Science of the Total Environment 867 (2023) 161511

Contents lists available at ScienceDirect

Science of the Total Environment

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

Mobile samplers of particulate matter – Flying omnivorous insects in detection of industrial contamination

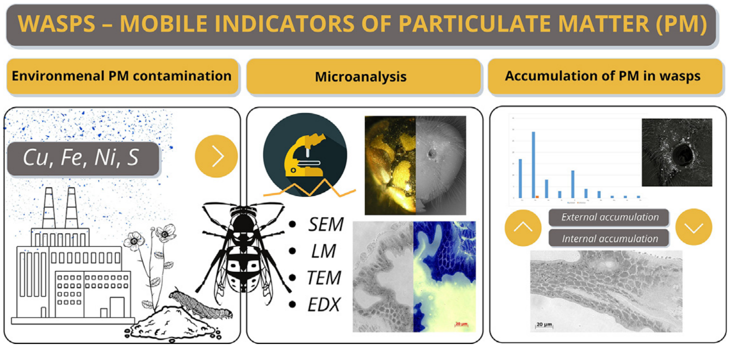
Oksana Skaldina [a,b,*], Adrian Łukowski [c], Jari T.T. Leskinen [d], Arto P. Koistinen [d], Tapio Eeva [a]

[a] Department of Biology, University of Turku, 20014, Turku, Finland
[b] Department of Environmental and Biological Sciences, University of Eastern Finland, Yliopistonranta 1E, 70211, Kuopio, Finland
[c] Faculty of Forestry and Wood Technology, Poznan University of Life Sciences, Wojska Polskiego 71E, 60-625 Poznań, Poland
[d] SIB Labs Unit, University of Eastern Finland, Yliopistonranta 1E, 70211 Kuopio, Finland

HIGHLIGHTS

- Omnivorous wasps for the first time assessed as mobile samplers of PM.
- SEM-EDX revealed elevated PM occurrence and size on the wasps in the smelter area.
- Surface accumulated PM reflected major local air pollutants – Fe, Ni, Cu, and S.
- Closely related wasp species trapped PM of different sizes.
- Number of metal-based granules in wasps' guts was higher in the industrial area.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Elena Paoletti

Keywords:
Atmospheric pollution
Bioindicators
Industrial emissions
electron microscopy
X-ray analyses
Vespula spp.

ABSTRACT

Flying insects are potential mobile samplers of airborne particulate matter (PM). However, current knowledge on their susceptibility to PM is limited to pollinators. Insects' capacity for particle surface accumulation depends on the lifestyle, structure of the body integuments, and behavioral patterns. Here, we investigate how two species of flying omnivorous insects from the genus *Vespula*, possessing direct interactions with air, soil, plants, and herbivores, indicate industrial pollution by accumulating coarse (PM_{10}) and fine ($PM_{2.5}$) particles on their bodies. The internal accumulation of particles in wasps' gut tissues is assessed considering heavy metals exposure to reveal and discuss the potential magnitude of ecotoxicological risks. Female individuals of *Vespula vulgaris* and *V. germanica* were sampled with a hand-netting near to Harjavalta *Cu-Ni* smelter and in the control areas in southwestern Finland. They were analyzed with light microscopy (LM), electron microscopy (SEM, TEM), and energy-dispersive X-ray spectroscopy (EDX) methods. Near to the smelter, wasps trapped significantly more particles, which were of bigger size and their surface optical density was higher. *Vespula vulgaris* accumulated larger particles than *V. germanica*, but that wasn't associated with morphological characteristics such as body size or hairiness. In both areas, accumulated surface PM carried clays and silicates. Only in polluted environments PM consistently contained metallic and nonmetallic particles (from high to moderate weight %) of *Fe*, *Ni*, *Cu*, and *S* – major pollutants emitted from the smelter. Wasps from industrially polluted areas carried significantly more granules in the columnar epithelial midgut cells. TEM-EDX analyses identified those structures were associated with metal ions such as *Cr*, *Cu*, *Ni*, and *Fe*. As epithelial gut cells accumulated metal particles, midgut confirmed as a barrier for metal exposure in wasps. External PM contamination in wasps is suggested as a qualitative, yet a natural and simple descriptor of local industrial emissions.

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* Corresponding author at: Department of Biology, University of Turku, 20014, Turku, Finland; Department of Environmental and Biological Sciences, Kuopio, Finland.
E-mail addresses: oksana.skaldina@uef.fi oksana.skaldina@utu.fi (O. Skaldina), lukowski@up.poznan.pl (A. Łukowski), jari.leskinen@uef.fi (J.T.T. Leskinen), arto.koistinen@uef.fi (A.P. Koistinen), teeva@utu.fi (T. Eeva).

http://dx.doi.org/10.1016/j.scitotenv.2023.161511
Received 7 October 2022; Received in revised form 5 January 2023; Accepted 6 January 2023

1. Introduction

Airborne particulate matter (PM) is a complex and heterogeneous mixture encompassing various chemical components and physical properties. It is classified by particle size as a coarse $PM_{10-2.5}$ (particle diameter is of 10 μm or less), fine $PM_{2.5}$ (particle diameter is of 2.5 μm or less), and ultrafine UFPs (particle diameter is of 0.1 μm or less) (Kelly and Fussell, 2012). Different types of PM induce variable and well-documented negative effects on humans (Dominici et al., 2014; Adams et al., 2015; Khaniabadi et al., 2017), and plants (Grantz et al., 2003; Rai, 2016; Łukowski et al., 2020). However, less is known for wildlife (Sanderfoot and Holloway, 2017; Grzyb and Pawlak, 2021; Li et al., 2021). PM toxicity strongly depends on the particle's morphology, chemical composition, dimension, their localization, and the individual susceptibility of the exposed organisms (Kelly and Fussell, 2012). Current methods of PM monitoring comprise ground-based measurements, satellite retrievals, and atmospheric chemistry models (Diao et al., 2019). The alternative approach to assess air quality is bioindication and biomonitoring (Käffer et al., 2012; Sæbø et al., 2012; Popek et al., 2017; Sorrentino et al., 2021). Using insects as bioindicators of PM is a relatively simple and inexpensive method (Papa et al., 2021). Development of new monitoring methods provides more versatile and comprehensive information on different parameters, strengthens environmental quality assessments, and increases environmental safety.

Flying insects such as honeybees *Apis mellifera* L. (Hymenoptera, Apoidea) are suggested as good mobile samplers of airborne PM (Negri et al., 2015) and microplastic particles (Edo et al., 2021). Pollinators possess specific morphological adaptation for pollen collection. During foraging, the pubescence on their body surfaces promotes the accumulation of electrical charge (Thorp, 1979). They often visit flowering meadows, accumulating large amounts of PM in landscapes with a limited number of trees (Przybysz et al., 2021). Pollen grains range in size from 10 μm to 200 μm and vary in shape, sculpturing, and aperture number (Pacini and Franchi, 2020). If body surfaces of these insects easily carry such large particles as pollen grains, they might be perfect sampling systems for smaller airborne particles. Thus, pollinators possess extensive potential for the efficient detection of atmospheric PM. Quantity and quality of PM can be assessed by using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) methods on their bodies (Negri et al., 2015; Capitani et al., 2021). Despite current knowledge on pollinators as active samplers of particulate matter, almost no information is available on the other flying insects interacting with more diverse environmental compartments.

Omnivorous insects are a key part of any ecosystem, representing 40 insect families and belonging to 12 orders (Coll and Guershon, 2002). They utilize both prey (proteins) and plant food (carbohydrates) to complete their life cycle (Krimmel, 2011). Many flying hymenopteran omnivores efficiently complement environmental monitoring programs (Hodkinson and Jackson, 2005). Vespid wasps (Hymenoptera, Vespidae) are good indicators of environmental heavy metal pollution (Urbini et al. 2006, Skaldina et al., 2020; Nadat et al., 2021). This is due to their ability to accumulate metals, survive in harsh environments, specific lifestyle (wasps are social insects and build carton nests underground, in rock caves, in the trees, or in humans' buildings), and diverse trophic interactions (wasps are omnivores using carbohydrates as adults and proteins as larvae). Wasps provide essential ecosystem services such as pest regulation, seed-dispersing, and decomposition of organic materials (Elizalde et al., 2020; Brock et al., 2021).

While evaluating Vespidae as indicators of airborne PM, possible contamination from soil particles should be considered. This is because wasps often build nests underground and forage for prey on the ground surface. From 55 to 68 % of *V. vulgaris* and *V. germanica* nests in New Zealand populations were soil-dwelling, followed by nests hanging on trees and made in anthropogenic constructions such as attics, storages, or barns (Donovan et al., 1992). Although wasps interact with soil, such interactions are often indirect or constitute only partial time of their daily activities. Even located underground, carton nests contain several layers of combs, covered with an envelope, which protects the whole colony from direct soil contamination. The central entrance of a nest usually matches with a ground hole, minimizing the potential contamination of individuals during their coming and going from the nest. In a prey examination survey, conducted for the German and common *Vespula* wasp species, the most common prey were flies (Diptera) and butterflies (Lepidoptera), while potentially soil-dwelling arthropods – spiders (Aranea) (*note*: not all spiders are soil-dwelling organisms) – were only in third place (Harris and Oliver, 1993). Thus, for *Vespula* spp. soil interactions might not exceed interaction with the aerial environment.

