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RESEARCH ARTICLE



Seasonal variability of potentially toxic and mineral elements in bee pollen collected from various Swiss agricultural landscapes

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ABSTRACT

Potentially toxic elements measured in bee pollen collected in Switzerland in 2024 from 16 apiaries in various agricultural settings were linked to the corresponding landscapes. Multielement analysis showed high temporal variability. Elements ranged in mg/kg: manganese (8–516), aluminium (8–214), iron (21–164), boron (8–110), zinc (24–80), copper (4–26) and molybdenum (0.1–3.5), and in µg/kg: cadmium (34–774), selenium (34–713), lead (34–284), chromium (166–370), cobalt (17–280) and arsenic (9–56). In spring, boron levels correlated with oilseed rape land use, ranging up to 130 mg/kg in fractionated pollen loads. Notably, clover pollen loads from an apiary near vineyards recorded Cu levels up to 52 mg/kg in June when Cu-containing products were used.

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Honey bees; pollen; elements; ICP-MS; ICP-OES; agrochemicals

1. Introduction

Monitoring environmental contamination is crucial for safeguarding human health, agriculture and ecosystems. The use of honey bees and their products has gained considerable attention in recent years due to their efficiency and cost-effectiveness [1]. Several studies on bee products have reported contaminants, such as pesticides [2,3], persistent organic pollutants, such as polychlorinated biphenyls [4], and potentially toxic elements (PTEs) [5–7].

When plants are widely available as food sources for pollinators, honey bees typically forage within a radius of up to 2 km around the apiaries, making them good indicators for a broader area (approximately 15 km²) [8]. During their foraging activities, they come into contact with vegetation, soil, air and water and can therefore be exposed to various contaminants, including PTEs. The determination of PTE levels in pollen is essential for risk assessment to bees [9]. Several studies investigated PTEs in pollen from Mediterranean climate zones [10,11], but studies on PTE levels in pollen collected in the central European region with different flora are sparse. Popular foraging plants for honey bees include fruit trees (*Prunus* sp. & *Pyrus* sp.) and crops, such as oilseed rape (OSR; *Brassica napus*), maize (*Zea mays*), sunflower (*Helianthus* sp.), as well as wild plants, such as dandelion (*Taraxacum officinale*), clover (*Trifolium* spp.), wild berries (*Rubus* sp.) and also trees (e.g. *Acer* sp. or *Salix* sp.) [12]. A honey bee usually collects pollen from the same plant species until the pollen load is complete [13].

PTE contamination can result from anthropogenic activities, such as agriculture, vehicular traffic and industrial processes, but it can also originate from natural sources, such as soil erosion, rock weathering and windblown dust [14]. In agriculture, PTEs may originate from plant protection products, such as those containing copper (Cu) and aluminium (Al) [15–17]. Alternatively, some plant protection products may contain contaminants, such as arsenic (As), chromium (Cr) and lead (Pb) [18,19]. Fertilisers and manure have been reported as further sources of PTEs, such as cadmium (Cd) in phosphate fertilisers [20] or Cu in manure [21]. In soils, PTEs can persist for decades [22] or even for centuries [23]. The uptake and internal translocation of these elements within plants vary substantially depending on plant species, bioavailability of PTE types and soil properties [9,24–26].

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Crops require mineral elements, such as phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), in comparatively high quantities for healthy growth and a good yield. Mineral elements, such as boron (B), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu), are applied in significantly lower quantities. PTEs, such as Al, As, Cd, Cr, Pb and nickel (Ni), may negatively affect plant health even at low concentrations. For honey bees, some elements act as beneficial macro- or micronutrients, but depending on their levels in pollen, they can be toxic, some even at low concentrations. For example, high Mn exposure may lead to a precocious switch from nurse to foragers and impair the bees' sense of direction, their learning, and memory abilities, as reviewed by Gekière et al. [9]. In addition, high Cu levels had negative consequences on larval development of honey bees, leading to a reduction in the weight of pre-pupae and pupae, and lowering the survival rate of both larvae and foragers [27]. Thus, in this study, not only As, Cd, Cr, Pb and Ni, but also the elements Al, B, Co, Cu, Fe, Mn and Zn were classified as potentially toxic to honey bees and were included hereafter in term PTEs.

Among the various bee products, bee-collected pollen is a particularly good environmental indicator, since its concentrations of PTEs are often above the levels measured in other bee products [28,29]. Furthermore, pollen collected at the hive entrance reflects contamination outside of the hive, while honey and wax can be exposed to in-hive contaminants related to products used for disease treatment of honey bees. Furthermore, the separation of pollen loads into various colour fractions allows the determination of the botanical origin of the pollen types and can be combined with residue analysis to identify the source of contamination. For honey bees, pollen is the protein, lipid and mineral source [30], but also the main exposure route for PTEs [5,29]. Newly emerged honey bees consume pollen to strengthen their flight muscles, while nurse bees consume pollen for the production of larval jelly.

Our aim was to study PTE exposure of honey bees foraging in a variety of agricultural landscapes. We measured the levels and seasonal variations of elements, which include mineral and potentially toxic elements, in pollen from 16 beekeeping locations with agricultural crop shares in the surroundings of the apiaries. Where possible, element levels were correlated with the share of agricultural crops around the apiaries and/or plant source. Finally, the determination of Cu, Cd and Cr levels in pollen allowed an estimation of the risk to honey bees.

2. Materials and methods

2.1. Chemicals, consumables and instruments

A list of chemicals, reagents and apparatus used in this study can be found in the supplementary material (SM; Tables S1 and S2).

2.2. Study sites and honey bee colonies

Beekeepers participated in this study. Their locations were chosen to represent a variety of agricultural environments while including sites without major agricultural activities. A total of 15 apiaries in various regions of Switzerland and an additional location in Liechtenstein were investigated, namely Bellechasse (BC), Bünzen (BU), Claro (CL), Fricktal (FR), Fully (FU), Gams (GA), Grandvaux (GR), Regensdorf (RG), Rengg (RE), Schaan (SA), Schenkon (SE), Schlosswil (SO), Thunstetten (TH), Walliswil (WL), Wangen b. Olten (WA) and Züberwangen (ZU). Among those, 14 apiaries were located north of the Alps, one apiary in the central (FU) and one in the southern flank of the Alps (CL). To protect the beekeeper's business interests, only the approximate locations of the studied apiaries are illustrated in the Supporting material Figure S1. QGIS (Version 3.34.4), a geographic information system, was used with the precise coordinates of the apiaries to analyse the cultivated crops in the surroundings of the studied apiaries. A large number of farmers participate in a controlled government programme for which they receive payments, but are obliged to document crops planted on each agricultural plot [31]. The land use data is provided by the relevant cantonal authorities and detailed information—including the areas of individual arable crops in the vicinity of the beehives—can be downloaded from Geodienste (www.geodienste.ch).

