











## From flowers to pollinators: Dietary exposure of honey bees, bumble bees and solitary bees to trace elements across European fields

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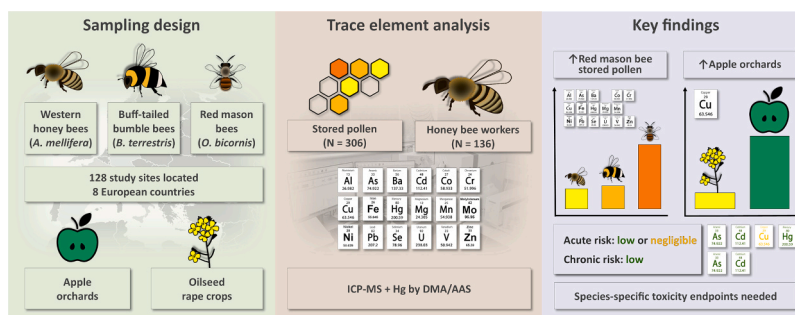
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## HIGHLIGHTS

- Pollen stores across Europe showed high variability in trace element composition.
- Trace element levels in pollen differed among bee species and cropping systems.
- Red mason bee pollen shows higher trace element levels than other bees' pollen.
- Elevated copper levels in apple orchard pollen likely reflect copper fungicide use.
- Limited toxicity data hinder assessment of sublethal trace element effects in bees.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Trace elements are persistent contaminants that enter bee diets through pollen, nectar, soil-derived particles and atmospheric deposition, yet field-based exposure data for pollinator taxa remain limited. We examined 18 trace elements (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, U, V, Zn) in pollen stored by managed western honey bees (*Apis mellifera*), buff-tailed bumble bees (*Bombus terrestris*), and red mason bees (*Osmia bicornis*) from 128 apple orchards and oilseed rape fields across eight European countries. We assessed species-, crop- and landscape-related drivers of element accumulation in stored pollen and honey bee workers, and used these data to estimate dietary exposure and risk. Stored pollen showed crop- and species-specific differences in trace element levels. Red mason bee pollen contained several-fold higher concentrations than honey bee and bumble bee pollen, suggesting that honey bees may not be a suitable model for pollen-based exposure studies. Stored pollen from apple orchards contained higher Cu than that from oilseed rape sites, likely linked to Cu-based fungicide use. Landscape characteristics, including crop and urban cover, influenced element concentrations in stored pollen and honey bee workers. Acute risks were assessed for As, Cd, Cu, and Hg, and chronic risks for As and Cd. For elements with available toxicity endpoints, estimated dietary risk was negligible or low; for elements lacking such endpoints, risk could not be quantified despite exposure estimates. Higher exposure of solitary bees, together with scarce toxicity data, indicates that future studies should focus on establishing species-specific toxicity endpoints.

## 1. Introduction

Pollinators play a crucial role in the reproduction of flowering plants, thereby supporting ecosystem balance and global food production [1,2]. Among these, bees have received significant attention due to their essential role as the most effective and ubiquitous pollinators of a wide diversity of wild and cultivated flowers, bushes, and trees [3,4]. However, bee populations worldwide have faced alarming declines driven by a multitude of stressors [5]. These include habitat loss, the emergence of pathogens, reduced diversity of flowering plants leading to nutritional deficiencies, climate change, exposure to agrochemicals [6], and other environmental contaminants [7]. Although a large body of research has focused on the impacts of synthetic agrochemicals in bee diets [6], the extent and effects of environmental exposure to elevated levels of trace elements have been poorly studied.

Bees are exposed to various trace elements in their foraging environments through dietary exposure from floral resources and water, airborne particulates, or direct contact with soil [8]. The bioavailability of trace elements and their homeostasis are crucial for maintaining proper physiological functions in bees. Essential trace elements such as copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) are necessary for various enzymatic reactions. However, an excess of these elements can adversely affect key physiological processes in insects [9,10]. Toxic trace elements, like arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), can accumulate in insect tissues, disrupting various biochemical processes [9], which can impair immune function, increase susceptibility to pathogens [11], lead to reproductive disorders [12], induce behavioral changes [13–17], and ultimately threaten the

fitness and survival of the colony [18,19]. Pollinator species also differ in their management status, which can influence their exposure routes and ecological interactions, for instance through differences between managed colonies and wild populations [5]. Western honey bees (*Apis mellifera*) are exclusively managed pollinators, whereas bumble bees (*Bombus* spp.) and solitary bees such as the red mason bee (*Osmia bicornis*) may occur as wild populations but are currently widely used as managed pollinators in agricultural systems [5]. These differences in ecology and management underline the importance of studying multiple bee taxa when evaluating environmental stressors [20].

Various human activities, including industry, road traffic, and intensive agriculture, are major sources of trace element emissions [21–23]. Consequently, areas with a higher proportion of certain land-use types, such as urban or intensively farmed areas, may show increased levels of selected elements in soil and biota [24,25]. Elements deposited in soil through anthropogenic activities can be absorbed by plants, accumulated in their organs [26,27], and translocated to floral resources, including nectar and pollen, making them accessible to pollinators [28]. Contamination of pollen from airborne particulates represents another pathway of bee exposure to toxic and trace elements [29]. Collected pollen, which serves as a food resource for both larvae and adult bees, is converted into bee bread by honey bees, and can be a dietary source of various metals and metalloids throughout bees' lives [30]. The ability of bees and bee-related matrices to accumulate elements from different environmental compartments makes them useful biomonitors of environmental contamination by selected trace elements, although the suitability of individual matrices may differ substantially [31–33].

Oilseed rape (*Brassica napus*) and apple (*Malus domestica*) are two intensively cultivated crops that are both grown across the world and hold significant economic value [34]. Oilseed rape, one of the most widely cultivated crops globally, grows across more than 40 million hectares as of 2021 [35]. Apples, important fruit crops grown in over 90 countries, have a worldwide cultivation area of approximately 7 million hectares in recent years [35]. Both crops offer abundant floral resources and rely to different extents on insect pollinators [36,37]. However, their intensive cultivation may negatively impact local ecosystems [38]. Notably, oilseed rape belongs to the Brassicaceae family which includes known species of metallophytes that tend to accumulate metals and metalloids [39]. Many oilseed rape varieties are resistant to metal stress and can accumulate specific metals from the soil, offering potential for phytoremediation of metal-contaminated sites [40].

Our study aimed to assess the levels of 18 trace elements, such as aluminum (Al), As, barium (Ba), Cd, cobalt (Co), chromium (Cr), Cu, Fe, Hg, Mg, Mn, molybdenum (Mo), nickel (Ni), Pb, selenium (Se), uranium (U), vanadium (V), and Zn, in the pollen collected by western honey bees (APS), buff-tailed bumble bees (*Bombus terrestris*) (BPS), and red mason bees (OPS) from nests situated in apple orchards and close to oilseed rape fields in eight European countries. Additionally, the levels of the studied elements in honey bee workers from the same sites were determined. The data were used to identify the principal factors influencing the variation in trace element levels, including bee species, crop type, and the relative amounts of cultivated and urban areas within the study sites. We also estimated the oral exposure of adult honey bees, bumble bees, and red mason bees to these trace elements and assessed the resulting potential acute and chronic risks to these pollinators.

## 2. Material and methods

### 2.1. Study design and sampling

Sample collection was performed within the PoshBee project (*Pan-European assessment, monitoring, and mitigation of stressors on the health of bees*) [41]. Samples of stored pollen from western honey bees, buff-tailed bumble bees, and red mason bees as well as western honey bee workers were collected from 128 sites across eight European countries (Estonia, Sweden, Ireland, United Kingdom, Germany, Switzerland, Italy, and Spain) covering four biogeographic zones (Boreal, Atlantic, Continental, and Mediterranean). Per country, we selected 16 sites split evenly between two cropping systems (apple and oilseed rape), with the 8 sites per cropping system distributed along a gradient of land-use intensity that also included one site dedicated to organic cultivation. At each site, three honey bee colonies, three bumble bee colonies, and three red mason bee nests were placed following a common protocol [34]. Red mason bees were not used in the United Kingdom and Ireland, as local subspecies could not be sourced, whereas they were available in the other countries. Although bee bread from honey bees and pollen stores from bumble bees and red mason bees have distinct characteristics and properties [42,43], for simplicity, we decided to use a single term, “stored pollen”, throughout the article.

