

Article

Particulate Matter Contamination of Bee Pollen in an Industrial Area of the Po Valley (Italy)

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Abstract: The global demand for bee pollen as a dietary supplement for human nutrition is increasing. Pollen, which comprises proteins and lipids from bees' diets, is rich in essential amino acids, omega fatty acids, and bioactive compounds that can have beneficial effects on human health. However, bee pollen may also contain contaminants due to environmental contamination. To date, data on bee pollen contamination by environmental pollutants refer almost exclusively to pesticides and heavy metals, and very little information is available on the potential contamination of bee pollen by airborne particulate matter (PM), a ubiquitous pollutant that originates from a wide range of anthropogenic sources (e.g., motor vehicles, industrial processes, agricultural operations). In the present study, pollen grains collected by forager bees living in an industrial area of the Po Valley (Northern Italy) were analyzed for contamination by inorganic PM. The morpho-chemical characterization of inorganic particles using SEM/EDX allowed us to identify different emission sources and demonstrate the potential risk of PM entering the food chain and exposing bees to its ingestion.

Keywords: bee pollen; particulate matter; pollen contamination; industrial pollution; vehicular traffic; SEM/EDX



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1. Introduction

Honeybees are important providers of ecosystem services by providing pollination to plants and delivering many products to humans (e.g., honey, pollen, propolis, and royal jelly). In recent years, the global demand for bee pollen as a dietary supplement in human nutrition has increased. Pollen, which represents the protein and lipid sources of a bee's diet, is rich in essential amino acids, omega fatty acids, and bioactive compounds [1–3]. Dietary antioxidant intake derived from bee pollen has been linked to the prevention and clinical treatment of several diseases, including liver and kidney fibrosis [4]. In addition, the incorporation of bee pollen into the diet may promote the inhibition of inflammation and oxidative stress [4]. However, bee pollen intake is also associated with health risks due to contamination by bacterial and fungal toxins, pesticides, and heavy metals. Previous data confirmed that bee pollen may also be contaminated by airborne particulate matter (PM) [5]. Airborne PM is a ubiquitous pollutant that may originate from either natural (e.g., soil erosion, volcanic eruptions, forest fires) or anthropogenic sources (e.g., motor vehicles, industries, agriculture). Particles are commonly classified according to their aerodynamic diameter, ranging from several micrometers (PM₁₀, i.e., PM with an aerodynamic diameter less than 10 μm) to a few micrometers (PM_{2.5}) or nanometers (PM_{0.1}). Airborne PM inhalation is associated with inflammation, cardiopulmonary diseases, and lung cancer [6], but exposure to airborne dust can also occur by swallowing inhaled particles or ingesting contaminated food, causing harmful effects on the digestive tract [7–10]. Recent studies

have demonstrated that, in heavily trafficked areas, bee pollen is contaminated by ultra-fine PM originating from vehicle braking systems, thus endangering the safety of food produced at traffic-influenced sites and the health of bees [5]. Pollen contamination by PM may also threaten the overall health of bees. Previous studies on the oral exposure of bees to airborne particles indicate cytological and histological modifications of the gut epithelium or alterations in the gut microbial community [11,12].

The study area of the present work is in the peri-urban area of the city of Parma (Po Valley, Northern Italy). The Po Valley is one of the most important industrial and agricultural areas in Europe, characterized by high levels of PM₁₀ and PM_{2.5} that mainly originate from traffic and agriculture [13–15]. A previous source apportionment study carried out in the area using honeybees has been used as an alternative sampling system for airborne PM. Agricultural operations and high-temperature combustion processes, probably linked to the Parma incineration plant, have been identified as a major sources of PM [16]. The honeybee (*Apis mellifera* L.), which is an important control in the biomonitoring of environmental pollutants [17–20], can act also as an efficient sampler of airborne dust [5,16,21–23]. The generation of static electricity on a bee's body during flight attracts airborne particles that can be analyzed by scanning electron microscopy (SEM) coupled with X-ray spectroscopy (EDX) to evaluate size, morphology, and chemical composition [21,22]. Such information can facilitate the identification of PM emission source(s) and potential health hazards [5,16,21,22].