Does PM possess ecotoxicological risks to economically and ecologically important insects? If yes, what are the magnitudes of those? Still, the known answers are scarce and inconsistent. During the last decade, airborne metal-based PM matter was only occasionally studied as an ecotoxicological threat to pollinators and insect predators (Skaldina and Sorvari, 2019; Feldhaar and Otti, 2020). However, during the past several years, the topic has been gaining notice (Thimmegowda et al., 2020; Capitani et al., 2021; Phillips et al., 2021; Papa et al., 2021). Feldhaar and Otti (2020) suggested that in pollinators, chemical particles stuck to the body surfaces or penetrated inside through tracheae inducing sublethal effects such as increased susceptibility to diseases and parasites. Recent research by Thimmegowda et al. (2020) describes the profound physiological and genotoxic effects of PM on giant honeybee *Apis dorsata* Fabr. (Hymenoptera, Apoidea) in India. PM also affects herbivores of different insect guild, like folivores, gall-inducing or leaf-mining insects (Khan et al., 2013; Łukowski et al., 2018; Vanderstock et al., 2019). Commonly observed negative effects on insects are increased mortality, body mass loss, and decreased fertility (Khan et al., 2013). A higher amount of PM on the body surface is lethal for insects, or at least causes their starvation or desiccation. Ingested PM leads to clogs in the digestive system and a decrease in the use of resources necessary for growth and development (Flanders, 1941). Also, insects must increase their production of metabolites involved in the biochemical immune response, which additionally weakens them (van Ooik et al., 2007). Ecotoxicological risk of PM may be related to the insect body size, as Osborne and Longcore (2021) revealed that the smaller larvae of the snout moth *Gloveria medusa* Strecker (Lepidoptera, Lasiocampidae) had elevated death risks when exposed to fugitive gypsum dust.

Insects possess several behavioral and physiological mechanisms to cope with external and internal chemical exposure including heavy metals. These mechanisms are avoidance of polluted food or an exposure source, limited uptake of contaminants, self-cleaning, metal detoxification or pathogen immobilization with melanin pigments, elimination, and excretion (Heikens et al., 2001). If immobilized, metals are frequently stored in granules within the integuments, digestive tract, fat body, genital organs, Malpighian tubules, and gut, which does not prevent damage to these tissues (Ballan-Dufrançais, 2002). In wasps, an excess of stored metal-based granules might cause adverse effects on the health condition of gut tissues (Polidori et al., 2018). Thus, the assessment of the quantity and density of immobilized granules and revealing the ultrastructural alterations in gut tissues will help to estimate the magnitude of potential ecotoxicological risks.

Here, we studied the potential of omnivorous wasps from the genus *Vespula* Thomson (Hymenoptera, Vespidae) as suitable indicators of industrial PM pollution. The specific objectives of this study were (*i*) to estimate total industry derived PM accumulation on head surfaces in two widely distributed wasp species; (*ii*) to reveal any difference in PM total number, size, number per surface area (density), and chemical composition in two wasp species regarding their morphological traits (body size and hairiness); (*iii*) to estimate the rate of internal gut contamination with metal-based particles by calculation number per surface area (density) of internal particles. Our observations and previous studies indicate a high potential for using wasps for bioindication. We set the following hypotheses: (*i*) both wasp species accumulate significantly higher amount of PM on their head surfaces in polluted rather than in non-polluted habitats; (*ii*) difference in particle number, size, occurrence, and chemical composition of surface

PM in two wasp species is significant between habitat types and is associated with the morphological characteristics; (*iii*) the rate of internal gut contamination with metal-based granules is different between contaminated and control habitats.

2. Materials and methods

2.1. Study areas

Field data were collected in the August 2019 in South-Western and Central Finland. The Boliden Harjavalta *Cu—Ni* smelter area (61° 19′ N, 22° 9′E) was chosen as an exposed (contaminated) site. It is one of the most heavily polluted zones in Finland, as the deposition of soil heavy metals is increased up to 30 km from the smelter (Kiikkilä, 2003). However, especially in 1990's, the installation of new filters and the construction of a taller smokestack reduced environmental deposition of pollutants (Berglund et al., 2015). Thus, emissions decreased from 1100 tons in the year 1985 to 4,3 tons in the year 2020 (data collected from various sources, Boliden Harjavalta - Boliden, n.d.).

The area is a part of the southern boreal coniferous zone, dominated by Scots pine *Pinus sylvestris* L. According to the classification of Finnish forest types, the forest varies from relatively barren *Vaccinium* to *Calluna* types (Cajander, 1949). The major soil type is ferric podzol. Two control areas represented similar habitat type close to Nakkila (61° 22′ N, 22° 00′E) and Tampere (61° 29′ N, 23° 47′E).

2.2. Study species

The study species were German wasp *Vespula germanica* (F.) and common wasp *Vespula vulgaris* (L.) (Hymenoptera, Vespidae), both species are commonly known as yellowjackets. *Vespula vulgaris* is widespread in Eurasia and invasive in many countries of the Southern Hemisphere. *Vespula germanica*, which is native to central and southern Europe, recently expanded its range to the northern areas and established its populations in Southern Finland since 2001 (Sorvari, 2018). It is also invasive in many other parts of the world. Thus, both species are highly invasive and share similar life-history traits such as sociality, annual life cycle, nesting behavior, and feeding preferences. They are omnivorous insects, interacting with soil (build their nests underground), plants (collect nectar from flowers), and arthropods (forage for protein-based food) (Richter, 2000). All collected individuals were identified as females.

2.3. Insects' sampling, preprocessing, and morphometrics

Altogether 20 yellowjackets samples were collected from polluted ($n = 15$) and control ($n = 5$) areas at the end of August 2019, during three days in both treatments. All wasps, exposed to industrial pollution, were sampled near the smelter – at 1–50 m from the fence of the smelter area. Nine individuals were *V. germanica* (six exposed and three control) and 11 individuals were *V. vulgaris* (nine exposed and two control). Only common wasp samples (*V. vulgaris*) were used for further histological analyses of gut tissues. Insects were collected with a hand-net flying in the wild, at sufficient distance (min 500 m) from each other to minimize the possibility that the individuals belong to the same colony. Insects were brought to the laboratory alive in plastic tubes (without ethyl acetate). Abdomens of *V. vulgaris* were dissected and placed into a Karnovsky's fixative (solution): glutaraldehyde (1.5 %) mixed with paraformaldehyde (2 %) in a Sörensen phosphate buffer 0.1 m, pH 7.4. These samples ($n = 11$) were stored in the fixative in a fridge under 4 °C for about two weeks before processing for histological analysis. Other samples were freeze-killed and stored −20 °C in clean Eppendorf tubes. Detailed preprocessing of the samples for light microscopy (LM) and transmission electron microscopy (TEM) is given in the sub-section (2.5). To estimate the effects of morphology on PM accumulation, we also assessed body size and hairiness.

Individual body size of wasps was assessed as a head width (HW) in millimeters scaled from digital photos taken with Olympus C-5050 5.1-megapixel camera attached to Nikon SM Z800 stereomicroscope using the ImageJ software. HW was measured as the maximum distance between concavities of the compound eyes or the maximum interocular distance according to existing methodologies (Karsai and Hunt, 2002). Head surface area (HA) was estimated in mm^2 from the same photos and program.

Hairiness (pilosity) was assessed as hair number (HaN: n), hair length (HaL: μm), and hair density (HaD: number of hairs/mm^2) from the SEM photos of the wasps' heads. These measurements were conducted according to the methodology suggested by Roquer-Beni et al. (2020).

2.4. Surface contamination: PM number, size, electron optical density (EOD), and chemical composition (SEM, EDX)

2.4.1. Revealing an indicator body part

To reveal the PM (all possible particles) number, size, and EOD density of the wasps' body surfaces, we used SEM and EDX methods. First, we processed dissected heads and thoraxes with wings. We aimed to select the most convenient indicator body part, where particles could be easily assessed both quantitatively and qualitatively. Previous studies on honeybees – insects similar in size and morphology to yellowjackets – revealed that the outer margins of wings and the legs accumulate large quantities of PM (Negri et al., 2015). Our data confirm similar trends for the wasps, as all body parts carried PM (Fig. 1).

However, we choose to further process heads and suggest those for biomonitoring purposes. In pollinators, heads are key surfaces of pollen exchange (Roquer-Beni et al., 2020). In wasps, interacting with more diverse environmental compartments (such as soil, plants, and arthropods) heads should be the proper surface to convey environmental information. Also, heads are easy to dissect and mound onto SEM sample stubs. All manipulations were made in gloves and with wooden and plastic material to avoid further metal contamination.

2.4.2. The analyses of surface-accumulated particles using SEM methods

The analyses were performed at the SIB Labs Unit of the University of Eastern Finland using a scanning electron microscope ZEISS SIGMA-HD|VP (Carl Zeiss NTS, Cambridge, UK). Metallic coating was not deposited on the samples. Heads were mounted onto stubs with double adhesive carbon tape. To estimate total industrially derived surface PM accumulation we at first studied the whole heads areas of both wasp species using nominal magnification 56 X. All samples (whole heads) were observed with SEM using electron acceleration voltage of 20 kV and operating with low vacuum environment in the chamber with nitrogen gas of 70 Pa. To recognize the trace element particles of nano-microscale (and contaminated areas) the most efficient way is to use a backscattered electron detector (BSED) as it reveals the atomic contrast (*Z* contrast) of a specimen. During the BSE imaging, the brightness of the specimen was adjusted between low atomic *Z* number surface such as chitin (dark) and the PM (bright). This gave BSE detection range a good dynamic contrast in order to observe chitin (dark grey), minerals (grey) and heavy metals (bright grey, white) distinctively. The further analyses were performed using Thermo Pathfinder 1.4 X-ray microanalyses (Thermo Scientific, Madison, WI, US) system. The BSED was used in order to seek the exact locations of higher atomic *Z* number. The PM of these locations revealed to be either natural mineral particles or heavy metals and were finally identified using EDX.