2.3. Pollen sampling

Bee pollen was collected by the participating beekeepers from four colonies of each apiary from the end of March to the end of August 2024. Pollen traps (manufactured in a local workshop) were installed at the entrance of the beehives. The grid ($\varnothing$ 5 mm) was large enough to let honey bees pass through while retaining the pollen pellets that bees carry on their hind legs (*corbiculae*). Pollen was collected on a single day, approximately every second week. To do this, the pollen traps were closed in the evening, and the pollen was collected approximately 24 h later from the drawer of the trap. The collected pollen was stored frozen at -20°C .

2.4. Sample preparation for elemental analysis

Honey bee colonies collect different amounts of pollen, depending on the season and pollen sources available. For each apiary, the same proportion of pollen (if possible, 5 g of pollen from each of the four colonies) collected on the same day was combined and homogenised. At certain sampling points, especially in summer, when pollen from fewer plants was available, only three honey bee colonies collected pollen in WA (cw 32), FR (cw 17), SA (cw 25, 28, 35), GR (cw 35) and RG (cw 28, 34), and only two colonies collected pollen in SA during cw 35 and in RG during cw 34, respectively. In GA, only three honey bee colonies collected pollen throughout the sampling season. A petri dish and an empty 50 mL Falcon tube were used as a pestle to grind the pollen by hand, resulting in 174 pooled samples. Next, the pollen was dried in a lyophiliser (Christ alpha 1–4, Kühner AG, Birsfelden, Switzerland) for 15 h at -50°C and 0.5 mbar and stored at -20°C until further use.

2.5. Microwave-assisted acid digestion of the samples

For extraction, 0.3 g of each pollen sample was weighed into a 15 mL quartz tube (purchased from a local glass manufacturer) and placed into a rack, before pipetting 30 μL of internal standard containing germanium (Ge; 60 $\mu\text{g/L}$), rhenium (Re; 6 $\mu\text{g/L}$) and rhodium (Rh; 6 $\mu\text{g/L}$) into each glass tube. Next, 8 mL of a mixture, containing 2.5 mL of nitric acid (HNO_3 , 69%) and 5.5 mL of H_2O MilliQ was added using a dispenser. Caps were then placed on each tube. A container was filled with 150 mL of H_2O MilliQ and 8 mL of a solution (5 mL of HNO_3 (69%) and 3 mL of H_2O MilliQ), matching the sample solution composition as closely as possible. Next, the rack containing the samples was placed in this container for microwave digestion. The temperature programme of the digestion unit was set to 235°C and 140 bar for 35 min and then held for 20 min at 235°C and 140 bar. The microwave settings for extraction are provided in SM Table S3. After cooling down the samples to room temperature, the liquid was transferred from the quartz tubes to 50 mL plastic tubes, which were filled up to 50 mL with MilliQ water and subsequently gently shaken by hand. For analysis, the samples were transferred into 15-mL tubes.

2.6. Elemental analysis by ICP-MS

Elemental analysis of Al, As, B, Cr, Mo, Ni, Co, Cd, Pb and Se was performed using a triple quadrupole inductively coupled plasma tandem mass spectrometer (iCAP TQ ICP-MS; ICP-MS, Thermo Fisher Scientific). The following isotopes were analysed in the kinetic energy discrimination (KED) mode: ^{27}Al , ^{11}B , ^{111}Cd , ^{59}Co , ^{98}Mo , ^{60}Ni , ^{206}Pb , ^{207}Pb , ^{208}Pb , ^{77}Se . By contrast, ^{75}As and ^{52}Cr were analysed using the O_2 -mode. ICP-MS operating parameters are provided in SM Tables S4–S6. Elements were quantified using external calibration. Element levels are listed in SM Table S7. As internal standards, ^{74}Ge was used for Al, As, B, Co, Cr, Mo, Ni and Se, ^{185}Re for Pb, and ^{103}Rh for Cd. The values were adjusted according to the levels of the internal standards.

2.7. Elemental analysis by ICP-OES

The elemental analysis of Ca, Cu, Fe, K, Mg, Mn, P, and Zn was carried out using ICP-OES. Operating parameters for the instrument are summarised in SM Tables S8–S10. The selected wavelengths (in nm) were Ca (315.887), Cu (327.395), Fe (238.204), K (766.490), Mg (279.078), Mn (257.610), P (213.618) and Zn

(206.200). A concentration level of 25 mg/L yttrium (Y) was used to assure instrument stability. The elements were quantified using external calibration. Element levels are listed in SM Table S11. The limits of quantification (LOQ) of all tested elements are provided in SM Table S12.

2.8. Certified reference material and fortification of a commercial pollen sample

ERM-CD-281 (rye grass) was included as a reference. As no certified pollen reference material was available, commercial pollen (Bienen Roth GmbH, Wila, Switzerland) was also included. The commercial pollen was fortified at four different levels (0.1, 5.0, 25 and 100 mg/kg) to cover various concentrations of elements. Recoveries of the fortified pollen were calculated for fortification levels exceeding the background concentrations of the commercial pollen. Prior to calculating recoveries, the background concentrations were subtracted from the measured values. In each extraction round, acid blanks, ERM-CD-281, commercial pollen samples, with and without fortification, were included, as well as a triplicate of one pollen sample. Recoveries of ERM-CD-281 are reported in SM Table S13. Element levels in pollen (Roth GmbH) used for spiking, and recoveries at various fortification levels are reported in SM Tables S14 and S15.

2.9. Determination of the botanical origin of the pollen

Some pollen samples collected at study sites with high B, Cu, or Mn levels were chosen for further analysis to determine the botanical origin of the pollen types, together with the element levels. Generally, samples were chosen from a single colony for which there was a sufficient amount of pollen for separation (6 g of pollen, for microscopic analysis, non-dried pollen was used). The pollen pellets were separated by hand into various fractions, according to their colour and shape of the pollen loads, as previously described [32,33]. To determine the botanical origin of each fraction, small parts of the pellets from each fraction were placed on microscope slides. The pollen was then mixed with liquid glycerol gelatine previously heated at 40 °C and covered with a cover slip. The slides were dried overnight at room temperature and subsequently stored at –20 °C until microscopic analysis to determine the botanical origin of the pollen types [34]. Additionally, the element levels in the pollen pellets were determined for each fraction as well as for the sample before separation using the analytical procedures described previously.