At each site, stored pollen of the three sentinel bee species was collected from all three colonies or nests in equal proportions to standardize the material tested by the laboratories. Sampling was performed only once for each species at each site. For honey bees and bumble bees, we sampled towards the end of the flowering period of the focal crop, while for red mason bees, sampling took place at peak flowering of the focal crop, representing maximum exposure to crop-specific pesticides and trace elements.

#### 2.1.1. Collection of stored pollen from honey bee colonies (APS)

Several pieces of combs containing cells filled with collected pollen were cut out from different frames (at least five out of ten frames in each hive) from all three hives per site. A minimum of 5 g of stored pollen per hive was obtained. Samples were cooled during field sampling and

stored at  $-20^{\circ}\text{C}$  on return to the laboratory. The stored pollen was then extracted from cells, separately from each hive, using a stainless-steel spatula (Fig. 1A). Extracted stored pollen from all three hives was pooled in equal proportions, yielding a sample of  $\sim 15$  g of pollen per site.

#### 2.1.2. Collection of stored pollen from bumble bee colonies (BPS)

At the end of the flowering season, the bumble bee colonies were terminated by freezing in polyethylene bags. Stored pollen was then extracted from the cells (Fig. 1B) using a stainless-steel spatula. The extracted stored pollen from all three colonies was pooled into a pre-weighed plastic container. Similarly, as for the stored pollen from honey bee colonies, the target quantity of the pooled sample was  $\sim 15$  g from each site.

#### 2.1.3. Collection of stored pollen from red mason bee nests (OPS)

Around the peak of the flowering season of the focal crop, approximately ten red mason bee nest tubes (single cardboard tubes) were collected per site. Roughly equal numbers of tubes were taken from each of three nests per site. The tubes were stored in polyethylene bags and placed in a chilled container during transport, and transferred to a freezer on return to the laboratory. Each nest tube was then split lengthwise (Fig. 1C) using a scalpel. After removing eggs and larvae, stored pollen was collected from each tube using a clean stainless-steel spatula. The extracted stored pollen was pooled from all nest tubes in equal proportions, with the required quantity of pooled sample per site being the same as for the other two bee species ( $\sim 15$  g).

#### 2.1.4. Stored pollen sample processing

Extracted stored pollen samples were stored in labelled polyethylene bags or polypropylene containers resistant to freezing. Samples were stored below  $-20^{\circ}\text{C}$  before shipment (frozen on dry ice) to the laboratory of the Department of Chemical Research of Food and Feed of the National Veterinary Research Institute (PIWet-PIB) in Puławy, Poland. At PIWet-PIB, the samples were homogenized by grinding in an LB20 laboratory homogenizer (Waring Laboratory Science, Stamford, USA) at 20,000 rpm for the short time necessary to obtain homogeneous samples. Sub-samples were prepared for elemental analysis (PIWet-PIB). Due to insufficient amounts of stored pollen to perform all of the mentioned analyses, we used a total of 123 out of 128 samples of APS, 96 samples of BPS, and 87 samples of OPS to determine trace element concentrations (Supplementary Table S3).

#### 2.1.5. Collection of honey bee workers

At each site, honey bee workers were collected and sent to the

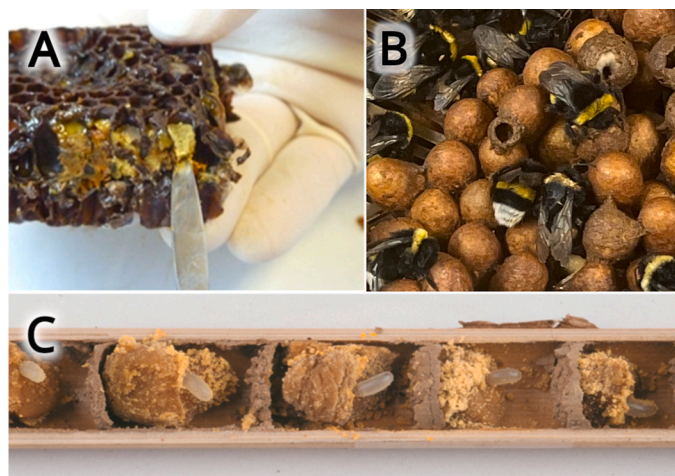


Fig. 1. Stored pollen of western honey bees (*Apis mellifera*) (A), buff-tailed bumble bees (*Bombus terrestris*) (B), and red mason bees (*Osmia bicornis*) (C).

Laboratory for Food Safety of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) for elemental analysis. A total of 20 internal workers were collected from each colony, resulting in 60 honey bees per site. The hives were opened, and worker bees were taken from border frames. After being frozen overnight, the samples were combined in a single polyethylene bag per site, aiming to collect an equal number of bees from each colony. The differences in the quantities sampled between colonies were kept within a maximum of 30%. If one colony did not yield the required number of specimens, the numbers from the remaining two colonies were increased to reach the total number requested. In the field, adult insects were placed in a chilled container and immediately frozen at  $-20\text{ }^{\circ}\text{C}$  on return to the laboratory. The samples of honey bee workers ( $N = 136$ ) were then shipped to ANSES on dry ice.

## 2.2. Landscape characteristics

Landscape elements at each sampling site were identified using the EUNIS habitat classification system [44]. These landscape elements were manually digitized at a scale of 1:2500 within a 1 km radius of the sampling sites using World Imagery (ESRI) for remote sensing data and ArcGIS Pro 2.4.1 (ESRI) for GIS analysis, as previously described in detail [45,46]. For the purposes of this study, we focused on the percentage of arable land and orchards in each landscape, collectively referred to as "crops", as well as the percentage of urban areas.

## 2.3. Analytical methods for trace elements determination in stored pollen

The elemental analyses of a total of 306 stored pollen samples were carried out at the PIWet-PIB, which serves as a National Reference Laboratory for toxic elements in food of animal origin and feed. Before analysis, homogenized stored pollen samples were digested using the microwave method described previously for biological samples [47,48]. Briefly, approximately 200 mg of homogenized stored pollen sample was weighed into pre-cleaned DAP-40 digestion vessels. Then, 3 mL of 67% nitric acid ( $\text{HNO}_3$ ) (NORMATOM®, VWR International, Leuven, Belgium) and 0.5 mL of 30% non-stabilized hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Suprapur®, Merck, Darmstadt, Germany) were added. After a pre-digestion step for about 30 min at room temperature, the vessels were sealed, placed in a Speedwave Four microwave digestion system (Berghof, Einingen, Germany), and digested using a previously optimized digestion program. Digested samples were cooled, transferred volumetrically into pre-cleaned 15 mL conical polypropylene (PP) tubes, made up to a final volume of 10 mL with MilliQ™ water, and stored at  $4\text{ }^{\circ}\text{C}$  until analysis. The concentrations of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, U, V, and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using a 7700x spectrometer (Agilent Technologies, Tokyo, Japan). Quantification was carried out by external calibration with five calibration levels between 0.5 and  $250\text{ }\mu\text{g L}^{-1}$ . Each standard was prepared freshly by dilution of a multi-element standard stock solution ( $10\text{ }\mu\text{g mL}^{-1}$ ) IV-ICPMS-71A (Inorganic Ventures, Christiansburg, VA, USA) with 3%  $\text{HNO}_3$  (v/v). The multi-element solution containing Bi, Li, Ho, In, Rh, Sc, Tb, and Y ( $200\text{ }\mu\text{g L}^{-1}$ ) was used as an internal standard to correct instrument drift and compensate for possible matrix effects. The accuracy of the calibration curve was verified using SRM-1643f Trace Elements in Water certified reference material (NIST, Gaithersburg, MD, USA).

The concentration of Hg in homogenized stored pollen samples was measured by atomic absorption spectroscopy with direct sample combustion and gold-amalgamation using a Tri-cell DMA-80® direct mercury analyzer (Milestone Srl, Sorisole (BG), Italy) as described in detail for animal liver samples by Durkalec et al. [49]. This method is routinely used at PIWet-PIB for food, feed, and biological matrices, requires small sample amounts, and provides suitable quantification limits. Both procedures were validated and accredited according to the PN-EN ISO/IEC 17025:2018 standard (ISO, 2018) and regularly verified in proficiency

tests organized by the European Union Reference Laboratory for Metals and Nitrogenous Compounds in Feed and Food (EURL-MN) in Kgs Lyngby, Denmark. The quality control of the measurements involved analyses of reagent blanks and the following certified reference materials: BCR-185r Bovine liver (IRMM, Geel, Belgium) and TORT-3 Lobster hepatopancreas (NRC, Ottawa, Canada). The recoveries of CRM analyses, method limits of detection (LODs) and limits of quantification (LOQs) are summarized in [Supplementary Table S1](#). All results of stored pollen analyses are expressed in  $\text{mg kg}^{-1}$  wet weight (ww).