2.4.3. The analyses of surface-accumulated PM number, size, and EOD

To analyze surface PM total accumulation (number), size, and density we used 8-bit BSED SEM images and the ImageJ program. First, we identified the exact head area with a polygon selection tool and cropped the image. SEM images are grey scaled pictures allowing contrast scaling such as the values 0 is white and 255 – black (or reverse if select a “dark background” option). In the BSED SEM images, particles are contrastingly visible as shiny spots, with a certain numerical threshold (175–206) opposite to the background. After the threshold set up, we used the option “analyze particles”, allowing the automated quantification for the number and size of the particles. To correctly reveal the PM size, we set up the

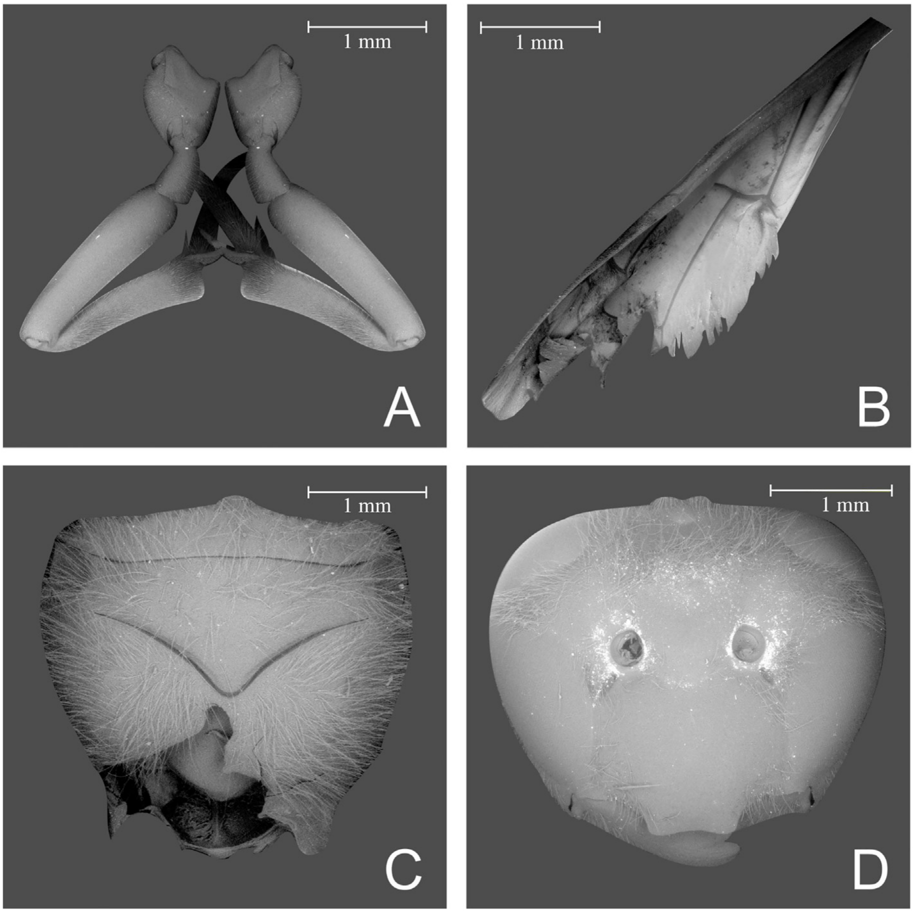

Fig. 1. Body parts of *V. germanica* such as legs (A), wing (B), prothorax (C), head (D) contaminated with particles. Shiny spots can be identified as high EOD (electron optical density) particles on BSED (backscattered electron detector) SEM micrographs.

scale prior to particle analyses such as 1 pixel was equal to 2.617 μm. PM density (PM/mm^2) was calculated as particle number divided by the head surface area. Images with the same magnification were used in the analyses.

2.4.4. Chemical composition of PM and point elemental analyses (EDX)

To conduct the point elemental analyses with EDX, we used Thermo 60 mm^2 EDX dual SDD-detector system (Thermo Scientific, Madison, WI, USA). We processed the heads' areas such as (*i*) front, (*ii*) mandibles, and (*iii*) & (*iv*) right/left antennae openings using magnification up to 500 X. The magnification settings offered the horizontal field of view from 1.0 to 5.4 mm for the SEM images. At the given area, we randomly selected and processed up to 13 points (particles or their aggregates) and compared those to one reference point (visually uncontaminated with at least fine $PM_{2.5}$ chitin cuticle surface seen under highest magnification). Ultrafine PM (< 0.1 μm) were not checked in the current study. That was performed to account the contribution of the chitin cuticle to the overall EDX spectra. Altogether 377 points were processed.

2.5. Internal gut contamination: particles and their chemical composition (LM, TEM, EDX)

Prior to LM and TEM, the post-fixation was conducted for the four *V. vulgaris* extracted gut samples (two exposed & two control samples). First whole guts were extracted from the abdomens with wooden sticks in the Karnovsky's fixative. Then midguts were dissected and post-fixated in 1 % sodium sulfide (Na_2S) in 0.1 M phosphate buffer (pH 7.4). After the initial step, the mid gut samples were washed in 0.1 M phosphate buffer (pH 7.4) and ethanol-dehydrated via 10 min series with the following concentrations: 70 %, 90 %, 94 % (one time), and 100 % (three times). The following dehydration step was with propylene oxide (CH_3CHCH_2O) for 15 min and after that for 10 min under stable room-temperature conditions. Infiltration was performed with a propylene oxide and LX-112 resin (epon) mixture (1:1) for two hours, and overnight with pure resin (epon). Further embedding was also conducted with epon; polymerization was performed for 48–72 h in the oven at +60 °C. To obtain cross sections from resin blocks, we used ultramicrotome Leica EM UC7. Semi-thin sections (1 μm) for LM were taken under a light microscope and stained with 1 % toluidine blue and 2.5 % sodium hydrogen carbonate 1:20.

Light microscopy was performed with a stereomicroscope Leica MZ75 connected to Nikon DS-Fi2 colour camera and analyzed with the Axio Vision program. Each cross section was processed with two setting types: (*i*) general view of the alimentary canal (magnification X 5); (*ii*) enlarged view of the cell walls: three pictures per sample (magnification X 40). Density per surface area of granules was further analyzed with the ImageJ software from digital RGB 8-bit pictures taken for each type of these settings. All together 40 photos (10 per individual of the (*ii*) setting type were processed for this purpose). Granules' density was estimated with the option “analyze particles” in the ImageJ software from the grey scaled photos as number spherites in gut tissues n/μm^2.

After LM, ultrathin sections for TEM (about 70 nm) were cut and used for TEM analyses without staining to allow EDX analyses. Analyses was performed in SIB Labs (University of Eastern Finland) by using high-resolution TEM, Jeol JEM-2100F (Jeol Co, Tokyo, Japan) equipped with a digital camera (Olympus Quemesa, Olympus Corp., Tokyo, Japan) and Radius v2.1 software (EMSIS GmbH, Münster, Germany). Thermo Noran System 7 EDX with LN2 cooled detector was used for elemental analyses in TEM.

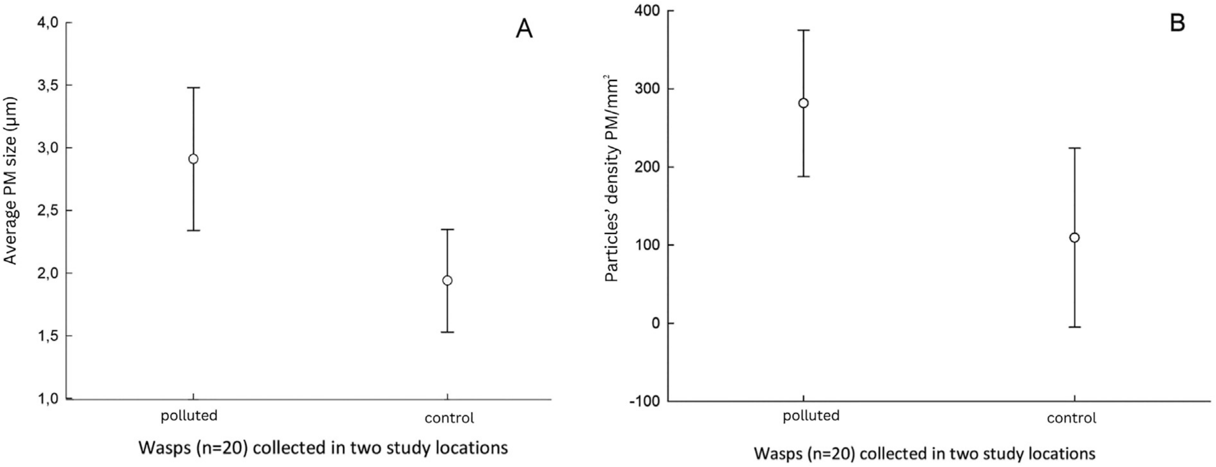

Fig. 2. Differences between mean values (Mean ± SD) of (A) particle size (μm) and (B) particle density (PM/mm^2) accumulated on the head surfaces of *V. vulgaris* and *V. germanica* in polluted and control habitats.

2.6. Statistics

To reveal the differences in the number, size, and density of PM (all possible particles including metallic) between treatments ($n = 2$) and study species ($n = 2$), we used *t*-Test (TTEST in SAS) for independent variables. The Polled approximation for the degrees of freedom was applicable when the demand for the equality of variances was met (variances didn't differ significantly). If there was a significant difference between variances, we applied Satterthwaite approximation of degrees of freedom. In the sets of these analyses, both PM-related variables such as PM number, size, or density and morphometrics variables such as head width (HW), and head area (HA), were assessed as continuous dependent variables. The treatment type (polluted and unpolluted) and the study species (*V. germanica* = spec. 1 and *V. vulgaris* = spec. 2) served as categorical classification variables. Similarly, internal density of granules in the midgut tissue was compared with the *t*-Test.