2.10. Calculation of the toxic exposure ratio for Cd, Cr and Cu

The toxicity exposure ratios were calculated for the PTEs, for which the lethal dose required to kill half of the tested population (LD_{50}) was available [35–38]. The potential risk of Cd, Cr and Cu for adult honey bees was calculated following the guidance document of the European Food Safety Authority [39]. The maximal daily PTE uptake per bee was calculated based on the measured maximal PTE concentrations in pollen, assuming a daily pollen consumption of 12 mg. The LD_{50} was subsequently divided by the maximal daily uptake of the PTE to obtain the toxicity exposure ratio (TER). The acute toxicity risk for honey bees is considered high at TER values of 1 or below, whereas levels above 10 are considered low risk (factor of 10 for an estimation of the chronic toxicity).

2.11. Data evaluation

Rstudio (Version 4.2.3) was used to visualise the results and create the heatmaps. Pearson correlation was calculated for B levels versus OSR shares and to compare P with K levels.

3. Results

3.1. Cultivated crops around the apiaries

The studied apiaries were located in landscapes characterised by a diverse mix of land uses, including cultivations of OSR, vegetables, grains, maize, sunflower, vineyards, berries, fruit trees and pasture. The proportions of these land-use types surrounding each site are shown in Table 1. All heatmaps and Table 1

Table 1. Agricultural crops within a 2-km radius of the studied apiaries.

	OSR	Veg.	Grains	Maize	Sunfl.	Berries	Fruits	Viney.	Pasture [†]	Other [‡]	Miscellaneous share [§]
Location	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
TH	3.5	4.5	15.8	5.9	1.1	0.01	1.9	0	33.5	0.3	33.6
BC	3.2	30.4	19.0	12.5	1.0	0.03	0.2	0	14.0	0.6	19.1
BU	2.7	4.9	12.6	8.5	0	0.05	0.2	0	17.6	0.9	52.6
WL	2.3	2.1	12.6	6.9	0.3	0.001	0.8	0	19.1	0.3	55.6
SE	2.0	1.6	23.5	10.9	0.1	0.21	0.2	0.2	35.8	0.4	25.1
FR	2.0	0.2	12.2	4.1	0.2	0	0.9	0.5	37.5	0.3	42.1
GR	1.1	0.02	6.7	1.4	0	0.003	0.02	20.7	14.2	0.1	55.8
ZU	0.9	0.4	3.3	4.1	0	0	0.3	0	53.1	0.1	38.0
RG	0.6	3.9	6.9	2.8	0.4	0.04	0.3	0.3	16.4	0.2	68.0
WA	0.6	0.7	1.9	2.3	0	0	0.9	0.01	20.5	0.1	72.9
SO	0.6	2.3	13.5	6.7	0.9	0	3.0	0	45.9	0.3	26.9
GA	0.4	8.2	3.5	8.0	0.2	0.33	0	0	45.5	1.1	32.9
RE	0.04	0.1	0.8	0.6	0	0	0	0	71.0	0	27.4
FU	0	1.5	0.1	0.4	0	1.04	15.6	11.6	8.1	0.009	61.6
CL	0	0.9	0.5	0.3	0	0	0	0.3	17.6	0.1	80.5

Abbreviations: OSR, oilseed rape; Veg., vegetables; Sunfl., sunflower; Viney., vineyards.

[†]Pasture: includes pastures, meadows, other permanent green areas and artificial lawns.

[‡]Other: includes areas such as flower strips, domestic gardens or wildflower meadow.

[§]Miscellaneous share includes forest, water bodies, settlement and industrial areas.

For study site SA, no data are available.

were vertically ordered according to OSR levels close to the apiaries, with the highest share on the top and the lowest or no OSR cultivation at the bottom. OSR was cultivated at all sites except CL and FU, with the highest proportions recorded at TH (3.5%), BC (3.2%), BU (2.7%), WL (2.3%), SE (2.0%) and FR (2.0%). Vegetables, grains and maize were present at all locations to various degrees. BC was dominated by vegetables (30.4%) and grains (19.0%). Grains were also prominent in SE (23.5%), TH (15.8%) and SO (13.5%). Notable shares of vegetables were also observed in GA (8.2%), BU (4.9%), TH (4.5%) and RG (3.9%). Fruit trees were most abundant in FU (15.6%), followed by SO (3.0%) and TH (1.9%). Vineyards were most abundant in GR (20.7%), FU (11.6%), FR (0.5%) and CL (0.3%). Pasture and meadow areas accounted for more than 30% of the surrounding landscape in RE, ZU, SO, GA, FR, SE and TH with the highest amounts in RE (71.0%) and ZU (53.1%).

3.2. Prevalences and maximal levels of elements in pollen

The prevalence of elements in pollen and their concentration ranges are shown in Table 2. All of the 18 elements tested were quantified (>LOQ) at least in one of the pooled pollen samples. Among these, 10 of the elements (Ca, Cu, Fe, K, Mg, Mn, P, Zn, B and Mo) were quantified in all samples (100%, $n = 174$). Four elements were present in more than 90% of all samples, namely Ni (99%, $n = 172$), Al (94%, $n = 164$), Co (93%, $n = 162$) and Pb (91%, $n = 158$). Cd and Se were present at prevalence levels of 57% ($n = 99$) and 55% ($n = 95$), respectively. Lower prevalences were observed for As and Cr, with prevalences of 31% ($n = 54$) and 11% ($n = 20$), respectively. The maximal element levels in at least one of the analysed pooled pollen samples

Table 2. Prevalences and concentration ranges of elements in pollen.

Element	Pre. ^a [%]	<i>n</i> ^b > LOQ	Conc. ranges of pos. samples ^c	Unit
Ca	100	174	0.6–2.8	g/kg
Cu	100	174	4–26	mg/kg
Fe	100	174	21–164	mg/kg
K	100	174	3.3–9.8	g/kg
Mg	100	174	0.4–2.9	g/kg
Mn	100	174	8–516	mg/kg
P	100	174	2.5–6.9	g/kg
Zn	100	174	24–80	mg/kg
B	100	174	8–110	mg/kg
Mo	100	174	0.1–3.5	mg/kg
Ni	99	172	0.2–2.8	mg/kg
Al	94	164	8–214	mg/kg
Co	93	162	17–280	µg/kg
Pb	91	158	34–284	µg/kg
Cd	57	99	34–774	µg/kg
Se	55	95	34–713	µg/kg
As	31	54	9–56	µg/kg
Cr	11	20	166–370	µg/kg

^aThe prevalences of elements in pollen [%] are calculated based on a total number of 174 pooled samples from the 16 apiaries. Elements are ordered accordingly.