## 2.4. Analytical methods for trace elements determination in honey bee workers

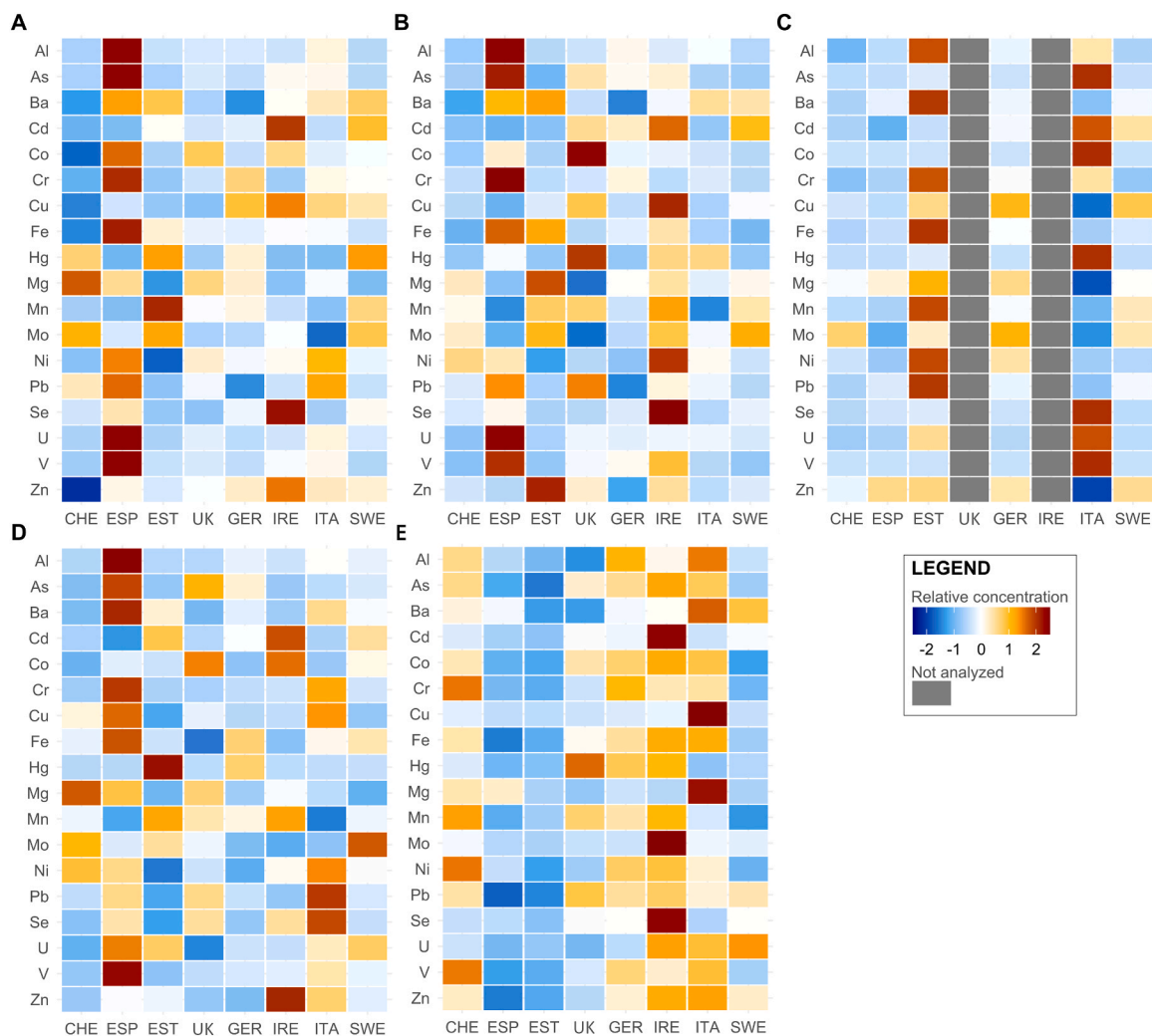
Multi-elemental analysis of honey bee workers was carried out at the Trace Elements and Nanomaterials laboratory of ANSES, which serves as the French National Reference Laboratory for trace metals in food of animal origin. The homogenized samples were analyzed using an in-house validated and accredited method (French Accreditation Body, COFRAC) with ICP-MS (7700 type from Agilent Technologies, Courtaboeuf, France) and acidic microwave digestion, as reported earlier [50]. Briefly, freeze-dried honey bee samples were homogenized using BM500 Benchtop Laboratory Ball Mill (Anton Paar, Graz, Austria). Approximately 0.5 g of sample (accurately weighed) was pre-digested first with 3 mL of 65% Suprapur®  $\text{HNO}_3$  (m/m) (Merck, Darmstadt, Germany) for 2 h. Then, 3 mL of MilliQ™ water was added and the sample was digested using a Multiwave Pro digestion system (Anton Paar, Graz, Austria). Digested samples were quantitatively transferred to calibrated 50 mL polypropylene (PP) tubes and an appropriate volume of internal standard solution (IS) containing scandium (Sc), indium (In), bismuth (Bi), ruthenium (Ru) and yttrium (Y) was added before the solution was filled up to 50 mL by MilliQ™ water to obtain the IS concentration of  $2.0\text{ }\mu\text{g L}^{-1}$  in the final solution. The samples were analyzed the same day when digested. A washing solution of  $\text{HNO}_3$  6%, (v/v) containing gold (Au) at  $10\text{ mg L}^{-1}$  was used to minimize the memory effects, mostly related to Hg. The determination of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, U, V, and Zn in digested samples of honey bees was performed using ICP-MS and the external calibration approach, a method routinely applied for the analysis of trace elements in foodstuffs. Since the chemical composition of bees may noticeably differ from the composition of food matrices, selected validation parameters of the method, including trueness, accuracy, precision, LODs, and LOQs were verified to confirm the method fitness for the purpose of bee analysis ([Supplementary Table S2](#)).

## 2.5. Trace elements in topsoil data

To characterize the geochemical background, we extracted trace element concentrations in agricultural topsoil (0–20 cm depth) from the GEMAS dataset [51,52]. For each study site, the closest GEMAS grid point was identified, and the corresponding soil values were assigned. These values were used only for comparative visualization of patterns (heat maps, [Fig. 2](#)), but not in the statistical models, to avoid spatial mismatch bias due to the  $50 \times 50\text{ km}$  grid resolution of the GEMAS survey.

## 2.6. Statistical analysis

The statistical analyses were performed using R version 4.3.2 [53] with RStudio [54]. The *dplyr* package [55] was used for data manipulation and calculation of descriptive statistics. Left-censored results occurred for selected elements whose concentrations were below the validated LOQs in part of the analyzed samples. Before the statistical analyses, the left-censored results (data below the LOQs) were replaced with plausible values using a nonparametric Kaplan–Meier technique [56] via the *multKM* command implemented in the *zCompositions* R package [57]. The Shapiro-Wilk test was employed to assess the



**Fig. 2.** Heat maps showing the relative levels of the studied trace elements in stored pollen collected by western honey bees APS (A), buff-tailed bumble bees BPS (B), red mason bees OPS (C), honey bee workers (D), and topsoil (E) across countries (CHE – Switzerland, ESP – Spain, EST – Estonia, UK – United Kingdom, GER – Germany, IRE – Ireland, ITA – Italy, and SWE – Sweden). The heat maps were built based on the median values of the elements, which were scaled using z-score normalization to allow for visual comparison. Topsoil trace element data were derived from the GEMAS database [51] available from the GEMAS Project Team (2021).

normality of data distribution [58]. As the data exhibited a non-normal distribution and log-transformation did not improve distributional properties, non-parametric and robust methods were applied.

To examine how crop type (APP vs OSR), bee species (APS, BPS, OPS), and the proportions of crop and urban areas at each sampling site related to trace element concentrations in stored pollen, we fitted robust linear-mixed effects models using the *rlmer* function from the *robustlmm* package [59]. A similar modelling approach was applied to assess the influence of crop type and the proportions of urban and crop areas on trace element concentrations in honey bee workers. Country was included as a random factor to account for substantial between-country variation observed in the data. Robust methods were used to reduce the influence of outliers and handle deviations from normality.