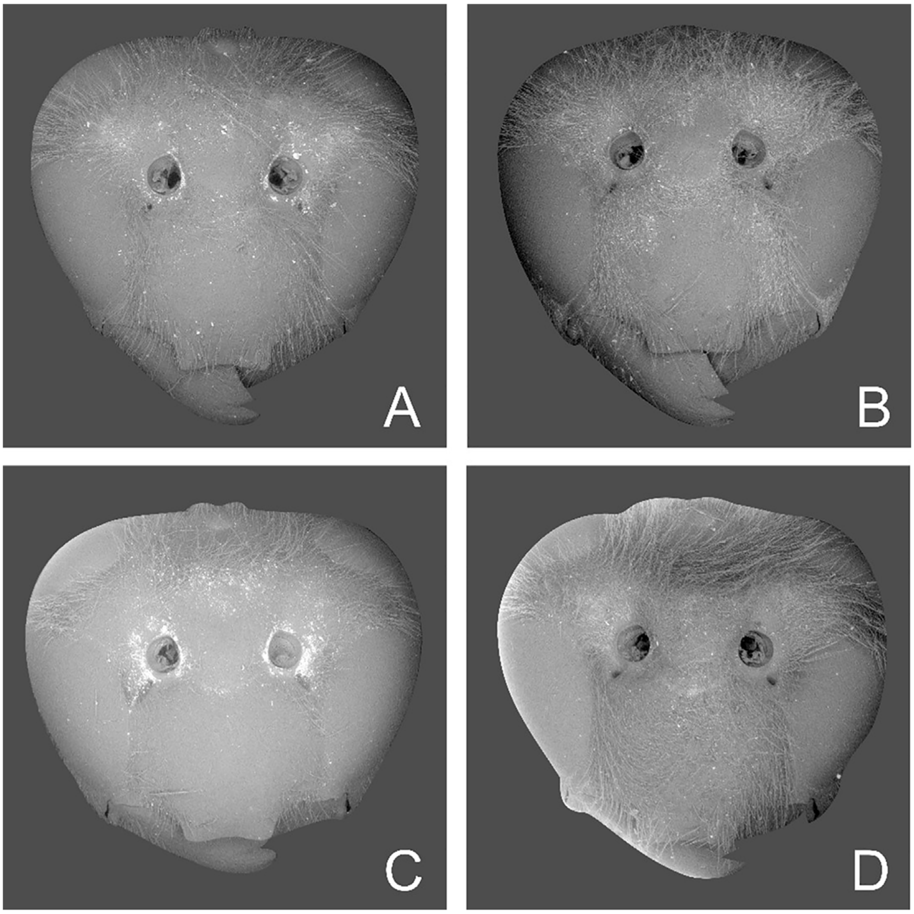

Fig. 3. SEM images of *V. vulgaris* (A & B) and *V. germanica* (C & D). Specimens shown at the A & C represented typical particle contamination of all individuals sampled near to Harjavalta *Cu—Ni* smelter. Wasps from the control areas (B & D) visually appeared to be less contaminated.

Effects of morphological traits such as size and hairiness on the accumulated PM in wasps were revealed with Linear Regression Models (REG proc. in SAS); HW, HA, HaL, and HaD been used as independent variables, while PM size was a dependent variable. Model residuals were normally distributed.

All statistical analyses were carried out in SAS 9.4 (SAS Institute, Cary; NC; USA).

3. Results

3.1. Differences in surface PM accumulation between polluted and control areas

There was a significant difference in the accumulated particles' number, size, and head surface density in both wasp species between polluted and control areas. Wasps collected near Harjavalta *Cu—Ni* smelter accumulated significantly more particles per head ($t = -2.14$, $df = 18$, $p = 0.04$; Mean ± SD PM number: polluted 2870.5 ± 1004.9; control 1045.8 ± 1097.8). In polluted areas wasps trapped larger particles ($t = -3.19$, $df = 18$, $p < 0.005$; Mean ± SD μm: polluted 2.91 ± 0.57; control 1.94 ± 0.41; $n = 20$; Fig. 2A) and their density was significantly higher ($t = -2.14$, $df = 18$, $p = 0.05$; Mean ± SD PM/mm^2: polluted 281.5 ± 93.8; control 109.4 ± 114.5, $n = 20$; Fig. 2B).

All wasps were similar in body size as neither wasps' head width (HW) nor head area (HA) was different between polluted and control habitats (HW: $t = -0.19$; $df = 18$; $p = 0.85$; Mean ± SD mm: polluted 3.19 ± 0.07; control 3.17 ± 0.23, $n = 20$; HA: $t = -0.67$, $df = 18$, $p = 0.52$; Mean ± SD mm^2: polluted 9.99 ± 0.51; control 9.68 ± 1.35, $n = 20$). In addition, head area surface (HA) affected neither average size of accumulated particles ($F_{1,18} = 0.93$, $p = 0.35$, $n = 20$) nor its density ($F_{1,18} = 2.25$, $p = 0.15$, $n = 20$). Thus, we assume no size-related effects on particle accumulation in the studied sample between polluted and control habitats.

3.2. Difference in surface PM accumulation between wasp species: Effects of morphological traits

Individuals of both wasp species collected from polluted environments accumulated more particles than those from the reference areas. That was confirmed by numerical investigations and by visual observations of SEM images. In both species, areas of compound eyes were relatively clean, while frons, central area of the heads, and especially the areas alongside antennae openings were heavily contaminated with particles (Fig. 3).

While studying the individual capacity of each species for trapping atmospheric PM, we found that *V. vulgaris* accumulated larger particles in comparison to *V. germanica*, but there was no interspecific difference in the PM number or density (Table 1. A). Among morphological parameters such as size or hairiness, only head width was different between species, being larger in *V. germanica* (Table 1. B).

In addition, the revealed difference in PM size between two wasp species was not associated with the wasp size (HW: $F_{1,18} = 0.03$, $p = 0.97$, $n = 20$; HA: $F_{1,18} = 0.52$, $p = 0.48$, n = 20) or hairiness (HaL: $F_{1,18} = 0.09$, $p = 0.76$, $n = 20$; HaD: $F_{1,18} = 1.22$, $p = 0.28$, $n = 20$).

3.3. Internal accumulation of particles in gut tissues

Analyses of LM midgut cross-sections revealed that wasps from both polluted (Fig. 4A) and control habitats (Fig. 4B) accumulated particles (granules) in columnar epithelial cells, primarily in the apical parts of those. However, the density of granules per gut tissue (n/μm^2) was significantly higher in wasps collected in the Harjavalta area in comparison to control habitats ($t = 7.7$, $df = 38$, $p < 0.0001$; Mean ± SD granules n/μm^2: polluted 0.0122 ± 0.001; control 0.004 ± 0.002, $n = 40$; Fig. 4C). Signs of microvilli degradation (they were soldered and stacked together) were revealed in one third of the processed midgut cross sections (Fig. 4D).

Table 1
A comparison between *V. germanica* and *V. vulgaris* in the (A) surface accumulated particles and (B) morphological characteristics. Mean values obtained from *t*-test (t-test procedure in SAS; $n = 20$). Significant *p* values are marked bold.

(A) Accumulated particles

	n	*t*	*df*	*p*	Mean ± SD
		Particles' number (n)			
V. germanica	9	0.55	18	0.59	2665.3 ± 1757.6
V. vulgaris	11				2209.1 ± 922.6
		Particles' size (μm)			
V. germanica	9	−3.02	13	**0.01**	2.1 ± 0.3
V. vulgaris	11				3.2 ± 0.8
		Particles' density (n/mm^2)			
V. germanica	9	0.33	18	0.74	252.8 ± 48.8
V. vulgaris	11				226.8 ± 81.6

(B) Morphological characteristics

	n	*t*	*df*	*p*	Mean ± SD
		Head width (mm)			
V. germanica	9	3.96	18	≤ **0.0001**	3.28 ± 0.08
V. vulgaris	11				3.09 ± 0.08
		Head area (mm^2)			
V. germanica	9	0.76	18	0.46	10.16 ± 0.76
V. vulgaris	11				9.72 ± 0.57
		Hair number (n)			
V. germanica	9	1.07	18	0.29	418.68 ± 182.76
V. vulgaris	11				348.39 ± 118.09
		Hair density (n/mm^2)			
V. germanica	9	0.59	18	0.56	40.46 ± 16.18
V. vulgaris	11				35.43 ± 11.45
		Hair length (μm)			
V. germanica	9	−1,5	18	0.16	492.51 ± 127.29
V. vulgaris	11				587.31 ± 82.11

3.4. External and internal chemical composition of accumulated particles

3.4.1. SEM-EDX

In all wasp individuals, the SEM-EDX analyses constantly revealed carbon (*C*), oxygen (*O*), and nitrogen (*N*) in every processed point, which was considered as related to background information (chitin structure). These elements were present in high weight % in every processed point. The point EDX analyses of potentially uncontaminated chitin also confirmed that the elements such as calcium (*Ca*), magnesium (*Mg*), potassium *(K)*, molybdenum (*Mo*), silicon (*Si*) and iron (*Fe*) were present at low (<1 wt%) or trace concentrations (<0.1 wt%). It is possible that chitin was contaminated with trace elements. However, in this study, we didn't check ultrafine PM contamination, and elements present in small, or trace concentrations were processed only as part of at least fine PM.