^bNumber of samples above the limit of quantification (LOQ).

^cConcentration ranges of positive samples (>LOQ).

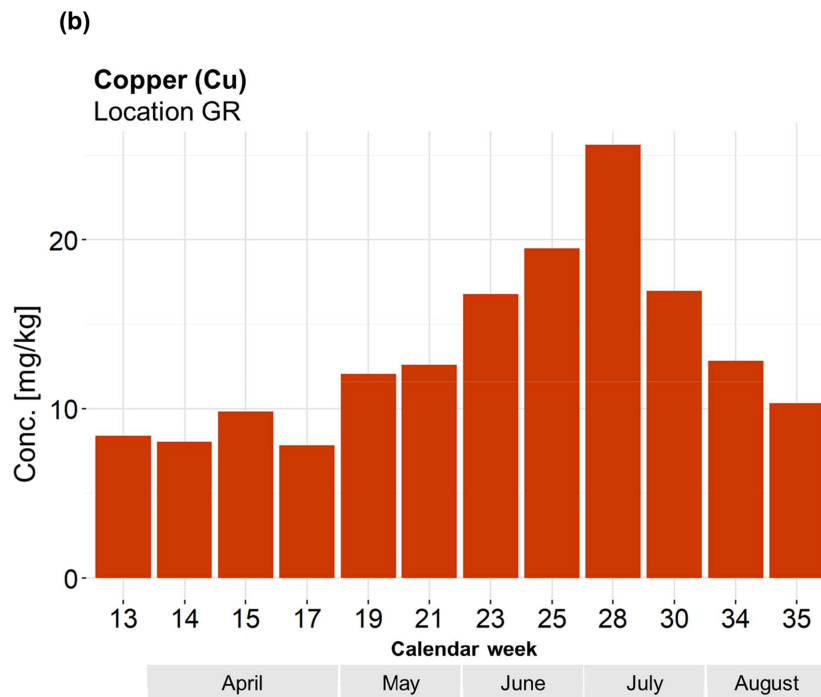
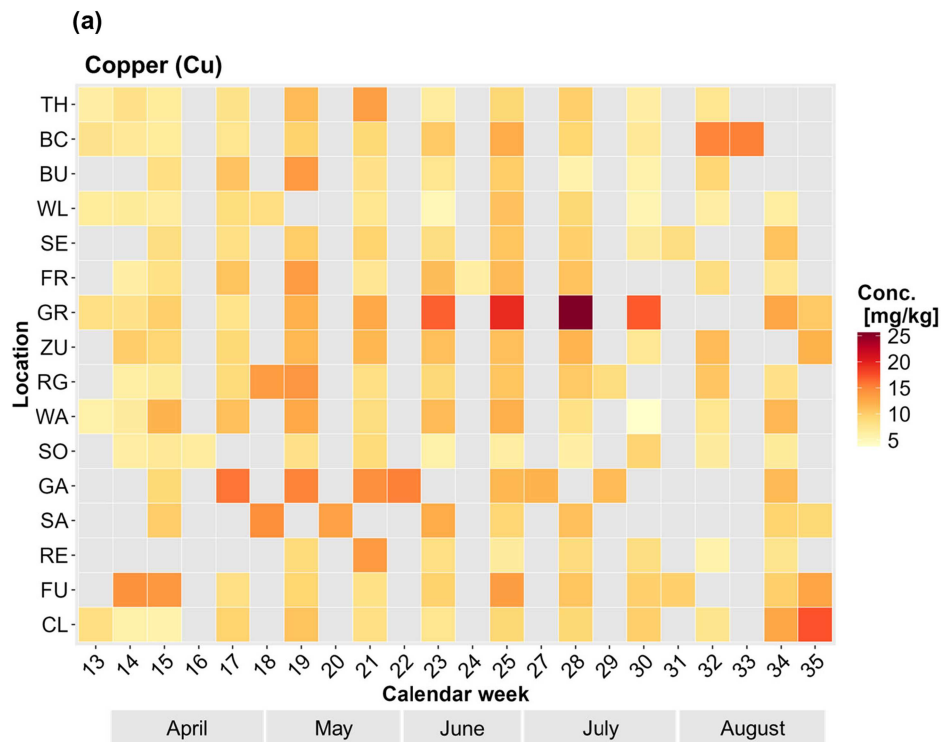
were found for mineral elements K (9.8 g/kg), P (6.9 g/kg), Mg (2.9 g/kg) and Ca (2.8 g/kg) (Table 2). The levels of other elements showed a decreasing order of Mn (516 mg/kg) > Al (214 mg/kg) > Fe (164 mg/kg) > B (110 mg/kg) > Zn (80 mg/kg) > Cu (26 mg/kg) > Mo (3.5 mg/kg) > Ni (2.8 mg/kg) > Cd (774 µg/kg) > Se (713 µg/kg) > Cr (370 µg/kg) > Pb (284 µg/kg) > Co (280 µg/kg) and As (56 µg/kg).

3.3. Temporal variations in Cu levels in pollen

Cu-containing products are authorised as fungicides or bactericides for the protection of orchards and vineyards, as well as vegetables [40]. In our study, the treatment period was known for GR, but not for the other study sites. Seasonal changes in Cu concentrations in pollen collected at the study sites are shown in Figure 1a. In early spring (calendar week [cw] 15), Cu levels in pollen were below 10 mg/kg for most study sites, except for FU and WA (14 mg/kg and 12 mg/kg, respectively). At those locations, the beehives were near orchards (15.6% and 0.9% of land use, respectively; Table 1), which might be treated during their blooming period in early April. In cw 19, Cu levels of up to 15 mg/kg were measured in pollen, for example, from BU, FR, RG and GA, possibly related to treatments of various crop types. A maximal Cu level of 26 mg/kg was recorded in late June/early July (cw 28) in pollen from GR. At this site, 20.7% of the land use around the apiary are vineyards (Table 1), which were treated for downy mildew with two Cu-based products, 'Flowbrix' (cw 22–30; rate: 0.6–0.8 L/ha) and 'Bordeaux mixture' (cw 21 and 32; rate: 1.0 and 1.5 kg/ha, respectively), resulting in the application of Cu of 0.2 kg/ha or 0.3 kg/ha per week (Figure 1b). Thus, the observed increase in Cu levels in pollen coincided with the application of copper-based products. By August, Cu levels in pollen had returned to approximately 10 mg/kg (cw 35).