In the present work, airborne PM contamination of pollen grains collected by honeybees in the study area was investigated by SEM/EDX, with the aim of characterizing the nature, morphology, and mineralogical composition of particles and evaluating their possible origin. Such analysis also allowed us to demonstrate the potential risk of PM entering the food chain and exposing bees to its ingestion.

2. Materials and Methods

2.1. Sample Collection and Preparation

In February 2017, a hive was placed in the peri-urban area of the city of Parma (44.834357, 10.353865), close to wheat and alfalfa crops and about 100 m from the A1 Milan–Naples highway. The highway exit and the Parma incinerator plant were located approximately 400 m from the hive (Figure 1).

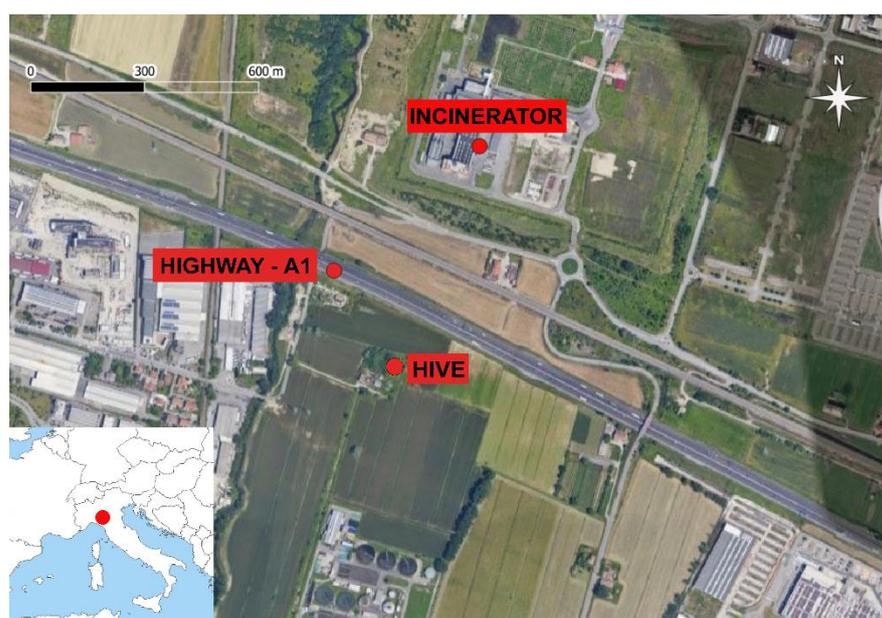


Figure 1. Area of investigation. Red dots represent the location of the hive with respect to the A1 highway and the incinerator plant [24].

To avoid possible contamination of pollen caused by using pollen traps, analyses were carried out directly on pollen grains attached to the hindlegs of worker bees (Figure 2).

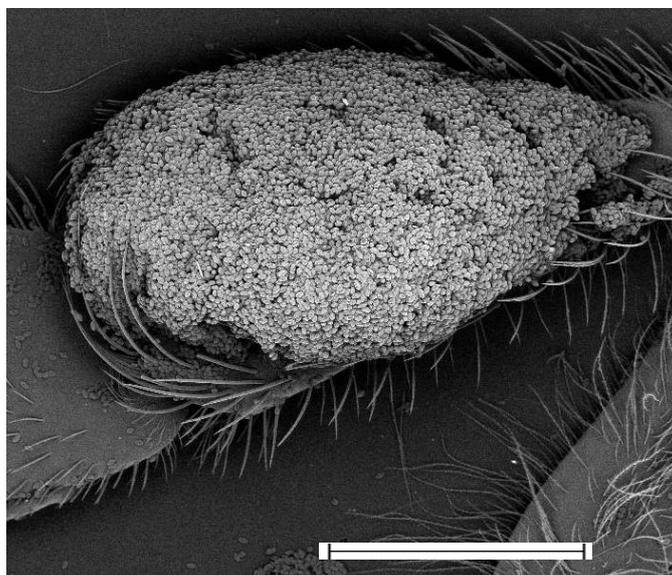


Figure 2. Example of a hindleg of a worker bee with packed pollen ready for the scanning electron microscope/energy dispersive X-ray (SEM/EDX) analysis. Bar = 1 mm.