The models were constructed as follows: element concentration in stored pollen or honey bee workers was taken as the dependent variable, while all explanatory factors, including crop type (APP vs OSR), bee species (APS, BPS, and OPS), percentage of crops within the sampling site, and the percentage of urban areas were considered. As noted above, country was treated as a random factor within each model.

Heatmaps were used to visualize patterns in element concentrations in stored pollen across bee species, honey bee workers, and topsoil samples from different countries. Median element values were subjected

to z-score normalization to enhance visual comparison of differences between countries. The data visualization was produced using the *ggplot2* [60] and *ggpubr* [61] packages.

### 2.7. Exposure and risk assessment

Dietary exposure, defined as ingestion of trace elements from contaminated stored pollen by adult honey bees, bumble bees, and mason bees over acute and chronic time scales, was assessed. Median and 90th percentile concentrations of trace elements determined in stored pollen samples were used for calculating chronic or acute exposure, respectively. The following pollen consumption data from the EFSA guidelines were taken into account: 11.6 mg bee<sup>-1</sup> day<sup>-1</sup> for adult honey bees, 11.7 mg bee<sup>-1</sup> day<sup>-1</sup> for adult bumble bees, and 0.6 mg bee<sup>-1</sup> day<sup>-1</sup> for adult solitary bees [20]. Adult honey bee acute oral LD<sub>50</sub> (µg bee<sup>-1</sup>) values were obtained for Cr (as Cr(NO<sub>3</sub>)<sub>3</sub> [62], Cu (as copper oxychloride) [63], Mn (as MnSO<sub>4</sub>) and Zn (as ZnSO<sub>4</sub>) [64]. LD<sub>50</sub> values were extrapolated from honey bees to bumble bees and solitary bees according to the latest EFSA recommendations [20] by applying toxicity extrapolation factors (TEF) of 2.4 for bumble bees, and 171 for solitary bees, based on honey bee toxicity. Direct LD<sub>50</sub> values of Cd and Cu for bumble bees were obtained from a recent study [65]. Honey bee, bumble

bee and solitary bee chronic toxicity threshold of 10-day median lethal dietary dose (LDD<sub>50</sub>) were obtained for As and Cd individually for each type of bee [66].

Acute dietary risk was assessed as a percentage of the estimated exposure in the adult acute oral LD<sub>50</sub> (%LD<sub>50</sub>) method proposed by Zioga et al. [67] and Kiljanek [68]. Acute dietary exposure was assumed to occur when bees orally ingest stored pollen contaminated with trace elements at the 90th percentile concentration level, thus reflecting a “realistic worst-case exposure scenario” assessed separately for each element [20].

Chronic dietary risk was expressed analogously to acute risk, as a percentage of the exposure relative to the respective toxicity endpoint. Chronic dietary risk for bees was assessed as a percentage of the estimated chronic exposure in the 10-day LDD<sub>50</sub> (μg bee<sup>-1</sup> day<sup>-1</sup>). Chronic dietary exposure was assumed to occur when bees orally consumed stored pollen contaminated with trace elements at the median concentration level.

### 3. Results and discussion

#### 3.1. Species, crop, and landscape effects on trace elements in stored pollen

Most of the 18 trace elements were quantified above the LOQ in stored pollen samples. Considerable proportions of left-censored data were found only for As, Hg, and U in APS and BPS (Supplementary Tables S3-S6). The analysis of stored pollen samples revealed distinct patterns in the elemental composition across different bee species and European countries (Fig. 2). The levels of trace elements in all analyzed matrices showed marked differences among bee species. Concentrations of most elements in APS (Fig. 2A) and BPS (Fig. 2B), as well as concentrations in honey bee workers (Fig. 2D), showed similar patterns, whereas levels in OPS (Fig. 2C) were considerably different.

Our results show considerably higher trace element concentrations in OPS compared to APS and BPS across all elements, except for Mo

**Table 1**  
Summary of robust linear mixed-effects model analyses of trace element concentrations in stored pollen, with “country” included as a random effect.

Dependent variable (element of interest)	Explanatory variables			
	Crop type (APP - reference; OSR)	Species (APS - reference; BPS; OPS)	Crop (%)	Urban areas (%)
Al	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
As	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Ba	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑*	↓*	↓ <sup>ns</sup>
Cd	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑ <sup>ns</sup>	↓*	↓ <sup>ns</sup>
Co	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Cr	OSR ↓ <sup>ns</sup>	BPS ↑ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Cu	OSR ↓*	BPS ↑ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Fe	OSR ↓ <sup>ns</sup>	BPS ↑ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Hg	OSR ↓ <sup>ns</sup>	BPS ↑ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Mg	OSR ↑ <sup>ns</sup>	BPS ↓*, OPS ↑*	↑ <sup>ns</sup>	↑ <sup>ns</sup>
Mn	OSR ↑ <sup>ns</sup>	BPS ↑*, OPS ↑*	↓*	↓*
Mo	OSR ↑*	BPS ↑*, OPS ↓ <sup>ns</sup>	↑*	↑*
Ni	OSR ↓ <sup>ns</sup>	BPS ↑*, OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Pb	OSR ↓ <sup>ns</sup>	BPS ↑ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Se	OSR ↓ <sup>ns</sup>	BPS ↑ <sup>ns</sup> , OPS ↑*	↑*	↑*
U	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
V	OSR ↓ <sup>ns</sup>	BPS ↓ <sup>ns</sup> , OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>
Zn	OSR ↓ <sup>ns</sup>	BPS ↑*, OPS ↑*	↓ <sup>ns</sup>	↓ <sup>ns</sup>

Notes: The table presents associations between trace element concentrations and crop type, bee species, and the percentages of crop and urban areas. APP (apple orchards) and APS (honey bee stored pollen) were used as reference levels for crop type and bee species, respectively. Bold text and asterisks (\*) indicate significant effects ( $|t| \geq 1.96$ ), whereas “ns” indicates non-significant effects. Directional arrows (↑ positive, ↓ negative) indicate whether element concentrations increased or decreased with continuous variables, or were higher or lower than the reference categories.