3.4. Temporal variations in B, Mn and Mo levels in pollen

Products containing B, Mn, or Mo are common fertilisers for various agricultural crops, B, for example, for OSR and Mn and B for sugar beets [41]. Seasonal variations of these elements in pollen collected from the study sites are visualised in Figure 2. Pronounced early-season peaks of B between the end of March and May were observed in pollen collected from all sites (except CL; no data for RE), followed by a decline from mid-May onward until August. Levels exceeding 100 mg/kg (up to 110 mg/kg; Table 2) were measured in pollen collected in April at TH, BU, SE and FR (SO in May). These are sites with 2%–3.5% OSR cultivations in the surroundings of the apiaries (Table 1). Seasonal B levels remained low in pollen from sites with lower agricultural activities (RE and CL). We noted a tendency towards higher B levels at study sites with OSR: for



	Calendar week								
	21	22	23	25	26	28	29	30	32
Cu [kg/ha]	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Figure 1. (a) Temporal variations of Cu concentration levels in pollen across our study sites. The heatmap shows the Cu concentrations in pollen collected from March to August 2024 at the 16 apiaries. Dark red coloured cells indicate high concentration levels, while light coloured cells indicate lower concentration levels. Grey cells denote missing or

(Caption on next page)

unanalysed samples. The colour scale indicates Cu concentrations in mg/kg. (b) Temporal variations of Cu concentrations (mg/kg) in pollen from GR. Products containing Cu (Flowbrix and Bordeaux Mixture) were applied in GR between calendar weeks 21–32, 2024.

example, a positive correlation of $r = 0.78$ was calculated for B levels in pollen collected in cw 14 and the percentage of OSR around the apiaries (Figure S2).

Mn levels remained generally low over the entire season (Figure 2), with isolated peaks in late June (cw 25) in pollen from TH (265 mg/kg), BC (315 mg/kg) and BU (329 mg/kg) and lower levels in pollen from WL (195 mg/kg), FR (126 mg/kg) and ZU (98 mg/kg). Sugar beets were cultivated near the apiaries of TH, BC, BU, WL and ZU according to the land use data (part of the vegetable shares listed in Table 1). A maximal level of 516 mg/kg was measured in pollen from RE in July (cw 28), a location without sugar beet cultivations, suggesting alternative sources. Elevated levels of Mo were observed between the end of June and August in pollen from ZU, RG, GA and SA, reaching the highest level of 3.5 mg/kg in July (cw 28) at ZU.

3.5. Temporal variations of additional elements in pollen

Mineral elements are essential for agricultural crops. In our study, pollen contained Ca, K, Mg and P in grams per kilogram range, whereas Fe, Zn and Al showed the order of 10–200 mg/kg, and Se levels were up to 713 µg/kg, as listed in Table 2. P and K exhibited a heterogeneous distribution throughout the season, with peak levels of 6.9 g/kg for P in pollen from BU (cw 32) and RG (cw 19) and a peak level of K of 9.8 g/kg in pollen from BU (cw 32), as shown in Table 2 and Figure S3. We observed a positive correlation ($r = 0.73$) between P and K in pollen collected in springtime (cw 13–22) (Figure S4). Ca levels in pollen also displayed a heterogeneous pattern, with peak concentrations observed in cw 25 at WL, FR and SA, ranging between 0.6 and 2.8 g/kg (Table 2 and Figure S3), while Mg levels showed a seasonal trend, with levels in pollen from multiple sites increasing between May and June (cw 18–23). During this period, Mg reached up to 2 g/kg at RG, BU, FR and ZU. The highest level was observed in cw 32 in pollen from BU, with 2.9 g/kg (Table 2).

Site-specific variations in Fe, Zn, Al and Se levels were observed, with peak levels of Fe and Al in pollen from FU (164 mg/kg and 214 mg/kg, respectively; cw 30), and Zn in pollen from CL (80 mg/kg; cw 28), and Se in pollen from GA (713 µg/kg; cw 17) (Table 2, Figures S3 and S5). Similarly, site-specific variations were noted for Ni, Co, Pb, Cd, As and Cr, with peak levels of Ni in pollen from FR (2.8 mg/kg; cw 17), Co in pollen from CL (280 µg/kg; cw 28), Pb in pollen from BU (284 µg/kg; cw 30), Cd in pollen from CL (774 µg/kg; cw 35), As in pollen from SA (56 µg/kg; cw 25) and FU (51 µg/kg; cw 30) and Cr in pollen from FU (370 µg/kg; cw 30) listed in Table 2 (Figures S3 and S5).

3.6. High B levels in OSR pollen

To confirm the botanical origin of the pollen types containing B, pollen samples (single colonies) were analysed from two study sites with high B levels (TH [cw 14] and FR [cw 15]; Figure 2) and an additional sample with lower B levels (FR [cw 19]; Figure 2). Pollen loads of these samples were separated into various colour fractions, which allowed us to determine the botanical origin of the various pollen types within the pollen samples. The samples from FR at cw 15 and cw 19 contained 59% and 22% OSR (*B. napus*) pollen, respectively (Figure 3a, c), and the sample from TH contained 31% OSR (Figure 3b). Element analysis was performed on the samples prior to separation, as well as on the colour fractions. Before fractionation, B levels in the three pollen samples were 84 mg/kg (sample corresponding to Figure 3a), 71 mg/kg (Figure 3b) and 29 mg/kg (Figure 3c) and are included in Table S16. Pure OSR pollen fractions contained B levels of 110 mg/kg, 130 mg/kg and 84 mg/kg, respectively (Figure 3). Levels in OSR pollen were higher than in the other pollen types of the same sample. Additional pollen types included cherry (*Prunus avium*; Figure 3a, b) and apple/pear (*Malus/Pyrus*; Figure 3b), as well as dandelion (Asteraceae T; Figure 3a, b), willow (*Salix* sp.; Figure 3b), maple (*Acer* sp.; Figure 3c) and pollen types belonging to the family of the rose family (Rosaceae; Figure 3c). In pollen samples from FR (cw 15; Figure 3a) and TH (cw 14; Figure 3b), relatively high B concentrations were also detected in Asteraceae T pollen (88 mg/kg and 103 mg/kg), likely due to the proximity of dandelion plants to the rapeseed fields. By contrast, pollen from trees, such as cherry, apple/