The external contamination was present in both exposed and control individuals; and besides difference in the PM number and size, the difference in chemical composition was found. Particles identified as clay minerals (containing *Al*, *Si*, *S*, *Cl*, *Mg*, and *P*) and quartz (higher peaks of *Si*) were revealed in both sample types. However, metal-rich (> 20 wt%) (Fig. 5A) and metal-moderate (10–20 wt%) (Fig. 5B) PM was found predominantly in wasps from the Harjavalta area. Some metal particles were occasionally found in control wasps from the control areas.

In both wasp species, metal-rich PM constituted 9.3 % and metal-moderate containing particles – 21 % from all particles processed ($n = 377$). The distributional analyses of chemical elements, which were found on the wasp surfaces (not at trace or small concentrations) revealed that the pollutants, which were the most common (found in ≥19 % of all particles) and dense (in terms of weight %) were *Cu, Fe, Ni,* and *S* (indicated bold Table 2).

3.4.2. TEM-EDX

TEM-EDX method confirmed the presence of granules in the epithelial columnar cells of the midgut tissues (Fig. 6). In those granules, *C* (up to 95 %), *O* (up to 3 %), and *P* (up to 1 %) were the most typical elements. In most of the processed cases, granules were associated with metal ions

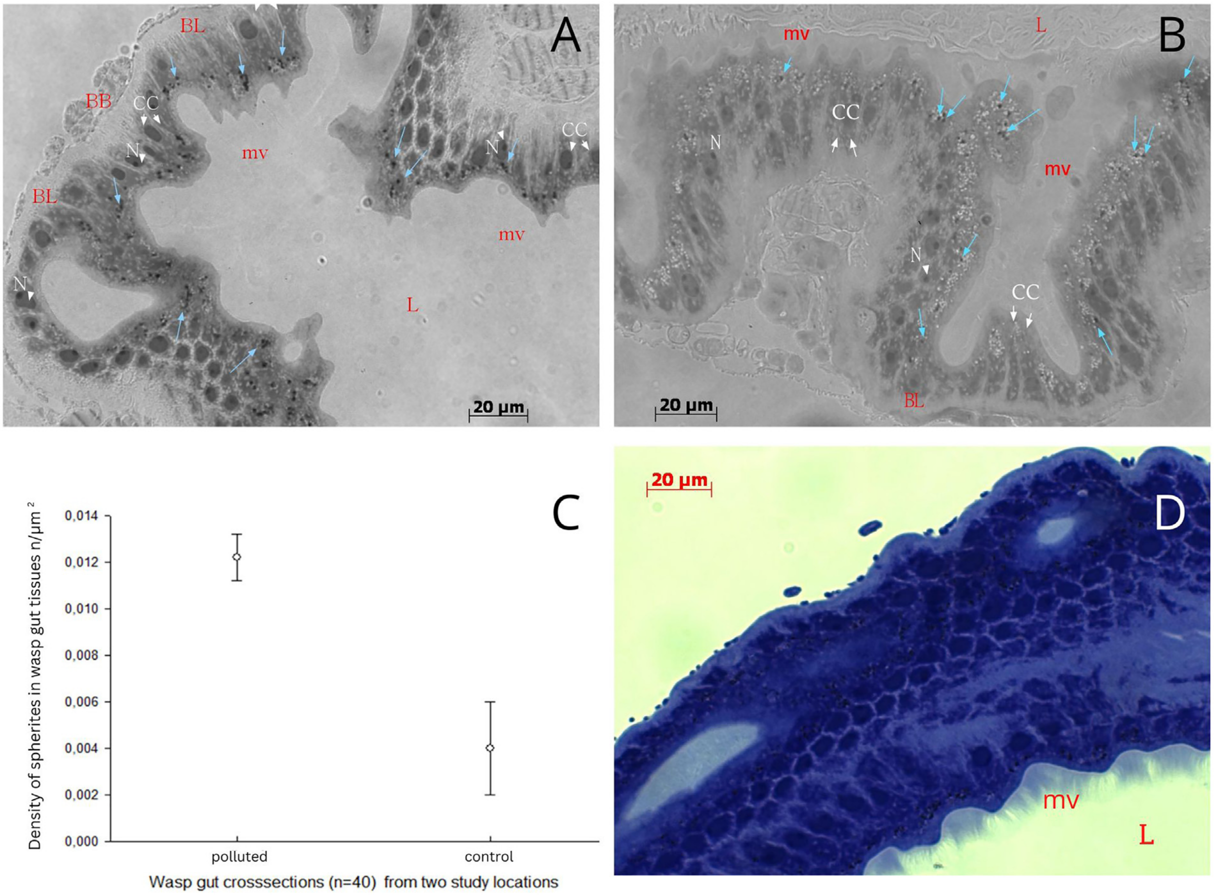

Fig. 4. Accumulation of granules (examples indicated with blue arrows) in the *V. vulgaris* midgut tissues revealed with LM. **A**. Wasp's midgut cross-section from polluted habitat (Harjavalta *Cu*—*Ni* smelter). **B.** Wasp's midgut cross-section from the control area (Nakkila). **C**. A comparison between spherite density in gut tissues n/μm^2 (means ± SE; $n = 40$). **D**. An example of a midgut tissue with slight signs of microvilli degradation (crumpled and soldered microvilli). BB – brush border; BL – basal lamina; L – lumen; CC – columnar epithelial cell (white arrows); N – nucleus; mv – microvilli.

such as *Cr*, *Fe*, *Ni*, and *Cu*. We assume, that ions of *Cu,* revealed in the samples, were not associated with a copper grid used in the TEM; as our previous study conducted for the same population of *V. vulgaris* with inductively coupled plasma mass spectrometry method (ICP-MS) revealed high body burdens of *Cu* (Skaldina et al., 2020). However, for TEM studies presence of *Cu* might be controversial due to possible contamination from the copper grid, which should be confirmed using the other methods. Metal containing granules were not found in the midgut tissues of wasps from control areas. To enable better EDX, we didn't use staining. Therefore, better morphology and types of granules might be revealed further.

4. Discussion

In both polluted and control habitats *Vespula* spp. wasps accumulate fine and coarse PM on their bodies. In an industrial area, surface accumulated particles are larger, their density is higher, and their chemical composition is associated with metals and other elements, emitted by the *Cu*—*Ni* smelter. These metals and elements are *Cu, Fe, Ni, S,* and, to a lower extent, − *As*, *Hg*, and *Sn*. Our previous ICP-MS analyses conducted for *V. vulgaris* in the same area, demonstrate that metal body burdens of *As*, *Cu* and *Ni* in wasps reliably reflect local pollution levels (Skaldina et al., 2020) approximately the same level as other organisms. Revealing the toxic components in the PM can be a challenging task due to the complex nature of particulate matter, "*which is much more complex than most other common air pollutants*" (Kelly and Fussell, 2012). EDX method enabled point identification and revealing of chemical elements, which can cause further toxicity.

In the control areas, yellowjackets also trap particles, which are smaller, and can be mostly defined as clays and silicates, while the occasional presence of metal-containing particles occurs. These results confirm, that similarly to honeybees, recommended as mobile PM samplers (Negri et al., 2015; Capitani et al., 2021), omnivorous wasps indicate local PM environmental contamination. PM accumulation capacity of mobile pollinating insects (Papa et al., 2021) and sessile plants (Leonard et al., 2016) is restricted by a narrow ecological niche and predefined area (blooming flowers for pollinators and distinct growing places for plants). Vespidae wasps are opportunistic generalists, utilizing different mechanisms during foraging and visiting a great variety of environmental compartments (Richter, 2000). Therefore, they can be recommended as mobile samplers of PM across diverse environments in biomonitoring surveys.

We found that *V. vulgaris* accumulated more PM_{10} than *V. germanica*, however no associated morphological differences, such as body size or hairiness, were revealed. Although the measures were relative, it might be logical to assume that size affects total surface PM – bigger individuals might trap more particles. Hairiness might also play a role, as, for example, in plants, presence of leaf hairs affects PM accumulation capacity (Leonard et al., 2016). However, in the case of flying omnivores wasps, the tendency toward PM sampling of different sizes may occur because of the other factors. Those can be (*i*) specific features of foraging behavior, (*ii*) distinctions in life strategies and nesting habits, and (*iii*) divergent selection between ecological niches in conspecifics. Pereira et al. (2016) found that both these wasp species possess asymmetrical foraging abilities – they respond differently to the presence of each other on the food. *Vespula germanica*