Four sampling sessions were carried out monthly from May to August. During each session, ten forager bees carrying pollen grains were sampled while returning to their hives. Bees were immediately placed in soda-glass-capped vials (Chromacol Limited, Welwyn Garden City, UK) stored on ice to inactivate them and quickly transferred to the laboratory for sample preparation. Hind legs with pollen grains (total number of pollen grains analyzed in this study = 40) were dissected under a laminar flow hood to avoid external contamination. Two pairs of legs were mounted onto SEM stubs using double-adhesive carbon tape for SEM/EDX analysis. Stubs were carbon coated, as previously described [16].

A few days after each sampling session, negative control bees carrying pollen grains were collected close to hives in a rural area located 10 km south of Parma (Northern Italy) near the bed of Parma creek and close to the foothills of the Apennine Mountains (44.690099, 10.336713). The control site was far from any known emitting source of PM (i.e., vehicular traffic, incinerators, agricultural lands).

2.2. Scanning Electron Microscopy with Energy Dispersive X-ray Spectrometry Investigation

The SEM/EDX investigation was carried out using both a Tescan VEGA TS5136XM (Brno-Kohoutovice, Czech Republic) and a Zeiss Gemini 500 field-emission instrument (Oberkochen, Germany), both equipped with an energy dispersive X-rays (EDX) analyzer [16]. Secondary electrons (SE) and backscattered electron (BSE) images, as well as EDX point analyses, were acquired in alternating sequence under the same conditions of 20 kV with a nominal beam current of approximately 1 nA. For each pollen grain, a panoramic BSE image was acquired. Airborne PM that could be distinguished from the pollen grain surface according to brightness and contrast was selected for the analysis [16]. Each analyzed PM was attributed to a phase according to the relative EDX spectrum, as well as the morphology of the particle itself, as previously described [21,22]. To account for contributions of pollen grains to the EDX spectra, point analyses were carried out directly on the surface of the pollen.

3. Results

During the monitoring period, forager bees were observed collecting pollen from wild and cultivated plants present in the surroundings (e.g., *Taraxacum officinale* or dandelion, *Prunus spinosa* or blackthorn, Brassicaceae, *Zea mays* or corn, *Medicago sativa* or alfalfa) in the industrial area. All pollen grains collected from both the control and the industrial site displayed contamination by inorganic particles, with controls appearing to be scarcely contaminated (no more than three particles for grain pollen were detected), while the pollen from the industrial site was heavily contaminated. Airborne PM detected on pollen from the control site was composed exclusively of natural mineral phases (i.e., clay minerals and calcite, Figures S1 and S2). All pollen samples from the industrial site were contaminated with clay minerals, calcite, iron oxides/hydroxides, barite, and quartz (Table 1, Figure 3).

Table 1. Mineralogical phases and other PMs contaminating bee pollen collected in different months.

Analyzed PM	Natural Source	Anthropogenic Source	May	June	July	August
Clay minerals	+	agriculture	+	+	+	+
Calcite	+	incinerator, agriculture	+	+	+	+
Quartz	+	incinerator, agriculture	+	+	+	+
Iron oxides/Hydroxides		traffic	+	+	+	+
Barite		traffic	+	+	+	+
Feldspars	+	agriculture	+			+
Fe-alloy (Fe-Cr; Fe-Cr-Mn)		traffic	+		+	
Spherical dioxide		incinerator	+			
Heavy metals (Zn, Pb, Sb, Sn)		traffic, incinerator	+			
Gold		incinerator		+		