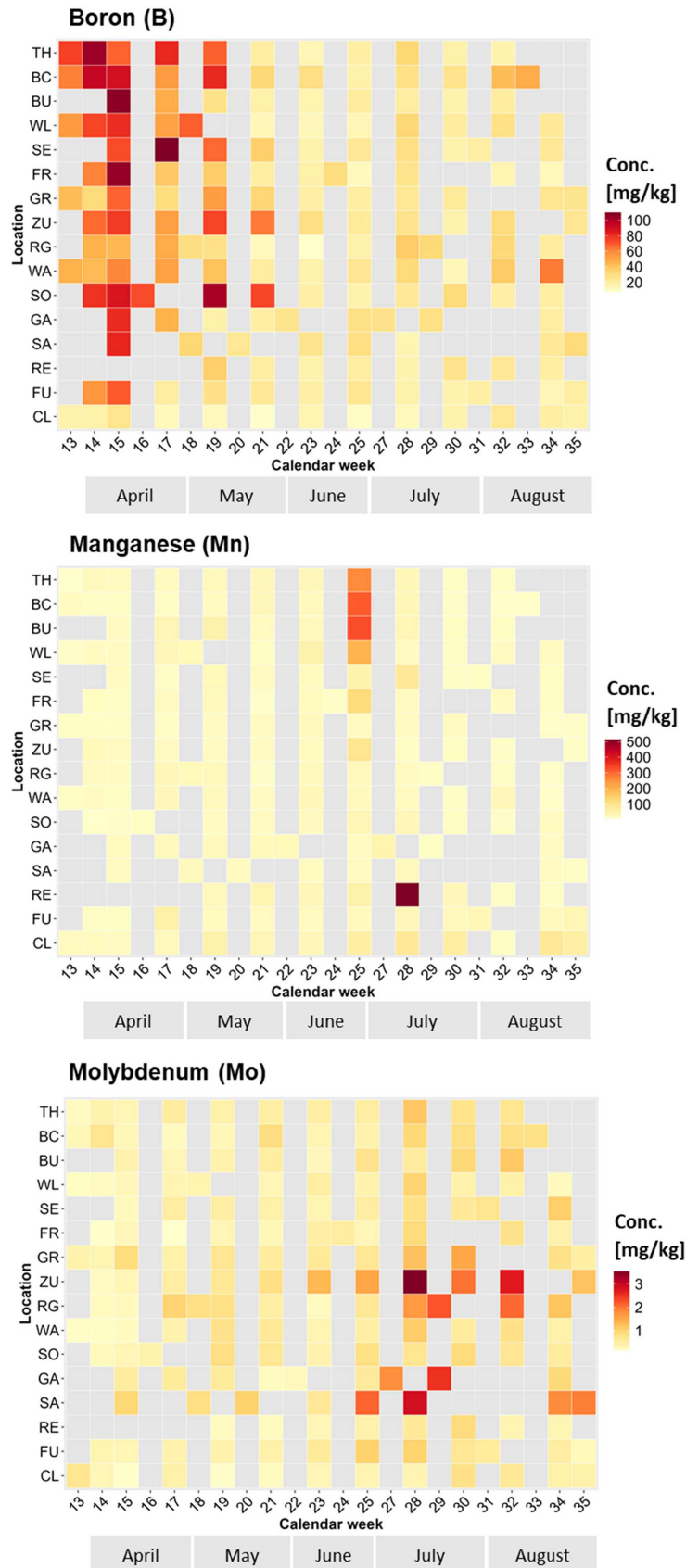


Figure 2. Seasonal variations in B, Mn and Mo concentration levels (mg/kg) in pollen from the 16 study sites for calendar weeks 13–35. Dark red coloured cells indicate high concentration levels, while light coloured cells indicate lower concentration levels. Grey cells denote missing or unanalysed samples. Locations on the y-axis are ordered by the share of oilseed rape within 2 km of each apiary, with the highest share at the top.