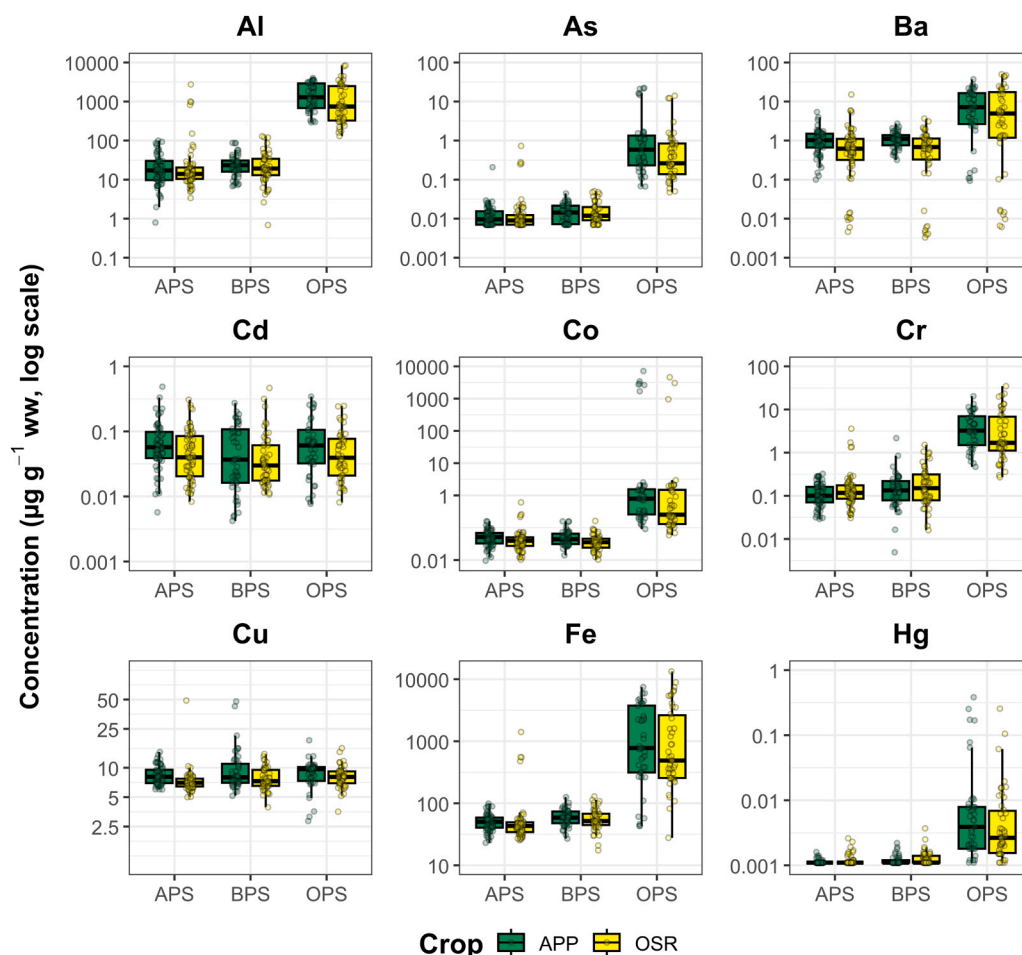
(Table 1, Figs. 3 and 4). For example, median concentrations of Al, As, Pb and V in OPS were 887, 0.398, 0.656 and 2.641 μg g<sup>-1</sup> ww, respectively, compared with 14.6, 0.009, 0.047 and 0.034 μg g<sup>-1</sup> ww in APS and 20.6, 0.013, 0.062 and 0.049 μg g<sup>-1</sup> ww in BPS. The median level of Al in OPS was over 40-fold higher compared to APS and BPS. Similar patterns were observed for As (29-fold), Cr (19-fold), Fe (12-fold), Pb (10-fold), and V (53-fold). Bumble bees, on the other hand, showed broadly similar trace element concentrations to honey bees across most elements, with only slightly higher levels of Mn, Mo and Ni and lower levels of Mg (Table 1, Figs. 3 and 4). These differences likely reflect differences in nesting ecology and foraging behavior rather than floral exposure alone. As a cavity-nesting species, the red mason bee uses mud to construct partitions separating brood chambers within the nest, which can introduce soil-borne trace elements directly into the stored pollen [69]. Although red mason bee stored pollen would be expected to reflect local soil composition more closely, given mason bee’s use of mud for nest construction and limited foraging range, the heatmaps (Fig. 2) did not indicate a consistent correspondence between stored pollen (Fig. 2C) and topsoil concentrations (Fig. 2E), potentially due to spatiotemporal mismatches in the available data. Therefore, soil contact should be considered a plausible additional exposure pathway for red mason bees, rather than a confirmed explanation for the observed differences. These findings highlight species-specific exposure pathways that should be considered when assessing environmental contamination risks for different pollinator taxa.

Beyond nesting materials, differences in foraging preferences may also contribute to the distinct exposure profile of red mason bees. Pollen analysis, based on the same sample material as used for this study, demonstrated clear species-specific foraging patterns across European agroecosystems: honey bees and bumble bees primarily collected pollen from focal crops (apple or oilseed rape), but with considerable differences in their preferences for alternative non-crop plants, whereas mason bees predominantly preferred non-crop pollen from field margins, hedgerows, and woody plants [70,71]. Non-crop vegetation may have different exposure histories and bioaccumulative properties compared to cultivated plants, potentially leading to higher trace element loads reaching red mason bees, and, to a lesser extent, bumble bees. Together with shorter foraging ranges and limited pollen mixing typical of the red mason bee, these factors may amplify local contamination effects and result in higher element concentrations.

Exposure of cavity-nesting pollinators to various substances present in the soil may result in a range of negative effects. Studies conducted at metal smelting sites have demonstrated declines in the abundance and diversity of solitary bees [72], as well as reductions in reproductive success and survival along with pollution gradients [12]. However, the levels of toxic elements, such as As, Cd, and Pb found in stored pollen in our study were much lower than those reported in the literature from different countries (Supplementary Table S9), likely because most previous studies targeted industrial, military, or urbanized areas, whereas our sampling sites were lowland agricultural landscapes without extensive industry or mining.

Robust linear mixed-effects model analysis showed that stored pollen collected from bees foraging in apple orchards (APP) tended to contain higher levels of Cu compared to stored pollen from oilseed rape fields (OSR) (Table 1). This pattern was also visible in species-specific median values, which were higher in APP than OSR for APS (8.08 vs 7.02 μg g<sup>-1</sup> ww), BPS (7.98 vs 7.28 μg g<sup>-1</sup> ww) and OPS (9.67 vs 8.05 μg g<sup>-1</sup> ww).

This pattern can be attributed to the extensive use of Cu-based fungicides to treat common diseases of apple trees, such as apple scab. It is worth noting that Cu-based fungicides are authorized for use in organic farming under certain conditions [73]. A recent survey conducted in 12 European countries showed that the use of Cu-based fungicides in apple cultivation was 16 times higher compared to their use in treating oilseed rape diseases [74]. The application of Cu-based fungicides leads to higher Cu accumulation in orchard soil and consequently in plant organs, which supports our findings. Similar to Cu, the levels of most trace



**Fig. 3.** Concentrations ( $\text{mg kg}^{-1}$ ) of Al, As, Ba, Cd, Co, Cr, Cu, Fe, and Hg in stored pollen of western honey bees (APS), buff-tailed bumble bees (BPS) and red mason bees (OPS). Colors indicate stored pollen collected from different crop types: apple orchards (APP, green) and oilseed rape fields (OSR, yellow). The horizontal line within each box represents the median, boxes indicate the interquartile range, whiskers represent 1.5 times the interquartile range, and dots represent individual observations. A  $\log_{10}$  scale was used to facilitate interpretation of the wide range of values.

elements tended to be higher in APP than OSR; however, these differences were not significant (Table 1).

An opposite pattern was observed for Mo, whose levels in APP were lower than those found in OSR (Fig. 4, Table 1). Molybdenum is essential for plant growth and metabolism and is a component of numerous Mo-dependent enzymes [75]. Given the known sensitivity of oilseed rape cultivars to Mo deficiency [76], it is likely that the slightly higher Mo concentrations in stored pollen samples from these crops are due to the use of Mo-enriched fertilizers. Such fertilizers are often applied as foliar sprays to oilseed rape, which may influence the accumulation of this element in the reproductive organs of the plant.

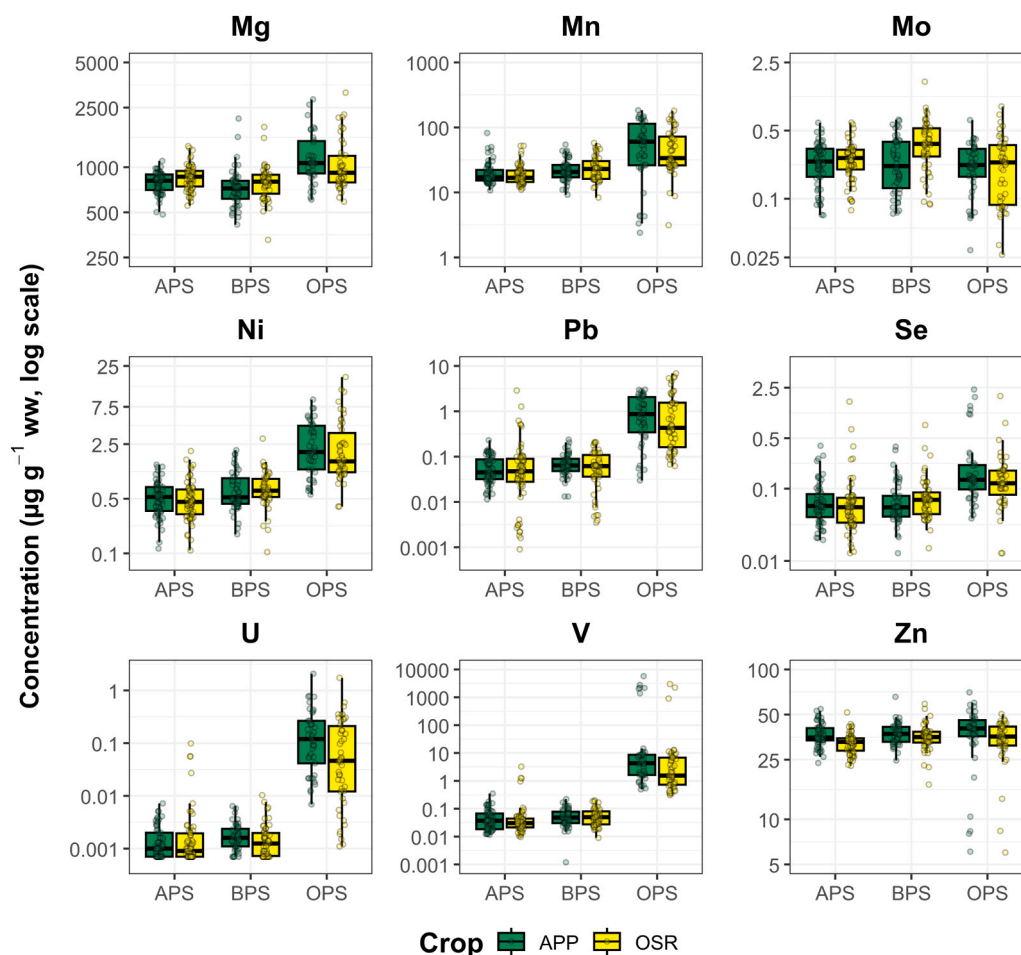
In addition to Mo, notable, but not significant, differences in Mg and Mn levels were observed between APP and OSR (Table 1, Fig. 4). These micronutrients are essential for the growth and productivity of oilseed rape and their inclusion in fertilizers is associated with improved yields. A positive relationship between these two elements was also observed in the stems and leaves of oilseed rape [77].