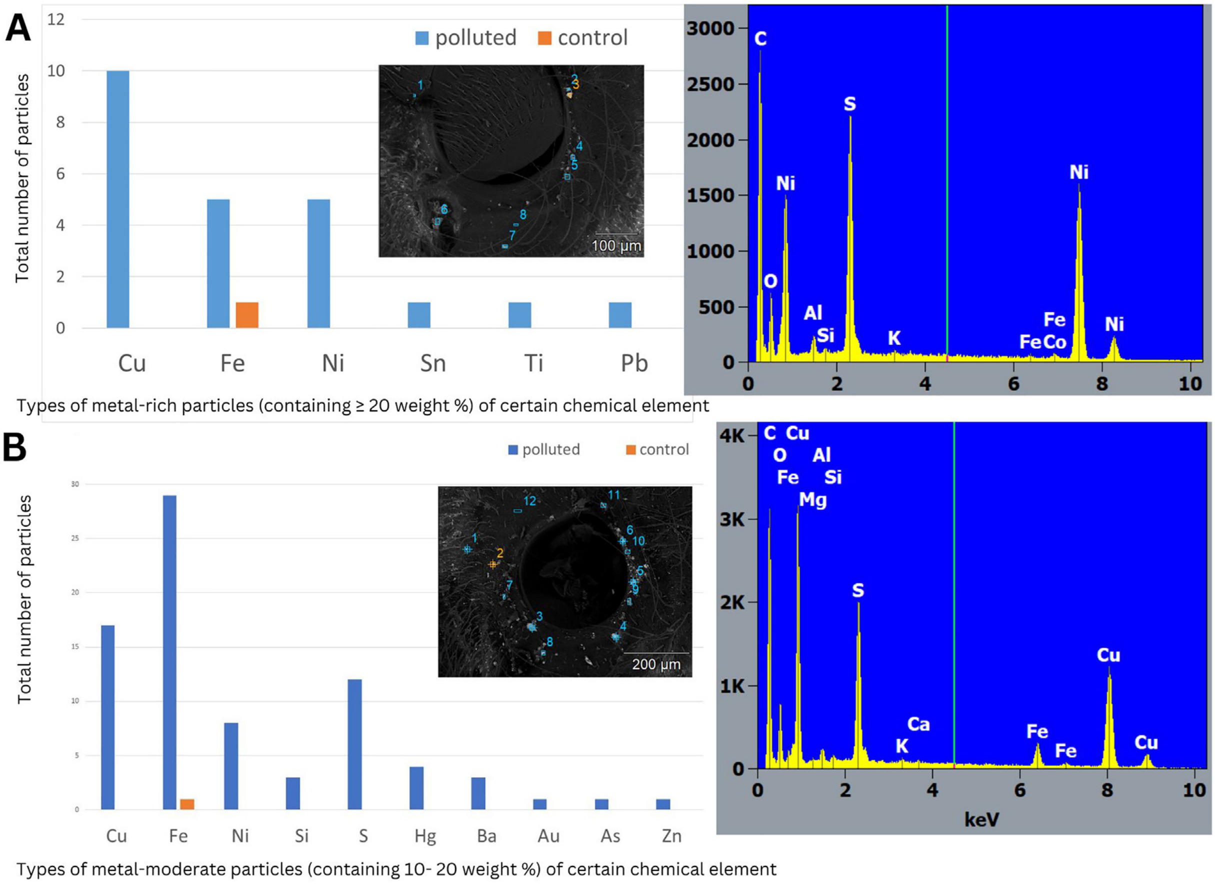

Fig. 5. Total number of metal-rich (A) and metal-moderate (B) PM revealed on the wasp heads in polluted and control areas. X axis represents type of particles, containing certain amount of a chemical element, Y axis – number of particles. Wasp individuals of different treatments (polluted or control) are indicated with colours. On the right, examples of EDX spectra obtained from metal-rich (above) and metal-moderate (below) particles. The height of the peak of each chemical element allows the quantification of its concentration in the examined particle.

avoid presence of *V. vulgaris*, while the last demonstrate indifferent response. In New Zealand honeydew beech forest (*Nothofagus* spp.), these wasps also show different foraging patterns. *V. germanica* mostly searches for protein food in the forest litter, while *V. vulgaris* is busy exploring shrubs and tree saplings (Harris et al., 1991). In sister species, microhabitat preferences might differ between geographic areas. Thus, in New Zealand, *V. vulgaris* prefers sunnier places for nesting, while *V. germanica* exhibits higher resistance for colder environments (Donovan et al., 1992). However, in Finland, the south-facing direction of a nesting slope was more important for the German than for the common wasp (Sorvari, 2018). An ability to accumulate larger particles might be associated with a local specify and a tendency of a species to build more nests underground. Again, an example from New Zealand population demonstrates that German wasps make their nests in soil more often than their common sister species. Such studies were not conducted for the northern area. Overall, we assume an ability of *V. vulgaris* to sample more PM_{10} might be associated with its foraging, nesting, or microhabitat preferences.

Concerning surface PM distribution, in honeybees, the presence of PM revealed primarily along the costal margin of the fore wings, the medial plane of the head, and the inner surface of the hind legs (Negri et al., 2015).

Table 2
Share of chemical elements in all processed particles (n = 377) in two wasp species, sampled in polluted and control areas. All values are related to mean concentrations of elements (weight %), except for the first row (which represents total % of particles, containing certain chemical elements, e.g. Al was present in 96 % of all (*n* = 377 particles processed).

	Share of chemical elements in the processed PM (point EDX)																
	Al	*As*	*Au*	*Br*	*Cu*	*Co*	*Cr*	*Hg*	*Fe*	*Mg*	*Mo*	*Ni*	*S*	*Si*	*Sn*	*Ti*	*Zn*
% of particles with certain element	96	1	0.5	0.8	**37**	3.7	1.6	1.9	**65.5**	44.8	11.14	**19**	**71.6**	74.5	1	6.6	6.6
Min	0.2	1	3.5	0.7	0.2	0.2	0.7	1	0.2	0.1	0.2	0.3	0.1	0.1	7.9	0.1	0.4
Max	18.7	6.6	8.6	1.1	28.6	0.7	3.9	13.2	36.5	1.7	1.7	26.8	15.5	11.8	23.3	21.1	14.1
Polluted																	
Mean	0.87	3.28	6.05	0.87	**5.24**	0.51	2.47	5.43	**4.23**	0.34	0.45	**4.63**	**2.53**	1.14	13.88	1.81	3.38
SD	1.31	2.10	2.55	0.21	**6.68**	0.19	2.61	5.12	**5.08**	0.67	0.26	**5.92**	**2.87**	1.75	6.91	4.91	4.17
Control																	
Mean	1.02				0.81				4.88	0.48	0.43		0.92	2.54		0.21	
SD	0.97				0.85				8.27	0.58	0.44		1.66	2.97		0.11	

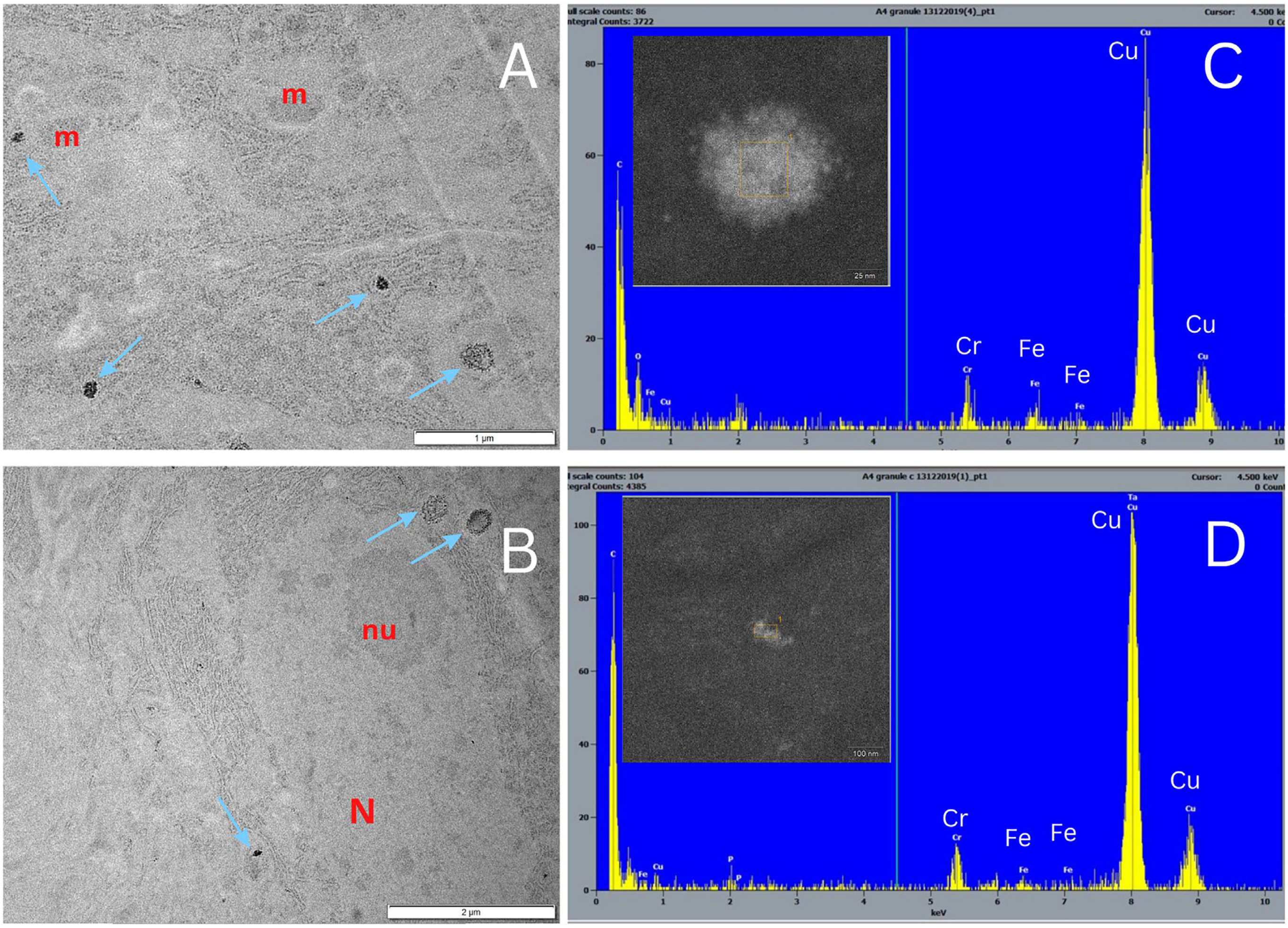

Fig. 6. Typical examples of metal-containing granules in our sample (indicated with blue arrows) in the columnar cells of the midgut tissues of *V. vulgaris* wasps from polluted habitats revealed with TEM-EDX method. A & B represent TEM pictures (without staining). C & D are examples of point particles processed with EDX and the relative spectra. N – nucleus; nu – nucleolus; m – mitochondria. The TEM-EDX spectra reveal some iron (Fe) and chromium (Cr) content on these locations. Copper content can be controversial as most of the emitted Cu X rays are from the copper grid of standard TEM specimen grid.