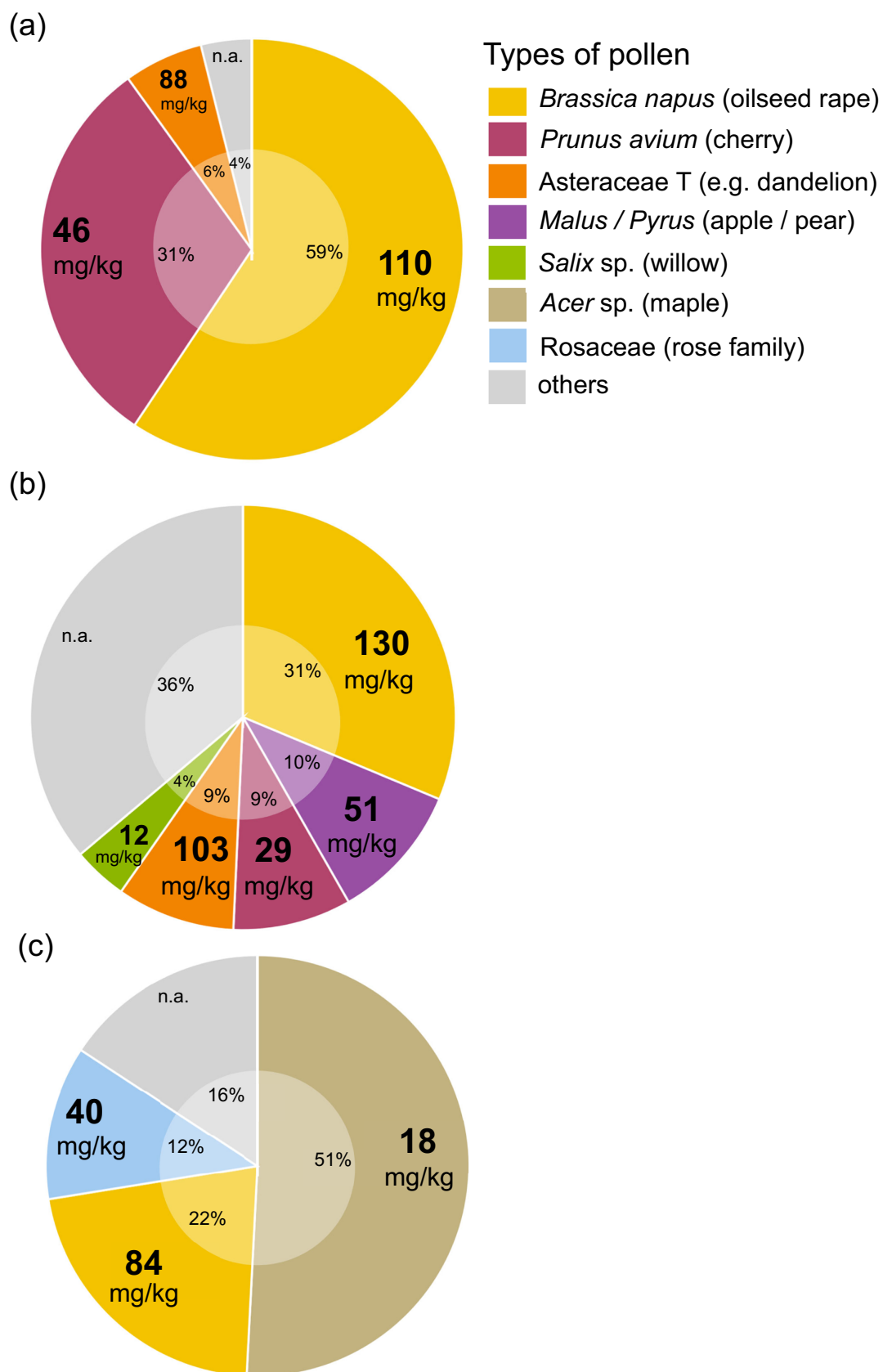


Figure 3. Elevated B levels in *Brassica napus* (oilseed rape) pollen loads compared to pollen types with other botanical origin in the same sample. The percentages (%) of the botanically different pollen types (inner circle) and their respective B concentrations as determined by element analysis (outer circle) are shown for three pollen samples collected at the apiaries in FR (a, c) and TH (b) in April (a, b) and May (c).

Table 3. Calculated TER values for acute oral toxicity of Cd, Cr and Cu to adult honey bees.

Element	Max. conc. in pollen [mg/kg]	Max. uptake ^a [µg/bee-day]	Active substance	LD ₅₀ ^b [µg/bee]	TER ^c
Cu	26	0.312	Copper oxychloride (Cu ₂ (OH) ₃ Cl)	12.1 [35]	39
			Bordeaux mixture (CuSO ₄ and CaO)	23.3 [35]	75
			Tribasic copper sulphate (Cu ₄ (OH) ₆ (SO ₄))	40 [35]	128
			Copper hydroxide (Cu(OH) ₂)	49 [35]	157
			Copper oxide (CuO)	116 [35]	372
Cd	0.774	0.00929	Cadmium sulphate (CdSO ₄)	2.34 [37]	252
			Cadmium chloride (CdCl ₂)	3.51 [37]	378
Cr	0.37	0.00444	Chromium nitrate (Cr(NO ₃) ₃)	25.5 [38]	5743

^a Maximal uptake based on the maximal PTE concentration in pollen and an assumed pollen consumption of an adult honey bee of 12 mg [39].

^b Acute (48 h) lethal dose (LD₅₀): PTE dose that kills 50% of the exposed honey bees. ^c The toxicity exposure ratio (TER): LD₅₀ of a PTE divided by the maximal uptake of the corresponding PTE.

pear and maple trees, exhibited much lower concentrations (<60 mg/kg). Two additional fractionated samples containing OSR together with B levels are presented in Table S16, as well as a sample without OSR for comparison.

3.7. The botanical origin of pollen containing Cu and Mn

In June (cw 25), the pollen composition of the pollen samples shifted towards wild plants that were not treated, such as clover (*Trifolium* sp.) and wild berries (*Rubus* sp.). Pollen of these wild plants exhibited comparatively high levels of Cu (52 mg/kg) and Mn (420 mg/kg), respectively (Figure S6), probably due to the proximity of these plants to the treated cultures. Consequently, we were not able to link Cu- or Mn-containing pollen types to possibly treated crop cultures. Although grapevine (*Vitis*) pollen is sometimes found in pollen collected near vineyards, it is not a major forage plant for honey bees. The plants that grow between the vineyard rows or close to the vineyard are often more popular for honey bees than the grapevine itself. In our study, honey bees did not collect grapevine pollen in GR in cw 25, but they collected pollen from clover (*Trifolium* sp.), dandelion (Asteraceae T) and bushes/trees (*Rhus typhina*, *Fraxinus ornus*, *Ligustrum* sp.) (Figure S6; GR cw 25). Sugar beet (*Beta vulgaris*), which is typically harvested in the first year before it reaches the flowering stage in the second year, has no relevance as a pollen and nectar source for honey bees. Instead, high Mn levels were measured in pollen from wild berries (*Rubus* sp.), probably growing near treated crops (Figure S6; BC cw 25).

3.8. Acute oral toxicity of Cd, Cr and Cu to adult honey bees

Based on our measured concentrations of Cd, Cr and Cu in pollen from the studied apiaries, a worst-case acute oral toxicity scenario was calculated for adult nurse bees. For the maximal Cu concentration measured in pollen collected from GR (26 mg/kg), an apiary surrounded by a large area with vineyards, the calculated TER values were well above 1 (Table 3). The LD₅₀ values for the different active substances of the tested Cu formulations varied to some degree (Table 3). The lowest TER, with a value of 39, was calculated based on an LD₅₀ value for copper oxychloride (Cu₂(OH)₃Cl), the active substance in Flowbrix, which was applied near the apiary in GR. The Bordeaux mixture, which was also used in the surrounding area of GR, contained copper sulphate (CuSO₄ and CaO). A TER value of 75 was obtained based on the corresponding LD₅₀ value. All TER values based on LD₅₀ values for Cd and Cr salts were well above the values obtained for Cu (e.g. TER of 252 for cadmium sulphate (CdSO₄) or TER of 5743 for chromium nitrate (Cr(NO₃)₃). Thus, we expected no increased acute oral toxicity to adult honey bees above the usual mortality levels for these elements.