Our results indicate that both agricultural fields and urban areas can contribute to elevated levels of some trace elements. Robust linear mixed effect model analysis showed a significant positive relationship between the levels of Mo and Se in stored pollen and the percentage of both crop and urban areas (Table 1). Molybdenum inputs are often linked to fertilizer use [76], whereas Se can derive from agricultural practices, atmospheric deposition or natural soil enrichment [78], which may explain their association with more intensively used landscapes. However, we also identified decreasing concentrations of trace elements

in stored pollen with the proportion of arable or urban land (Table 1), such as for Cd. The bioaccumulation of Cd in the generative parts of plants depends on a number of factors, such as the specific taxon and the physicochemical parameters of the soil. A recent study by Pietrelli et al. [79] showed that despite significantly higher Cd contents in soils of urban areas compared to rural areas, the bioaccumulation of Cd in flowers of some herbaceous plants may show the opposite pattern, demonstrating the complexity of this phenomenon.

As for Cd, a negative association was found between Ba, Mn and the percentage of crops, as well as for Ba and the percentage of urban areas (Table 1). This pattern is consistent with management-driven changes in soil chemistry, as agricultural liming increases soil pH and decreases the solubility and plant availability of Mn and Ba [80,81]. In addition, Ba availability can decline through precipitation of barium sulfate (barite) in soils enriched with sulfate from fertilizers [82].

Airborne particles from natural and anthropogenic sources can contaminate pollen and bee bodies. For example, pollen collected by bees may contain mineral particles, iron oxides, polymetallic alloys [29, 83], and microplastics [84]. Traffic-related emissions are commonly associated with elements such as Al, Cu, Cr, Fe, Ni, Pb and Zn [85]. Episodic local emission sources may also affect bee exposure; Giampaoli et al. [86] reported increased elemental concentrations in honey bees after a landfill fire event, together with oxidative stress and metabolomic responses. Our study showed that Pb and Zn, which are typically associated with traffic and industrial pollution, showed a positive relationship with the percentage of urban areas, but the levels of Al, Cu, Cr,



**Fig. 4.** Concentrations ( $\mu\text{g kg}^{-1}$ ) of Mg, Mn, Mo, Ni, Pb, Se, U, V and Zn in stored pollen of western honey bees (APS), buff-tailed bumble bees (BPS), and red mason bees (OPS). A color scale was used to distinguish stored pollen collected from different crop types: apple orchards (APP, green) and oilseed rape fields (OSR, yellow). The line within the box represents the median, the box represents the interquartile range, the whiskers represent 1.5 times the interquartile range, and the dots represent individual observations. A  $\log_{10}$  scale was used to facilitate interpretation of the wide range of values.

Fe, Ni had opposite patterns. However, the relationships we observed were not significant. These contrasting patterns highlight the complexity of trace element transfer pathways in agricultural and urban landscapes and underscore the need to consider multiple exposure sources in pollinator risk assessments.

### 3.2. Crop and landscape effects on trace elements in honey bee workers

Most of the studied trace elements could be quantified in honey bee workers, with the exception of Hg, whose levels were significantly lower than the method LOQ in 98% of the tested samples; thus, this metal was excluded from the robust regression analysis. Additionally, a considerable proportion of left-censored data was observed for Ni and U (21% each), followed by Cr (4%) and Se (2%) (Supplementary Table S7). Trace elements in honey bee workers may reflect both internal accumulation and external deposition on the body surface. In a study comparing washed and unwashed bees from Polish urban and rural sites, Al, As, Cr, Co, Cu, Pb, and Zn were predominantly associated with external deposition, whereas Cd was mainly accumulated internally [87]. Because the bee workers analyzed in our study were reared outside the study sites and then exposed as adults, unwashed samples were used to retain the signal of both internal residues and external deposition from the foraging environment. This approach is consistent with Conti et al. [31], who considered whole, unwashed bees as passive samplers of atmospheric particulate matter. The levels of trace elements in honey bee workers in our study were often lower compared to those reported

by other authors (Supplementary Table S11). The mean concentration of As observed in our study ( $0.030 \mu\text{g g}^{-1} \text{ dw}$ ) was lower than that reported from different sites in Mongolia and Russia [88] and from apiaries located in rural and urban sites in central Poland [87]. The median Cd level observed in our study ( $0.247 \mu\text{g g}^{-1} \text{ dw}$ ) fell within the range reported in other studies ( $0.06\text{--}1.12 \mu\text{g g}^{-1}$ ). Mercury was mostly left-censored (97.8%) with a median concentration of  $0.002 \mu\text{g g}^{-1} \text{ dw}$ , which aligns with findings from the USA, where mean levels of this metal in honey bees from different locations ranged from  $0.0017$  to  $0.0036 \mu\text{g g}^{-1}$  [89]. Other studies have reported Hg below the LOD [90–92]. Furthermore, the median concentration of Pb in our study was  $0.054 \mu\text{g g}^{-1} \text{ dw}$ , which was lower than concentrations reported elsewhere (Supplementary Table S11).

We found that the concentrations of the majority of trace elements (As, Ba, Cd, Cu, Fe, Mn, Mo, Pb, Se, V, and Zn) were higher in apple sites (APP) compared to those from oilseed rape fields (OSR; Table 2, Fig. 5). For example, median concentrations of Cd, Cu, Fe, Mn and Zn in honey bee workers from APP sites were  $0.273$ ,  $5.60$ ,  $64.6$ ,  $55.2$  and  $42.6 \mu\text{g g}^{-1} \text{ dw}$ , respectively, compared with  $0.164$ ,  $4.86$ ,  $55.2$ ,  $45.9$  and  $36.5 \mu\text{g g}^{-1} \text{ dw}$  in workers from OSR sites. Comparing the results for stored pollen (Table 1) and honey bee workers (Table 2), we found that the accumulation patterns of As, Ba, Cd, Cu, Fe, Pb, Mn, V, and Zn in honey bee workers between APP and OSR mirrored those found in the stored pollen of honey bees, suggesting the same sources of exposure to these elements.

These similarities are also evident in the heatmap plots showing the

**Table 2**

Summary of robust linear mixed-effects model analyses of trace element concentrations in honey bee workers, with country included as a random effect.