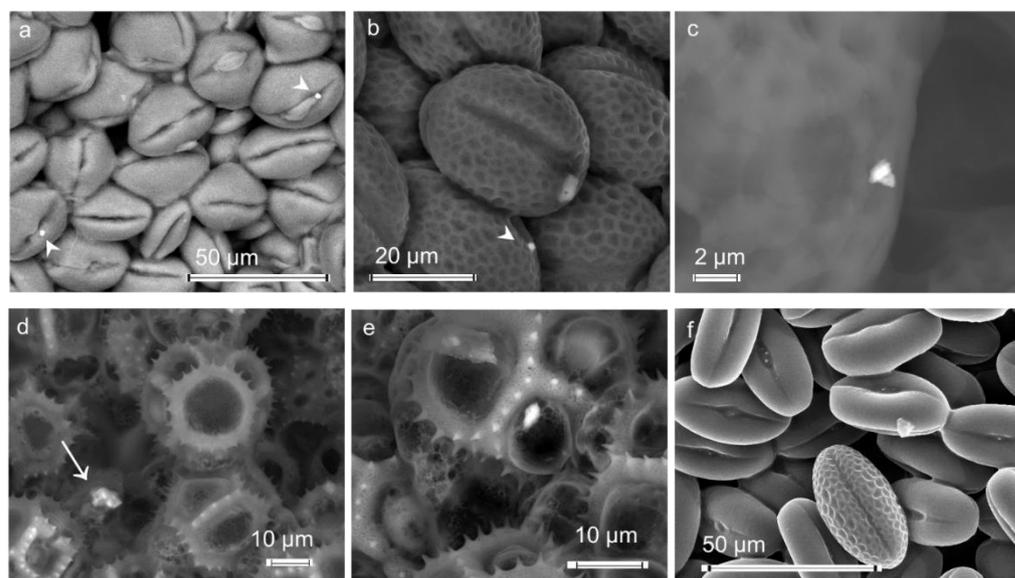


Figure 3. Airborne PM collected on all pollen grains. (a) Two particles composed of iron oxides/hydroxides (arrowheads; 1.5 and 1.4 µm) on alfalfa pollen. (b) A particle composed of iron oxides/hydroxides (arrowhead; 1.5 µm) on pollen of a Brassicaceae. (c) PM of barite (1.2 µm). (d) A particle of quartz (10 µm) on pollen grains of *Taraxacum officinale*. (e) PM of a clay mineral (6.9 µm) on a pollen grain of *T. officinale*. (f) Pollen grains of *Medicago sativa* and a Brassicaceae contaminated by PM of calcite (4.7 µm). Corresponding EDX spectra are provided in the Supplementary Materials.

Pollen grains contaminated by feldspar (Figure 4a), and Fe alloys (Fe-Cr and Fe-Cr-Mn) appeared less frequently (Table 1, Figure 4b–d).

