c]	Legal limits[d]
	A	B	C		med	max	average	
Cr	116	130	0	60	22	234	42	150
Mn	1241	465	155	–	382	6480	418	–
Ni	–	–	–	42	14	2560	18	120
Cu	88	112	0	55	12	239	14	120
Zn	217	322	72	104	48	2270	62	150
Ba	233	305	63	–	65	1700	362	–
Pb	130	232	0	124	15	886	25	100

[a] This study.
[b] Canedoli et al. (2020).
[c] Alloway (2013).
[d] Decree Law 3/4/2006, n. 152 "Norme in materia ambientale," Gazzetta Ufficiale n. 88, 14/4/2006 - Supplement n. 96.

may provide additional information on PM emission sources in daily-life scenarios and the actual population exposure.

Author contribution statement

Marco Pellecchia and Ilaria Negri designed research; Giulia Papa, Marco Pellecchia, and Ilaria Negri performed research; Giancarlo Capitani and Ilaria Negri contributed analytic tools; Giulia Papa, Giancarlo Capitani, Marco Pellecchia, Mario Barbato, and Ilaria Negri analyzed data; Giancarlo Capitani, Marco Pellecchia, Mario Barbato, and Ilaria Negri wrote the manuscript. All authors reviewed the manuscript.

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.

Data availability

Data will be made available on request.

Acknowledgments

We thank Dr. Paolo Gentile for his expertise and support in SEM/EDX analysis and Christian Mavris for his comments that greatly improved our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121885.

References

Alloway, B.J., 2013. Sources of heavy metals and metalloids in soils. In: Alloway, B. (Ed.), Heavy Metals in Soils, Environmental Pollution, vol. 22. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-4470-7_2.

Adamiec, E., Jarosz-Krzemińska, E., Wieszała, R., 2016. Heavy metals from non-exhaust vehicle emissions in urban and motorway road dusts. Environ. Monit. Assess. 188, 369. https://doi.org/10.1007/s10661-016-5377-1.

Baensch-Baltruschat, B., Kocher, B., Stock, F., Reifferscheid, G., 2020. Tyre and road wear particles (TRWP) - a review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. Sci. Total Environ. 733, 137823 https://doi.org/10.1016/j.scitotenv.2020.137823.

Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. 22, 35–67. https://doi.org/10.1007/s11356-014-3332-7.

Brown, J.S., Gordon, T., Price, O., Asgharian, B., 2013. Thoracic and respirable particle definitions for human health risk assessment. Part. Fibre Toxicol. 10, 12. https://doi.org/10.1186/1743-8977-10-12.

Canedoli, C., Ferrè, C., El Khair, D.A., Padoa-Schioppa, E., Comolli, R., 2020. Soil organic carbon stock in different urban land uses: high stock evidence in urban parks. Urban Ecosyst. 23, 159–171. https://doi.org/10.1007/s11252-019-00901-6.

Capitani, G., Papa, G., Pellecchia, M., Negri, I., 2021. Disentangling multiple PM emission sources in the Po Valley (Italy) using honey bees. Heliyon 7, e06194. https://doi.org/10.1016/j.heliyon.2021.e06194.

Carrero, J.A., Arana, G., Madariaga, J.M., 2014. Chapter 6. Use of Raman spectroscopy and scanning electron microscopy for the detection and analysis of road transport pollution. In: Douthwaite, R., Duckett, S., Yarwood, J. (Eds.), Spectroscopic Properties of Inorganic and Organometallic Compounds. Royal Society of Chemistry, Cambridge, pp. 178–210. https://doi.org/10.1039/9781782621485-00178.

Dashtipour, K., Liu, M., Kani, C., Dalaie, P., Obenaus, A., Simmons, D., Gatto, N.M., Zarifi, M., 2015. Iron accumulation is not homogenous among patients with Parkinson's disease. Parkinson's Dis. 2015, 1–8. https://doi.org/10.1155/2015/324843.

Devillers, J., Pham-Delegue, M.-H. (Eds.), 2002. Honey Bees: Estimating the Environmental Impact of Chemicals. CRC Press. https://doi.org/10.1201/9780203218655, 0 ed.

Edo, C., Fernández-Alba, A.R., Vejsnæs, F., Van Der Steen, J.J.M., Fernández-Piñas, F., Rosal, R., 2021. Honeybees as active samplers for microplastics. Sci. Total Environ. 767, 144481. https://doi.org/10.1016/j.scitotenv.2020.144481.