Element	Crop type (APP - reference; OSR)	Crop (%)	Urban areas (%)
Al	OSR ↓ns	↑ns	↓ns
As	OSR ↓*	↑ns	↓ns
Ba	OSR ↓*	↑ns	↑ns
Cd	OSR ↓*	↓ns	↓ns
Co	OSR ↑ns	↑ns	↑ns
Cr	OSR ↓ns	↑ns	↑ns
Cu	OSR ↓*	↑ns	↑ns
Fe	OSR ↓*	↑ns	↑ns
Hg	-	-	-
Mg	OSR ↓ns	↑ns	↓ns
Mn	OSR ↓*	↓*	↓ns
Mo	OSR ↓*	↑ns	↑ns
Ni	OSR ↑ns	↑ns	↑ns
Pb	OSR ↓*	↑*	↑ns
Se	OSR ↓*	↑ns	↑ns
U	OSR ↓ns	↑ns	↑ns
V	OSR ↓*	↑*	↓ns
Zn	OSR ↓*	↓*	↑ns

Notes: APP, apple orchards; OSR, oilseed rape fields. APP was used as the reference level for crop type. Asterisks (\*) indicate statistically significant effects ( $|t| \geq 1.96$ ), whereas ns denotes non-significant effects. Directional arrows (↑ positive, ↓ negative) indicate the direction of the effect, i.e. whether element concentrations increased or decreased with continuous variables or were higher or lower relative to the reference category.

patterns of trace elements in honey bee stored pollen (Fig. 2A) and honey bee workers (Fig. 2D). Interestingly, Mn and Mo exhibited opposite patterns, which may indicate different sources for these two metals in stored pollen and honey bee workers.

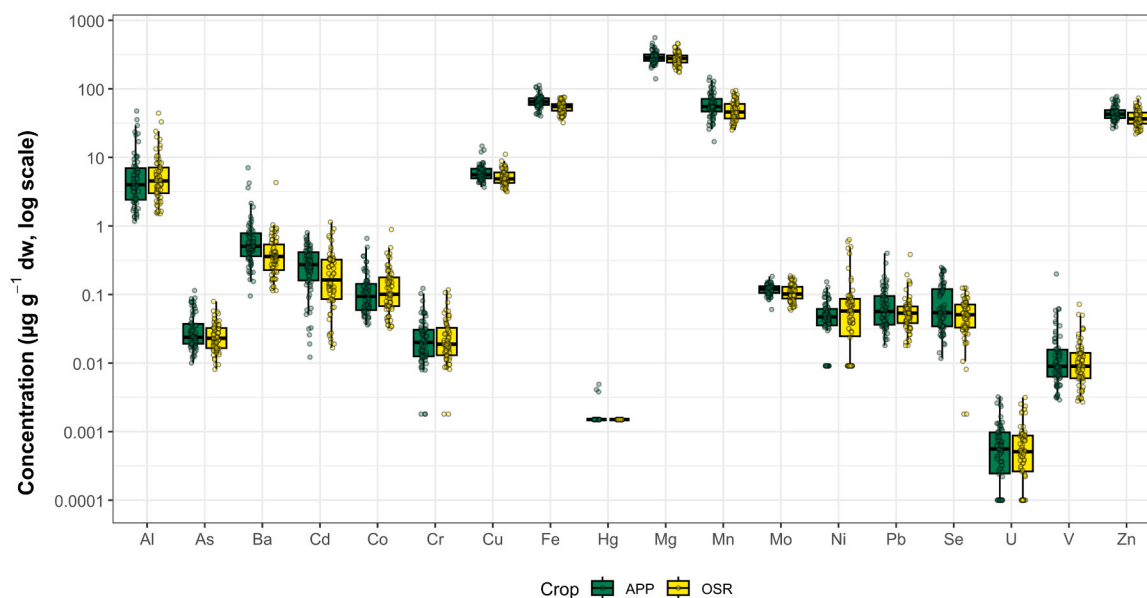
With respect to land-use effects, we identified a negative relationship between the percentage of crops and the concentrations of Mn and Zn, and a positive association between percentage of crops and the concentrations of V and Pb (Table 2). Surprisingly, Pb levels in honey bee workers and stored pollen showed an opposite relationship with the proportion of crops. This discrepancy may result from multiple, partly independent exposure pathways. Airborne particulates containing trace elements can adhere both to bee body surfaces [87,93] and to pollen

grains, either directly on flowers or during collection, transport, and storage, thereby contributing to the trace element content of stored pollen independently of plant uptake [29,83]. However, the lack of correspondence between Pb patterns in stored pollen and in bee bodies may also arise from differences in how trace elements are absorbed, processed, and excreted by the bees. Element-specific toxicokinetic and homeostatic regulation can create non-linear relationships between dietary exposure and internal residues [9,10].

Another important aspect relates to the bees that were analyzed. The honey bee workers were sampled from border frames and represented in-hive cohorts performing late-stage tasks rather than active foragers. These individuals were approximately three weeks old and had already completed the nurse phase, when pollen consumption is most intense, meaning they were unlikely to have ingested the stored pollen collected during the focal crop bloom. Therefore, the lack of a direct correlation between trace element concentrations in stored pollen and those in worker bodies is biologically plausible under our sampling design. Previous studies have also shown that Pb exposure in bees can occur through several routes, including atmospheric deposition, ingestion of contaminated nectar or water, and contact with soil or hive materials, which complicates the identification of the dominant source [94]. Future studies might therefore analyze both stored pollen and nurse or forager bees collected simultaneously during crop bloom to better understand how external contamination, dietary exposure, and internal processing interact to shape trace element profiles in honey bees.

### 3.3. Dietary exposure and risk characterization

While it is possible to assess both acute and chronic dietary exposure of bees to trace element contamination via stored pollen, it is difficult to carry out a comprehensive risk assessment due to the lack of toxicological reference values. Unlike pesticides, the toxicity of trace elements to bees is poorly understood. While it was possible to obtain toxicity values for compounds containing metal atoms in their structure by searching publicly available databases such as US EPA Ecotox [95], EFSA OpenFoodTox [96] or Pesticide Properties Data Base [97], their further detailed verification showed that they often turned out to be plant protection products for which direct exposure and risk assessments are more appropriate. Some of these substances also have very specific uses and properties that cannot be extrapolated to the general forms of



**Fig. 5.** Concentrations of trace elements in western honey bee (*Apis mellifera*) workers ( $\mu\text{g g}^{-1}$  dw) collected from sites located in apple orchards (APP, green) and oilseed rape fields (OSR, yellow). The horizontal line within each box represents the median, boxes indicate the interquartile range, whiskers represent 1.5 times the interquartile range, and dots represent individual observations. A  $\log_{10}$  scale was used to facilitate interpretation of the wide range of values.

elements found as a result of pollen analysis. Often, these were also values for substances that had been withdrawn from use as plant protection products many years ago (e.g. As or Hg compounds), and it cannot be assumed that the presence of these elements in the collected pollen is due to the historical use of such pesticides.

Acute exposure of honey bees, bumble bees and solitary bees resulting from their consumption of pollen containing high concentrations of trace elements was calculated for all analyzed trace elements. Acute risk, expressed as the percentage of estimated exposure relative to the median oral lethal dose LD<sub>50</sub> was estimated only for Cr, Cu, Mn and Zn in all bee species. For Cd, acute risk was determined only for bumble bees, for which relevant toxicity data were available. The results of the acute dietary exposure and associated risk assessment for honey bees, bumble bees and mason bees are summarized in Table 3.

Generally, acute risk related to intake of highly contaminated pollen by honey bees and bumble bees can be considered negligible. For the solitary mason bees, the risk was higher. When analyzing the results of acute risk assessment in terms of using LD<sub>50</sub> values determined directly for a given bee species, which were available for honey bees exposed to Cr, Cu, Mn and Zn, and for bumble bees exposed to Cd and Cu, it should be noted that the highest risk of 1% of the LD<sub>50</sub> value was found for honey bees exposed to Cu present in the stored pollen. Significantly higher acute risk values were estimated when LD<sub>50</sub> values interpolated from honey bees to other bee species were used. The maximum acute risk of 1.9 and 22.8% of the interpolated LD<sub>50</sub> value was estimated for bumble bees and solitary bees exposed to Mn, respectively. A significant source of uncertainty in this regard appears to be the high TEF interpolation coefficient value of 171 for solitary bees. Therefore, determining acute toxicity LD<sub>50</sub> values of trace elements directly for solitary bees would be important.

With regard to the previously indicated positive correlations between higher Cu content in pollen collected by bees from apple orchards, it should be noted that the Cu concentrations observed in our study are not expected to pose a risk to bees. According to the U.S. EPA BeeRex model, the level of concern in acute risk assessment corresponds to a Risk Quotient (RQ) of 0.4, which equals exposure of bees to 40% of LD<sub>50</sub> [98]. The level of concern defined as 40% of LD<sub>50</sub> was not exceeded in this acute dietary risk assessment.