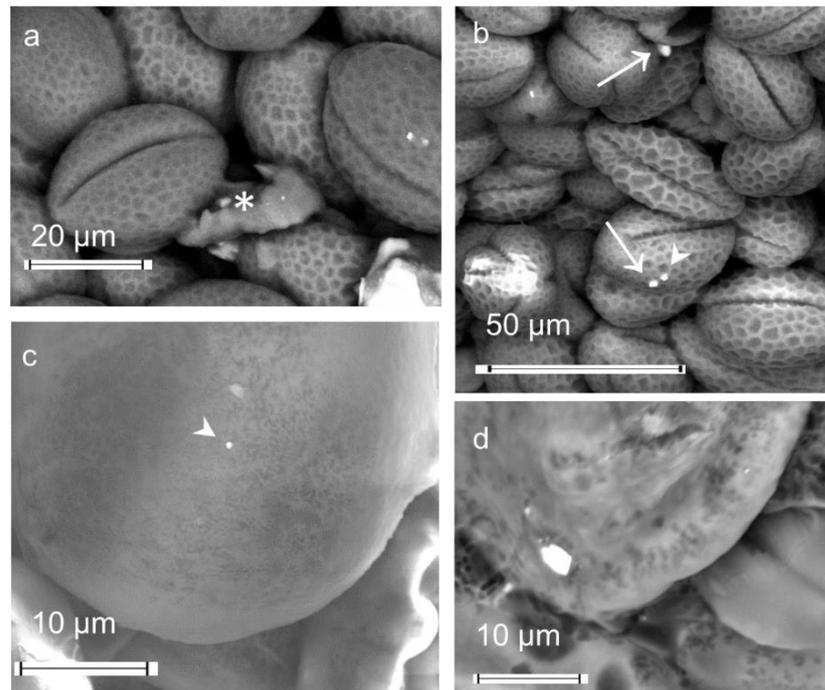


Figure 4. (a) Particulate matter (PM) of feldspar (asterisk). (b) Particles of Fe-Cr-Mn (arrows, 1.3 μm and 2.7 μm) and Fe oxides/hydroxides (arrowhead, 1 μm). (c) a submicrometric PM of Fe-Cr (arrowhead, 200 nm). (d) a particle of Fe-Cr-Mn (6.3 μm). Corresponding EDX spectra are provided as Supplementary Materials.

PM containing Zn, Pb, and Sb (Figure 5a–c) and spherical PM composed of silicon dioxide (Figure 6d,e) were only occasionally present on bee pollen.

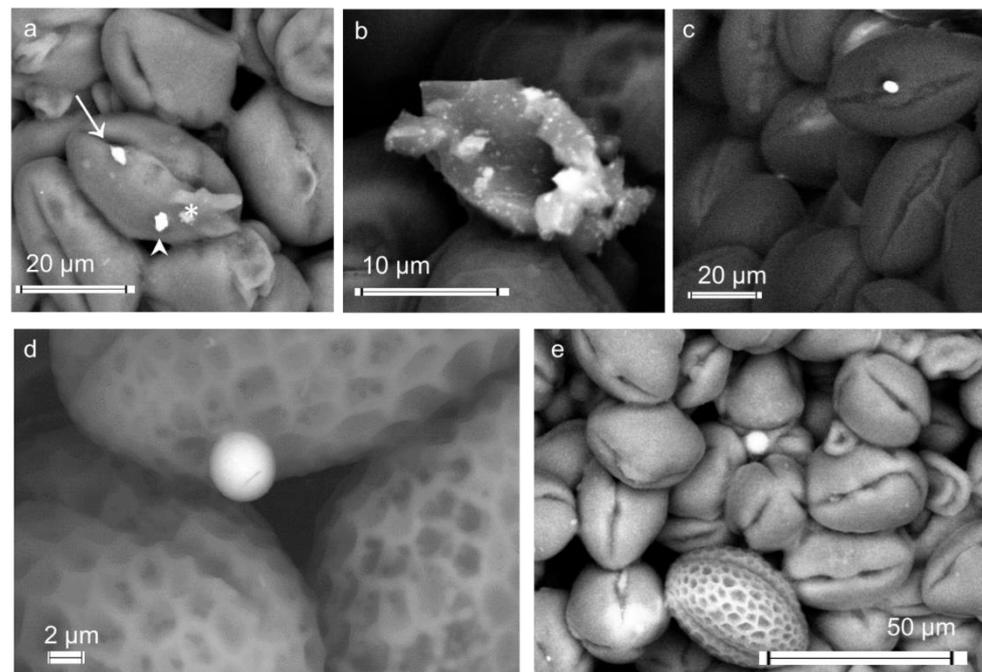


Figure 5. (a) Pollen grains of *Medicago sativa* contaminated with PM of quartz (arrow, 4.2 μm), clay mineral (asterisk, 2.9 μm), and metallic Zn (arrowhead, 3.8 μm). (b) PM containing lead (brighter areas). (c) PM of antimony oxide (2.7 μm). (d,e) Round-shaped SiO_2 particles (4.2 μm and 4.3 μm).