Grigoratos, T., Martini, G., 2015. Brake wear particle emissions: a review. Environ. Sci. Pollut. Control Ser. 22, 2491–2504. https://doi.org/10.1007/s11356-014-3696-8.

Ingo, G.M., D'Uffizi, M., Falso, G., Bultrini, G., Padeletti, G., 2004. Thermal and microchemical investigation of automotive brake pad wear residues. Thermochim. Acta 418, 61–68. https://doi.org/10.1016/j.tca.2003.11.042.

Jan, R., Roy, R., Bhor, R., Pai, K., Satsangi, P.G., 2020. Toxicological screening of airborne particulate matter in atmosphere of Pune: reactive oxygen species and cellular toxicity. Environ. Pollut. 261, 113724 https://doi.org/10.1016/j.envpol.2019.113724.

Järlskog, I., Jaramillo-Vogel, D., Rausch, J., Perseguers, S., Gustafsson, M., Strömvall, A. M., Andersson-Sköld, Y., 2022. Differentiating and quantifying carbonaceous (tire, Bitumen, and road marking wear) and non-carbonaceous (metals, minerals, and glass beads) non-exhaust particles in road dust samples from a traffic environment. Water Air Soil Pollut. 233 https://doi.org/10.1007/s11270-022-05847-8.

Kukutschová, J., Moravec, P., Tomášek, V., Matějka, V., Smolík, J., Schwarz, J., Seidlerová, J., Šafářová, K., Filip, P., 2011. On airborne nano/micro-sized wear particles released from low-metallic automotive brakes. Environ. Pollut. 159, 998–1006. https://doi.org/10.1016/j.envpol.2010.11.036.

Maher, B.A., Ahmed, I.A.M., Karloukovski, V., MacLaren, D.A., Foulds, P.G., Allsop, D., Mann, D.M.A., Torres-Jardón, R., Calderon-Garciduenas, L., 2016. Magnetite pollution nanoparticles in the human brain. Proc. Natl. Acad. Sci. U.S.A. 113, 10797–10801. https://doi.org/10.1073/pnas.1605941113.

Matějka, V., Perricone, G., Vlček, J., Olofsson, U., Wahlström, J., 2020. Airborne wear particle emissions produced during the dyno bench tests with a slag containing semi-metallic brake pads. Atmosphere 11, 1220. https://doi.org/10.3390/atmos11111220.

Negri, I., Mavris, C., Di Prisco, G., Caprio, E., Pellecchia, M., 2015. Honey bees (*Apis mellifera*, L.) as active samplers of airborne particulate matter. PLoS One 10, e0132491. https://doi.org/10.1371/journal.pone.0132491.

OECD, 2020. Non-exhaust Particulate Emissions from Road Transport: an Ignored Environmental Policy Challenge. OECD. https://doi.org/10.1787/4a4dc6ca-en.

Österle, W., Griepentrog, M., Gross, Th, Urban, I., 2001. Chemical and microstructural changes induced by friction and wear of brakes. Wear 251, 1469–1476. https://doi.org/10.1016/S0043-1648(01)00785-2.

Panko, J., Kreider, M., Unice, K., 2018. Review of tire wear emissions. Non-Exhaust Emiss. 147–160. https://doi.org/10.1016/b978-0-12-811770-5.00007-8.

Pant, P., Harrison, R.M., 2013. Estimation of the contribution of road traffic emissions to particulate matter concentrations from field measurements: a review. Atmos. Environ. 77, 78–97. https://doi.org/10.1016/j.atmosenv.2013.04.028.

Papa, G., Capitani, G., Capri, E., Pellecchia, M., Negri, I., 2021b. Vehicle-derived ultrafine particulate contaminating bees and bee products. Sci. Total Environ. 750, 141700 https://doi.org/10.1016/j.scitotenv.2020.141700.

Papa, G., Capitani, G., Pellecchia, M., Negri, I., 2021b. Particulate Matter Contamination of Bee Pollen in an Industrial Area of the Po Valley (Italy). Applied Sciences 11, 11390. https://doi.org/10.3390/app112311390.

Papa, G., Di Prisco, G., Spini, G., Puglisi, E., Negri, I., 2021a. Acute and chronic effects of Titanium dioxide (TiO2) PM1 on honey bee gut microbiota under laboratory conditions. Sci Rep 11, 5946. https://doi.org/10.1038/s41598-021-85153-1.

Papa, G., Maier, R., Durazzo, A., Lucarini, M., Karabagias, I.K., Plutino, M., Bianchetto, E., Aromolo, R., Pignatti, G., Ambrogio, A., Pellecchia, M., Negri, I., 2022. The honey bee *Apis mellifera*: an insect at the interface between human and ecosystem health. Biology 11, 233. https://doi.org/10.3390/biology11020233.