Results of the chronic dietary exposure and risk assessment are shown in Table 4. Chronic dietary risk was assessed only for As and Cd,

for which 10-day LDD<sub>50</sub> toxicity thresholds were available. Chronic risk (%LDD<sub>50</sub>) was found to be negligible regardless of whether it was assessed for honey bees, bumble bees or red mason bees. It should be emphasized that As and Cd, together with Hg and Pb, are considered to be among the most toxic elements for living organisms. Despite this, no chronic risk was identified from exposure of honey bees, bumble bees and solitary bees to As and Cd present in pollen. The chronic risk was estimated in relation to the LDD<sub>50</sub> toxicity values determined directly for all three bee species without any interpolation. These results highlight substantial gaps in toxicological data for trace elements in non-*Apis* bees, which currently limit the robustness of environmental risk assessments for these contaminants.

#### 4. Conclusions

Our study provides a large-scale assessment of trace element accumulation in stored pollen and honey bee workers across diverse European landscapes. Differences in elemental composition between stored pollen from western honey bees, buff-tailed bumble bees, and red mason bees may reflect species-specific foraging behaviors and floral preferences, distinct nesting ecologies and potential exposure pathways. Stored pollen collected from nests located in apple orchards contained higher Cu concentrations, likely due to Cu-based fungicides in apple production, whereas Mo was higher in oilseed rape sites. Although all bee species were exposed in the same landscapes, the elemental composition of their pollen stores differed markedly, especially for red mason bees.

No substantial differences were found between stored pollen collected by honey bees and that collected by bumble bees. Solitary bees, however, provision their brood cells with pollen that is separated by mud or soil partitions, which may represent an additional route of exposure to trace elements. Although our results do not directly demonstrate soil contamination, the consistently higher concentrations observed in solitary bee pollen suggest that soil contact could be an influencing factor. The observed species-specific differences in elemental profiles also suggest that honey bees may be a limited model organism for evaluating trace element exposure in other pollinating insects, particularly solitary species with distinct nesting ecologies and foraging ranges.

This study showed that the trace element content in pollen stores can

**Table 3**

Acute dietary exposure of bees, calculated as the ingestion of stored pollen contaminated with trace elements at the 90th percentile concentration level, and the corresponding acute risk assessment expressed as a percentage of the respective acute oral LD<sub>50</sub> (%LD<sub>50</sub>). Acute oral LD<sub>50</sub> values were obtained directly for honey bees or bumble bees where available, or extrapolated (\*) from honey bees to bumble bees or red mason bees using toxicity extrapolation factors.

Element	oral LD <sub>50</sub> for honey bees (μg bee <sup>-1</sup> )	Honey bees		oral LD <sub>50</sub> for bumble bees (μg bee <sup>-1</sup> )	Bumble bees		oral LD <sub>50</sub> for red mason bees (μg bee <sup>-1</sup> )	Red mason bees	
		Acute Exposure (μg bee <sup>-1</sup> )	Acute Risk (% LD <sub>50</sub> )		Acute Exposure (μg bee <sup>-1</sup> )	Acute Risk (% LD <sub>50</sub> )		Acute Exposure (μg bee <sup>-1</sup> )	Acute Risk (% LD <sub>50</sub> )
Al	-	0.71	-	-	0.67	-	-	2.1	-
As	-	0.00026	-	-	0.00034	-	-	0.0040	-
Ba	-	0.022	-	-	0.022	-	-	0.014	-
Cd	-	0.0016	-	1.6	0.0017	0.1	-	0.00010	-
Co	-	0.0010	-	-	0.0090	-	-	0.23	-
Cr	20.5	0.0031	0.02	8.5*	0.0057	0.07	0.12*	0.0071	5.9
Cu	12.1	0.12	1.0	31.54	0.15	0.5	0.071*	0.0065	9.1
Fe	-	0.79	-	-	1.0	-	-	3.3	-
Hg	-	0.000016	-	-	0.000018	-	-	0.000022	-
Mg	-	12	-	-	12	-	-	1.1	-
Mn	61.1	0.34	0.6	25.4*	0.49	1.9	0.36*	0.082	22.8
Mo	-	0.0045	-	-	0.0069	-	-	0.00025	-
Ni	-	0.011	-	-	0.015	-	-	0.0039	-
Pb	-	0.0015	-	-	0.0017	-	-	0.0017	-
Se	-	0.0016	-	-	0.0017	-	-	0.00023	-
U	-	0.000045	-	-	0.000042	-	-	0.00026	-
V	-	0.0013	-	-	0.0014	-	-	0.22	-
Zn	125.3	0.50	0.4	52.2*	0.52	1.0	0.73*	0.029	3.9

**Table 4**

Chronic dietary exposure of bees calculated as the ingestion of stored pollen contaminated with trace elements at the median concentration level, and the corresponding chronic risk assessment expressed as a percentage of the respective oral 10-day LD<sub>50</sub> (%LDD<sub>50</sub>). Oral 10-day LD<sub>50</sub> values were obtained directly for honey bees, bumble bees and red mason bees.

Element	oral 10-day LD <sub>50</sub> for honey bees (µg bee <sup>-1</sup> )	Honey bees		oral 10-day LD <sub>50</sub> for bumble bees (µg bee <sup>-1</sup> )	Bumble bees		oral 10-day LD <sub>50</sub> for red mason bees (µg bee <sup>-1</sup> )	Red mason bees	
		Chronic Exposure (µg bee <sup>-1</sup> )	Chronic Risk (%LD <sub>50</sub> )		Chronic Exposure (µg bee <sup>-1</sup> )	Chronic Risk (%LD <sub>50</sub> )		Chronic Exposure (µg bee <sup>-1</sup> )	Chronic Risk (%LD <sub>50</sub> )
Al	-	0.17	-	-	0.24	-	-	0.53	-
As	2820	0.00010	0.000004	15400	0.00015	0.000001	1240	0.00023	0.00002
Ba	-	0.0092	-	-	0.011	-	-	0.0034	-
Cd	1030	0.00058	0.0001	13600	0.00039	0.000003	1000	0.000027	0.000002
Co	-	0.00050	-	-	0.00044	-	-	0.00029	-
Cr	-	0.0013	-	-	0.0017	-	-	0.0016	-
Cu	-	0.086	-	-	0.088	-	-	0.0052	-
Fe	-	0.53	-	-	0.63	-	-	0.41	-
Hg	-	0.000013	-	-	0.000013	-	-	0.0000019	-
Mg	-	9.6	-	-	8.8	-	-	0.55	-
Mn	-	0.20	-	-	0.24	-	-	0.027	-
Mo	-	0.0029	-	-	0.0037	-	-	0.00013	-
Ni	-	0.0056	-	-	0.0071	-	-	0.0011	-
Pb	-	0.00055	-	-	0.00073	-	-	0.00039	-
Se	-	0.00065	-	-	0.00070	-	-	0.000078	-
U	-	0.000012	-	-	0.000016	-	-	0.000054	-
V	-	0.00039	-	-	0.00058	-	-	0.0016	-
Zn	-	0.40	-	-	0.42	-	-	0.023	-

vary depending on the dominant crop surrounding their nesting sites. The example of Cu and Mo suggests that plant protection and crop fertilization practices can influence concentrations of these metals in stored pollen.

Given the current state of knowledge on the toxicity thresholds of trace metals or metalloids to bees, the dietary risk associated with the ingestion of stored pollen is considered low or negligible for elements with available toxicity endpoints. However, substantial gaps remain in our understanding of the toxicity of metals to pollinating insects, which limits the capacity for comprehensive risk assessments. Further studies should focus on establishing species-specific toxicity endpoints, particularly for solitary bees, and on evaluating sublethal effects and interactions among elements under environmentally realistic exposure scenarios.

### Environmental implications

This study shows that trace elements in agricultural landscapes are transferred from plants to pollinators under realistic field conditions, with exposure shaped by species-specific ecology and farming practices. Higher concentrations in pollen provisions of solitary bees compared with social bees indicate that honey bee-based exposure assessments may underestimate risks for other pollinators. Crop-specific management, such as copper fungicide use in orchards, further influences exposure patterns. Although estimated dietary risks were low, the lack of toxicological endpoints for many trace elements limits hazard characterization, highlighting the need to improve risk assessment frameworks for terrestrial non-target organisms.

### CRedit authorship contribution statement

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### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT to improve the grammar and readability of the text. After using this tool, the author(s) reviewed and edited the content as needed and take full

responsibility for the content of the published article.

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## 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.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2026.142644](https://doi.org/10.1016/j.jhazmat.2026.142644).

## Data availability

Data will be made available on request.

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