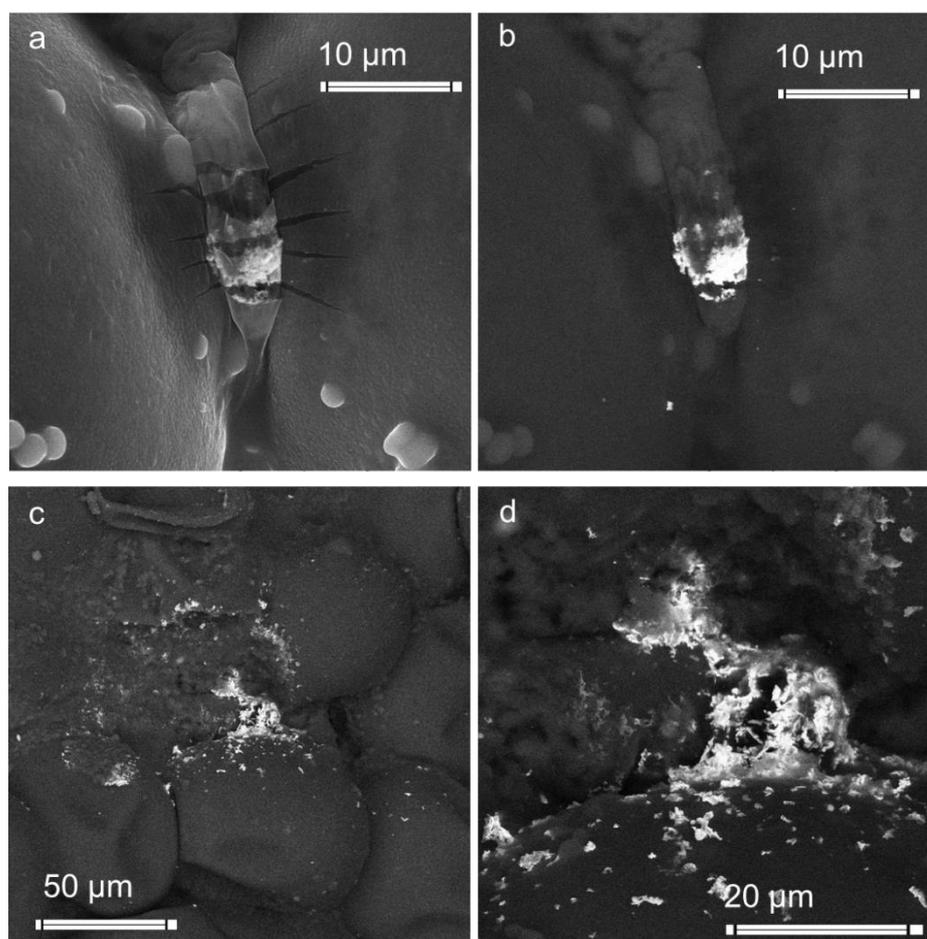


Figure 6. Fine and ultrafine gold PM found on the pollen surfaces. (a) Secondary electron micrograph of corn pollen contaminated by gold particles. (b) Corresponding backscattered electron (BSE) micrograph of corn pollen, highlighting bright spots that correspond to gold particles. (c) BSE micrograph showing PM of gold scattered on several pollen grains. (d) Higher magnification of gold PM on pollen grains showing the presence of fine and ultrafine PM.

Interestingly, some grains collected in May appeared to be contaminated with gold particles (Figure 6).

4. Discussion

Airborne PM of natural and anthropogenic origin was observed on pollen grains collected by worker bees from the monitoring station. Naturally derived minerals included calcite, clay minerals, quartz, and feldspars, which are abundant in the neighboring soils [16]. Natural erosion and agricultural activities, such as sowing and harvesting that may have occurred in the adjacent crop fields, may have contributed to the environmental spread of soil minerals. Regarding calcite, this mineral is also widespread in incinerator bottom ash and represents one of the most common products of ash weathering [25]. Even if our data suggest that handling ash may represent a critical step for dust leakage, further work is needed to assess specific contributions of the waste-treatment process. The incinerator plant may also be the source of the spherical dusts of SiO₂ contaminating bee pollen [16]. The spherical morphology is, indeed, a well-known marker of high-temperature combustion, such as that occurring at the incinerator plant [25,26].

Furthermore, the unexpected presence of gold particles on bee pollen could also be attributed to the incineration plant, as bottom ash may contain economically significant levels of this mineral [27,28]. According to the literature, the presence of precious metals

is mainly derived from waste of electrical and electronic equipment and many efforts are currently being made to recover precious metals in bottom ash [27,28]. Airborne PM of quartz contaminating bee pollen may also have originated from the incineration plant, as SiO₂ fragments are often abundant in bottom ash [25,29].