We found the same trend for the wasps. Although, on the wasps' heads, particles were often aggregated alongside antennae openings. Such localization might be explained by wasp self-cleaning behavior, as these parts are the hardest to reach for cleaning. Insects exhibit three types of self-cleaning behavior: maxillae nibbling, one-directional scraping of one structure by another, and rubbing back and forth while respective parts are constantly in contact (Jander, 1976). While some taxa have restrictions, most of the Hymenoptera use all three types of self-cleaning. Wasps, like other hymenopterans, often clean antennae and heads with their forelegs and further forelegs are cleaned with the mouthparts (Jander, 1976). Particles stuck to body surfaces can easily be ingested by insects. Insect antennae play a pivotal role in odor perception (Sache and Krieger, 2011), and external contamination might impair insects' performance and perception of olfactory signals. Thus, in polluted environments omnivorous insects might intake pollutants with food, water, and via a direct contact with air or soil – when they further ingest surface-deposited PM.

Our LM, TEM and EDX results confirmed the presence and higher internal density of granules, associated with metals, in midgut tissues in wasps from polluted areas. Insects (and their different life stages) store immobilized chemical elements in various organs and types of granules. These granules are (*i*) *spherocrystals* – relatively small (up to 5–6 μm) concretions, composed of organic stroma and mineral strata of varying thickness and chemical composition, possessing concentric layers, (*ii*) *lysosomal granules* – concretions without organized, concentric strata and with irregular profile, (iii) *pigment granules* – membrane-bound structures, usually containing calcium (*Ca*) (Ballan-Dufrançais, 2002). Spherocrystals or spherites were previously identified in hymenopterans (Jeantet et al., 1977), including wasps (Polidori et al., 2018). In *Formica* sp. ants, the midgut was confirmed to be an effective ionic barrier, immobilizing potentially toxic chemical elements in spherocrystals (Jeantet et al., 1977). However, no signs of tissue or cellular damage were revealed (only slightly enlarged ER and lysosomes) in response to chemical agricultural and industrial pollution. Our results also suggest midgut tissue serve as an efficient metal barrier for the wasps. However, more studies needed to confirm the frequency of different granule type in gut epithelial cells in *Vespula*. Only small number of processed samples had signs of slightly degraded microvilli in columnar cells, while no other indicators of developmental noise found. Contrary, Polidori et al. (2018), revealed evident ultrastructural alterations such as degraded microvilli, elevated heterochromatin, and mitochondrial disruptions in the midgut epithelium of *Polistes* sp. wasps in the urban area. We assume, that ecotoxicological risks for the wasps themselves might be associated with the individual tolerance of the species, rate of exposure, the age of an insect (for how long it was present in the environment).

While immobilized metals in gut tissues might not cause direct toxic effects to wasps, they can pass through higher trophic levels and expose other organisms to pollution (Gall et al., 2015). Even though wasps are visually and chemically well protected, birds (Raw, 1977), bats (Jeanne, 1970), and mammals (O'Hare, 2005) hunt and consume Vespidae as larvae and imago in different geographical areas. Thus, being a metal-tolerance mechanism, the internal gut contamination in wasps might cause further ecotoxicological risks to higher trophic levels.

5. Conclusions

Microanalysis reveals flying omnivorous *Vespula* sp. wasps as representative bioindicators of local industrial PM. In polluted area, *Vespula* sp. accumulate more PM of larger size and its surface density is higher. Wasps sample particles in both industrial and control areas. However, only closer to *Cu-Ni* smelter, most of the particles consistently contain chemical element such *Cu, Fe, Ni,* and *S,* which are major local pollutants. Two conspecific wasp species tend to accumulate PM of different size. *Vespula vulgaris* samples more PM$_{10}$, while *V. germanica* – more PM$_{2.5}$. That isn't associated with morphological traits such as size or hairiness and might be explained with distinctions in foraging and microhabitat preferences. In wasps, surface PM is accumulated in the places, which are hard to reach for the self-cleaning, for example, alongside antennae openings. Wasps frequently clean their body surfaces with forelegs in the mouthparts, which creates an exposure (ingestion) pathway in addition to direct ingestion of water, protein, or carbohydrate food. Midgut is confirmed as a metal barrier for the *Vespula* sp. wasps. In polluted habitats, the midgut tissue concentration of metal-containing granules is much higher compared to the control areas. External and internal accumulation of metal PM might cause some ecotoxicological exposure risks to higher trophic levels.

CRediT authorship contribution statement

Conceptualization (OS, AK, AŁ); Data curation (OS, JL); Formal analysis (OS, JL); Funding acquisition (OS, AŁ); Methodology (JL, AK, TE); Project administration (OS); Resources (JL, AK); Validation (AK, TE); Roles/Writing – original draft (OS, AŁ); Writing – review & editing (JL, AK, TE).

Oksana Skaldina (OS), Adrian Łukowski (AŁ), Jari Leskinen (JL,) Arto Koistinen (AK), Tapio Eeva (TE).

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Oksana Skaldina reports financial support was provided by Jenny and Antti Wihuri Foundation, Maja and Tor Nessling Foundation, National Science Centre, Poland.

Acknowledgments

The study was financially supported by Jenny and Antti Wihuri Foundation to OS [grants 00180353 and 00190336] during data collection and analyses and by Maj and Tor Nessling Foundation [grant 202200067] during manuscript writing stage. Adrian Łukowski's participation was co-financed by National Science Centre, Poland [2020/39/D/NZ9/00969]. Special thanks to SIB Labs and Virpi Miettinen for her kind assistance with sample preparation for electron microscopy. Special thanks to bachelor trainees Oliver Welling and Jarkko Hanninen for their help with gut samples analyses and granules quantification, to Mati Saag for the accommodation arrangements during field work, and to Senni Skaldina for help with samples collection. We are sincerely grateful to anonymous reviewers, whose valuable comments helped to make this manuscript better!

References

Adams, K., Greenbaum, D.S., Shaikh, R., van Erp, A.M., Russell, A.G., 2015. Particulate matter components, sources and health: systematic approaches to testing effects. J. Air Waste Manag. Assoc. 65 (5), 544–558.

Ballan-Dufrançais, C., 2002. Localization of metals in cells of pterygote insects. Microsc. Res. Techn. 56, 403–420.

Berglund, Å.M.M., Rainio, M.J., Eeva, T., 2015. Temporal trends in metal pollution: using bird excrement as indicator. PLoS ONE 10 (2), e0117071.

Boliden Harjavalta - Boliden, n. www.boliden.com/operations/smelters/boliden-harjawalta. (Accessed 29 September 2022).

Brock, R.E., Cini, A., Sumner, S., 2021. Ecosystem services provided by aculeate wasps. Biol. Rev. 96 (4), 1645–1675.

Cajander, A.K., 1949. Forest types and their significance. Acta Forest. Fenn. 56 71 pp..

Capitani, G., Papa, G., Pellecchia, M., Negri, I., 2021. Disentangling multiple PM emission sources in the Po Valley (Italy) using honeybees. Heliyon 7, e06194.

Coll, M., Guershon, M., 2002. Omnivory in terrestrial arthropods: mixing plant and prey diet. Annu. Rev. Entomol. 47, 267–297.

Diao, M., Holloway, T., Chois, S., O'Neill, S.M., Al-Hamdan, M., van Donkelaar, A., Martin, R.V., Jin, X., Fiore, A.M., Henze, D.K., Lacey, F., Kinney, P.L., Freedman, F., Larkin, N.K., Zou, Yu, Kelly, J.T., Vaidyanathan, A., 2019. Methods, availability, and applications of PM2.5 exposure estimates derived from ground measurements, satellite, and atmospheric models. J. Air Waste Manag. Assoc. 69 (12), 1391–1414.

Dominici, F., Greenstone, M., Sunstein, C.R., 2014. Particulate matter matters. Science 344, 257–259.

Donovan, B., Howie, A.M.E., Schroeder, N.C., 1992. Comparative characteristics of nests of *Vespula germanica* (F.) and *Vespula vulgaris* (L.) from Christchurch City, New Zealand. N. Z. J. Zool. 19, 61–71.

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 microplastic. Sci. Total Environ. 767, 144481.

Elizalde, L., Arbetman, M., Arnan, X., 2020. The ecosystem services provided by social insects: traits, management tools, and knowledge gaps. Biol. Rev. 95 (5), 1418–1441.

Feldhaar, H., Otti, O., 2020. Pollutants and their interaction with diseases of social Hymenoptera. Insects 11 (3), 153.

Flanders, S.E., 1941. Dust as an inhibiting factor in the reproduction of insects. J. Econom. Entomol. 34, 470–472.

Gall, J.E., Boyd, R.S., Rajakaruna, N., 2015. Transfer of heavy metals through terrestrial food webs: a review. Environ. Monit. Assess. 187 (4), 1–21.

Grantz, D.A., Garner, J.H.B., Johnson, D.W., 2003. Ecological effects of particulate matter. Environ. Int. 29 (2–3), 213–239.

Grzyb, J., Pawlak, K., 2021. Impact of bacterial aerosol, particulate matter, and microclimatic parameters on animal welfare in Chorzów (Poland) zoological garden. Environ. Sci. Pollut. Res. 28, 3318–3330.