Papa, G., Power, K., Forestieri, B., Capitani, G., Maiolino, P., Negri, I., 2023. Effects of oral exposure to brake wear particulate matter on the springtail *Orthonychiurus folsomi*. Environ. Pollut. 329, 121659. https://doi.org/10.1016/j.envpol.2023.121659.

Pellecchia, M., Negri, I., 2018. Particulate matter collection by honey bees (*Apis mellifera*, L.) near to a cement factory in Italy. PeerJ 6, e5322. https://doi.org/10.7717/peerj.5322.

Piscitello, A., Bianco, C., Casasso, A., Sethi, R., 2021. Non-exhaust traffic emissions: sources, characterization, and mitigation measures. Sci. Total Environ. 766, 144440 https://doi.org/10.1016/j.scitotenv.2020.144440.

Plutino, M., Bianchetto, E., Durazzo, A., Lucarini, M., Lucini, L., Negri, I., 2022. Rethinking the connections between ecosystem services, pollinators, pollution, and health: focus on air pollution and its impacts. IJERPH 19, 2997. https://doi.org/10.3390/ijerph19052997.

Rausch, J., Jaramillo-Vogel, D., Perseguers, S., Schnidrig, N., Grobéty, B., Yajan, P., 2022. Automated identification and quantification of tire wear particles (TWP) in airborne dust: SEM/EDX single particle analysis coupled to a machine learning classifier. Sci. Total Environ. 803, 149832 https://doi.org/10.1016/j.scitotenv.2021.149832.

Rietveld, H.M., 1969. A profile refinement method for nuclear and magnetic structures. J. Appl. Crystallogr. 2, 65–71. https://doi.org/10.1107/S0021889869006558.

Satta, A., Verdinelli, M., Ruiu, L., Buffa, F., Salis, S., Sassu, A., Floris, I., 2012. Combination of beehive matrices analysis and ant biodiversity to study heavy metal pollution impact in a post-mining area (Sardinia, Italy). Environ. Sci. Pollut. Res. 19, 3977–3988. https://doi.org/10.1007/s11356-012-0921-1.

Soltani, N., Keshavarzi, B., Sorooshian, A., Moore, F., Dunster, C., Dominguez, A.O., Kelly, F.J., Dhakal, P., Ahmadi, M.R., Asadi, S., 2018. Oxidative potential (OP) and mineralogy of iron ore particulate matter at the Gol-E-Gohar Mining and Industrial Facility (Iran). Environ. Geochem. Health 40, 1785–1802. https://doi.org/10.1007/s10653-017-9926-5.

Sommer, F., Dietze, V., Baum, A., Sauer, J., Gilge, S., Maschowski, C., Gieré, R., 2018. Tire abrasion as a major source of microplastics in the environment. Aerosol Air Qual. Res. 18, 2014. https://doi.org/10.4209/aaqr.2018.03.0099. –2028.

Vaknin, Y., Gan-Mor, S., Bechar, A., Ronen, B., Eisikowitch, D., 2000. The role of electrostatic forces in pollination. Plant Systemat. Evol. 222, 133–142. https://doi.org/10.1007/BF00984099.

Valavanidis, A., Fiotakis, K., Vlachogianni, T., 2008. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. Journal of Environmental Science and Health, Part C 26, 339–362. https://doi.org/10.1080/10590500802494538.

Venkatesh, S., Murugapoopathiraja, K., 2019. Scoping review of brake friction material for automotive. Mater. Today: Proc. 16, 927–933. https://doi.org/10.1016/j.matpr.2019.05.178.

Weinbruch, S., Worringen, A., Ebert, M., Scheuvens, D., Kandler, K., Pfeffer, U., Bruckmann, P., 2014. A quantitative estimation of the exhaust, abrasion and resuspension components of particulate traffic emissions using electron microscopy. Atmos. Environ. 99, 175–182. https://doi.org/10.1016/j.atmosenv.2014.09.075.

World Health Organization, 2020. World Health Statistics 2020: Monitoring Health for the SDGs, Sustainable Development Goals. Geneva.

Zarić, N., Ilijevic, K., Stanisavljevic, L., Grzetic, I., 2018. Use of honeybees (*Apis mellifera* L.) as bioindicators of spatial variations and origin determination of metal pollution in Serbia. J. Serb. Chem. Soc. 83, 773–784. https://doi.org/10.2298/JSC171110018Z.