Other mineral phases frequently found in bee pollen included Fe oxides/hydroxides, metallic Fe, and Fe alloys (e.g., Fe, Cr, ±Mn, ±Sn). In natural soils, Fe oxides/hydroxides are common in mafic and ultramafic rocks, in lateritic horizons, and following alteration of sulfide ores, but they are rare in the alluvial deposits that characterize the area of investigation [16]. Thus, the natural origin of such dusts should be excluded. In addition, an anthropogenic origin should be considered for metallic Fe and Fe alloys. These compounds are present in several manufactured goods, especially in the automotive industry [30,31]. According to previous studies, Fe compounds and their alloys may therefore originate from the wearing of mechanical parts or the braking systems of vehicles [5,16,22,31].

Antimony oxides contaminating pollen grains may also be derived from the braking system. This compound is known to originate from the oxidation of antimony sulfide used to improve friction stability at elevated temperatures [32]. One possible origin of metallic Zn is vehicular traffic, as pure Zn is widely used in the automotive industry to protect steel from corrosion (International Zinc Association, www.zinc.org, 19 January 2021).

Another mineral phase frequently found in pollen grains was barite (BaSO₄). Barite occurs naturally in hydrothermal deposits; therefore, this mineral should not be present in the area of investigation. However, barite (BaSO₄) is widely used in vehicle tires and brakes as it improves the thermal and wear resistance and reduces noise [33–35]. An anthropogenic origin for TiO₂ particles found on bee pollen was suggested: titanium dioxide occurs in nature as the mineral rutile, usually concentrated in the heavy fraction of sediments; however, synthetic titanium dioxide (TiO₂) is widely used as a filler and a whitening agent in plastics, paints, and vehicle components.

It is widely acknowledged that waste incineration may be an important source of heavy metals, including Pb [36]. However, Pb is environmentally persistent and its presence in airborne dust may be the consequence of its common use in the past as a gasoline additive [36].

5. Conclusions

Our data provide evidence of the contamination of bee pollen by specific airborne PM of natural or anthropogenic origin. The morpho-chemical characterization of the dusts allowed us to clearly distinguish among different anthropogenic sources, namely vehicular traffic and incineration plants. Among the markers of traffic are particles originating from the wearing of mechanical parts and brakes (Fe-based compounds, metallic Zn, barite, and antimony oxide) of vehicles travelling on the A1 motorway. Emissions from the brake system may indeed be exceptionally high in areas close to motorway exits. However, it is also possible that the incineration plant may be responsible for the environmental presence of peculiar PM (gold and spherical SiO₂) contaminating bee pollen, as this PM is characteristic of bottom and fly ash. PM containing lead is persistent in the environment and may derive from different past and present anthropic activities; further studies involving Pb isotopic analyses may help constrain their relative contributions [37]. In addition, the presence of TiO₂ should be further investigated, given that this compound is used in a wide range of manufactured objects. The presence of calcite, clay minerals, quartz, and feldspars on the bee pollen may be due to both the erosion of soils and agricultural operations; however, further work is needed to assess the specific contributions of the waste-treatment process (e.g., handling of bottom ash) that may be responsible for the environmental spread of quartz and calcite.

In a previous work, similar contamination was found on the wings of worker bees living in the area [5,16], confirming that airborne PM pollutants are widespread and people living in the area may be chronically exposed to specific airborne PM. The present research also suggests a potential risk for PM entering the food chain and exposing honeybees

and other pollinators to its ingestion. Therefore, ecotoxicological studies are urgently needed to specifically evaluate the exposure levels and health effects on humans and ecosystem services.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/app112311390/s1>, Figure S1: Representative EDX spectra of particles of iron oxides/hydroxides, barite, quartz, and of a clay mineral; Figure S2: Representative EDX spectra of a feldspar, a Fe-Cr alloy, a Fe-Cr-Mn alloy, and metallic Zn; Figure S3: representative EDX spectra of a particle containing Pb, antimony oxide, and gold.

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