Harris, R.J., Oliver, E.H., 1993. Prey diets and population densities of the wasps *Vespula vulgaris* and *V. germanica* in scrubland pasture. New Zeal. J. Ecol. 17 (1), 5–12.

Harris, R.J., Thomas, C.D., Moller, H., 1991. The influence of habitat use and foraging on the replacement of one introduced wasp species by another in New Zealand. Ecol. Entomol. 16 (4), 441–448.

Heikens, A., Peijnenburg, W.J.G.M., Hendriks, A.J., 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environ. Pollut. 113 (3), 385–393.

Hodkinson, I.D., Jackson, J.K., 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. Environ. Manag. 35 (5), 649–666.

Jander, R., 1976. Grooming and pollen manipulation in bees (Apoidea): the nature and evolution of movements involving the foreleg. Physiol. Entomol. 1, 179–194.

Jeanne, R.L., 1970. Note on the bat (*Phylloderma stenops*) preying upon the brood of a social wasp. J. Mammal. 51, 624–625.

Jeantet, A.Y., Ballan-Dufrançais, C., Martoja, R., 1977. Insects resistance to mineral pollution. Importance of spherocrystal in ionic regulation. Rev. d'Ecolog. Biolog. Sol. 14, 563–582 [in French].

Käffer, M.I., Lemos, A.T., Apel, M.A., Rocha, J.V., de Azevedo Martins, S.M., Vargas, V.M.F., 2012. Use of bioindicators to evaluate air quality and genotoxic compounds in an urban environment in Southern Brazil. Environ. Pollut. 163, 24–31.

Karsai, I., Hunt, J.H., 2002. Food quantity affect traits of offspring in the paper wasp *Polistes metricus* (Hymenoptera, Vespidae). Environ. Entomol. 31 (1), 99–106.

Kelly, F., Fussell, J., 2012. Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. Atmos. Environ. 60, 504–526.

Khan, T.A., Ramegowda, G.K., Dar, M.Y., 2013. Effect of road dust pollution in mulberry on silkworm performance in Kashmir valley, India. Res. J. Agric. Sci. 4 (4), 501–506.

Khaniabadi, Y.O., Goudarzi, G., Daryanoosh, S.M., Borgini, A., Tittarelli, A., de Marco, A., 2017. Exposure to PM$_{10}$, NO$_2$, and O$_3$ and impacts on human health. Environ. Sci. Pollut. Res. 24, 2781–2789.

Kiikkilä, O., 2003. Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu-Ni smelter, SW Finland. Silva Fenn. 37 (3), 399–415.

Krimmel, B.A., 2011. Omnivorous insects: evolution and ecology in natural and agricultural ecosystems. Nat. Educ. Know. 3 (10), 52.

Leonard, R.L., Arthur, M.C., Hochuli, D.F., 2016. Particulate matter deposition on roadside plants and the importance of leaf trait combinations. Urban For. Urban Green. 20 (1), 249–253.

Li, M., Nabi, G., Sun, Y., et al., 2021. The effect of air pollution on immunological, antioxidative and hematological parameters, and body condition of Eurasian tree sparrows. Ecotoxicol. Environ. Saf. 208, 111755.

Łukowski, A., Popek, R., Jagiełło, R., et al., 2018. Particulate matter on two *Prunus* spp. decreases suvival and performance of the folivorous beetle *Geonioctena quinquepunctata*. Environ. Sci. Pollut. Res. 25, 16629–16639.

Łukowski, A., Popek, R., Karolewski, P., 2020. Particulate matter on foliage of *Betula pendula*, *Quercus robur*, and *Tilia cordata*: deposition and ecophysiology. Environ. Sci. Pollut. Res. 27, 10296–10307.

Nadat, Y.T., Kylin, H., Sithole, R., Lesch, V., Bouwman, H., 2021. The wasp as terrestrial indicator of environmental metal composition: evidence from Zimbabwe. Environ. Toxicol. Chem. 40 (60), 1726–1739.

Negri, I., Mavris, C., Di Prisco, G., Caprio, E., Pellecchia, M., 2015. Honeybees (*Apis mellifera*, L.) as active samplers of airborne particulate matter. PLoS ONE 10 (7), e0132491.

O'Hare, M., 2005. Does anything eat wasps?: And 101 other questions. New Sci. Hachette UK, 224 pp.

van Ooik, T., Rantala, M.J., Saloniemi, I., 2007. Diet-mediated effects of heavy metal pollution on growth and immune response in the geometrid moth *Epirrita autumnata*. Environ. Pollut. 145, 348–354.

Osborne, K.H., Longcore, T., 2021. Effect of gypsum dust on lepidopterous larvae. Ecotoxicol. Environ. Saf. 228, 113027.

Pacini, E., Franchi, G.G., 2020. Pollen biodiversity – why are pollen grains different despite having the same function. A review. Bot. J. Linn. Soc. 193 (2), 141–164.

Papa, G., Capitani, G., Capri, E., Pellecchia, M., Negri, I., 2021. Vehicle-derived ultrafine particulate contaminating bees and bee products. Sci. Total Environ. 750, 141700.

Pereira, A.J., Pirk, G.I., Corley, J.E., 2016. Foraging behavior interactions between two non-naitive social wasps, *Vespula germanica* and *V. vulgaris* (Hymenoptera, Vespidae): implications for invasion success? J. Insect Sci. 16 (1), 78.

Phillips, B.B., Bullock, J.M., Gaston, K.J., et al., 2021. Impacts of multiple pollutants on pollinator activity on road verges. J. Appl. Ecol. 58 (5), 1017–1029.

Polidori, C., Pastor, A., Jorge, A., Pertusa, J., 2018. Ultrastructural alterations of midgut epithelium, but not greater wing fluctuating asymmetry, in paper wasps (*Polistes dominula*) from urban environments. Microsc. Microanal. 24 (2), 183–192.

Popek, R., Łukowski, A., Bates, C., Oleksyn, J., 2017. Accumulation of particulate matter, heavy metals, and polycyclic aromatic hydrocarbons on the leaves of *Tilia cordata* Mill. in five Polish cities with different levels of air pollution. Int. J. Phytoremediation 19 (12), 1134–1141.

Przybysz, A., Popek, R., Stankiewicz-Kosyl, M., Zhu, Ch.Y., Małecka-Przybysz, M., Maulidyawati, T., Mikowska, K., Deluga, D., Griżuk, K., Sokalski-Wieczorek, J., Wolszczak, K., Wińska-Krysiak, M., 2021. Where trees cannot grow – particle matter accumulation by urban meadows. Sci. Total Environ. 785, 147310.

Rai, P.K., 2016. Impacts of particulate matter pollution on plants: implications for environmental biomonitoring. Ecotoxicol. Environ. Saf. 29, 120–136.

Raw, A., 1977. Avian predation on individual neotropical social wasps (Hymenoptera, Vespidae) outside their nests. Ornitol. Neotrop. 8, 89–92.

Richter, M.R., 2000. Social wasps (Hymenoptera: Vespidae) foraging behaviour. Annu. Rev. Entomol. 45, 121–150.

Roquer-Beni, L., Rodrigo, A., Arnan, X., Klein, A.-M., Fornoff, F., Boreux, V., Bosch, J., 2020. A novel method to measure hairiness in bees and other insect pollinators. Ecol. Evol. 10 (6), 2979–2990.

Sache, S., Krieger, J., 2011. Olfaction on insects. Neuroforum 2 (3), 49–60.

Sæbø, A., Popek, R., Nawrot, B., 2012. Plant species differences in particulate matter accumulation on leaf surfaces. Sci. Total Environ. 427, 347–354.

Sanderfoot, O.V., Holloway, T., 2017. Air pollution impacts on avian species via inhalation exposure and associated outcomes. Environ. Res. Lett. 12 (8), 083002.

Skaldina, O., Sorvari, J., 2019. Ecotoxicological effects of heavy-metal pollution on economically important terrestrial insects. In: Kesari, K. (Ed.), Networking of Mutagens in Environmental Toxicology, pp. 137–144.

Skaldina, O., Ciseck, R., Peräniemi, S., Kolehmainen, M., Sorvari, J., 2020. Facing the threat: common yellowjacket wasps as indicators of heavy metal pollution. Environ. Sci. Poll. Res. 27 (1), 29031–29042.

Sorrentino, M.C., Capozzi, F., Wuyts, K., et al., 2021. Mobile biomonitoring of atmospheric pollution: a new perspective for the moss-bag approach. Plants 10 (11), 2384.

Sorvari, J., 2018. Habitat preferences and spring temperature-related abundance of German wasp *Vespula germanica* in its northern range. Ins. Conserv. Divers. 11 (4), 363–369.

Thimmegowda, G.G., Mullen, S., Sottilare, K., Sharma, A., Mohata, S.S., Brockmann, A., Dhandapancy, P.S., Olsson, S.B., 2020. A field-based quantitative analysis of sublethal effects of air pollution on pollinators. PNAS 117 (34), 20653–20661.

Thorp, R.W., 1979. Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. Ann. Mo. Bot. Gard. 66 (4), 788–812.

Urbini, A., Sparvoli, E., Turilazzi, S., 2006. Social wasps as bioindicators: a preliminary research with *Polistes dominulus* (Hymenoptera, Vespidae) as a trace metal accumulator. Chemosphere 64 (5), 697–703.

Vanderstock, A.M., Latty, T., Leonard, R.J., Hochuli, D.F., 2019. Mines over matter: effects of foliar particulate matter on herbivorous insect, *Helicoverpa armigera*. J. Appl. Entomol. 143 (1–2), 77–87.