4. Discussion

We observed significant seasonal and site-specific fluctuations in PTE concentrations in pollen from apiaries located near agricultural environments. Thus, honey bees in Switzerland are exposed to a multitude of PTEs

throughout the agricultural season. In line with previous research [42,43], we have found the highest levels of Cu in pollen from apiaries located near vineyards. At the study site with the most grapevines (21% of the land use) around the bee hive, the increase in Cu levels in pollen in June/July reflected the application of copper-containing products in the vineyards. Additionally, we have measured elevated Cu levels in pollen collected in the vicinity of orchards and vegetable fields. In this study, we linked PTE pollen levels to the recently available detailed land use data, which included the areas of individual arable crops around the beehives. A positive correlation was observed between B levels in pollen and the share of OSR cultivations around the apiaries. The separation of pollen loads into various colour fractions combined with chemical analysis allowed to attribute the maximal B levels to the OSR pollen loads. Thus, our study showed that it is possible to trace the origin of the PTEs back to the relevant crop type, especially if crops are attractive foraging plants for honey bees. Furthermore, elevated Mn levels were measured in pollen from apiaries near sugar beet cultivations, suggesting a possible use of Mn-containing fertilisers. Finally, we expect no acute toxicity to adult honey bees for Cd, Cr and Cu with respect to their calculated TER values. In accordance with these calculations, we did not find any unexpectedly high numbers of dead bees in front of the flight entrance or colony losses during the winter.

4.1. Cu and Al levels in pollen compared to other studies and their use in agriculture

Pesticide products containing Cu or Al are widely used for the plant protection of agricultural crops. Cu concentrations in pollen in our study ranged between 4–26 mg/kg, which aligns with ranges reported for pollen collected in the several Mediterranean countries, such as Spain (4–16 mg/kg), and Greece (4–22 mg/kg), or in European countries of other climate zones, such as Finland (7–19 mg/kg) and Poland (6–24 mg/kg), as well as levels reported in Asia (China, 8–25 mg/kg) and South America (Brazil, 3–25 mg/kg), as reviewed by Pohl et al. [24]. Similar to our study, observations of seasonal variation of Cu levels were found in the Abruzzo region of Italy, where higher Cu levels in wild plants near vineyards were observed in July (12.5 mg/kg) versus April (5.5 mg/kg) and linked to Cu sulphate applications against the downy mildew of grapevine [44]. Flammini et al. reported maximal Cu levels in pollen (up to 14.1 mg/kg) from apiaries near vineyards [42]. Markedly higher Cu levels in bees (97 mg/kg) were detected from apiaries near French vineyards [43]. Apart from vineyards, Cu is authorised for use on fruit trees (scab, leaf curl diseases), berries (stem, leaf spot and leaf fall diseases) and vegetables (downy mildew and late blight) [40]. The Cu levels observed in the present study in pollen from BC and GA with vegetable cultures (30% and 8%, respectively) or FU with fruit trees (16%) and berries (1%) plausibly reflect Cu applications in these crops.

Al concentrations in our study ranged between 8–214 mg/kg, comparable to Brazil (10–314 mg/kg), Serbia (9–112 mg/kg) and Turkey (12–109 mg/kg) [24]. Elevated Al levels were particularly observed in FU, where fruit trees (16%), vineyards (12%) and berries (1%) dominated land uses in the surroundings of the apiary. Al-based fungicides, such as aluminium fosetyl ($C_6H_{18}AlO_9P_3$), are authorised against grapevine downy mildew, root rot in strawberries and pear blossom blight [40]. Additionally, kaolin ($Al_2Si_2O_5(OH)_4$) is approved as an insecticide/acaricide in fruit cultivation to control pests, such as *Drosophila suzukii* and pear psylla [40]. Such applications in summer might be the reason for the elevated levels of Al in FU.

4.2. Levels of B, Mn and Mo in pollen compared to other studies and use in agriculture

Assigning elements in pollen for use as fertilisers is more difficult than assigning products used as plant protection. The potential element sources are diverse: elements may originate from anthropogenic activities, such as agriculture, vehicular traffic and industrial processes, but also from natural sources, such as soil erosion, rock weathering and windblown dust [14]. Furthermore, the element uptake may differ between plant types [45] and also depends on soil factors such as organic matter content, soil type and pH [25,26,46]. In addition, climatic conditions such as wind or drought can influence element concentrations [47,48]. In France, for example, higher Pb concentrations were found in pollen during dry periods in summer and fall [49].

Fertilisers containing B might be used for OSR [50] or sugar beet cultivation [51]. In our study, we found a correlation between OSR and B levels in springtime. OSR pollen loads contained high B levels, suggesting an enhanced uptake of B from the soil into the plant or foliar applications of B-containing products.

Application of B on other crops and as drift on OSR can also not be excluded. In our study, B levels (8–110 mg/kg) are comparable to the ranges reported by Pohl et al. for pollen in Turkey (0.6–61 mg/kg) [24]. Levels of B in pollen from Greece ranged between 19 and 300 mg/kg [52]. B is a key micronutrient in OSR, supplied via soil and foliar fertilisation [53]. Tölle et al. [54] showed that adequate B supply in OSR significantly increases pollinator visitation, reinforcing the link between B fertilisation, floral quality, and the likelihood of B-rich pollen in bee pollen loads.

Sugar beet cultivation is sensitive to Mn deficiency [41]. Mn levels in pollen in this study ranged from 8–516 mg/kg, concentrations that are similar to reported ranges in pollen from Poland (7–367 mg/kg) and China (13–430 mg/kg) [24]. Elevated Mn levels in pollen from study sites where sugar beets are cultivated may reflect liquid Mn foliar fertilisation. Manure is an alternative source of Mn, as documented by Sager [21], who reported Mn levels from 312 mg/kg to 807 mg/kg in dung from cattle, poultry and pigs. Our recorded pollen Mo concentrations ranged from 0.1–4 mg/kg, consistent with findings by Sattler et al. [55] in Brazil (0.2–5 mg/kg).

4.3. Levels of further mineral and potentially toxic elements

Our measured P (3–7 g/kg) and K (3–10 g/kg) levels ranged within the internationally reported ranges, which span approximately 1–9 g/kg for P and 2–12 g/kg for K across Europe (e.g. Poland and Greece), Asia (China) and South America (Brazil and Argentina) [24]. Both macronutrients are among the essential primary nutrients for plants. P and K are the most abundant naturally occurring mineral elements of pollen [56]. They are also commonly used as fertilisers for agricultural crops.

P is a component of photosynthesis, maintaining cell structure, building proteins and carbohydrates, and cell division and the transport of assimilates, so that growth disorders can occur in the event of P deficiency [41]. On the other hand, K regulates the plant's water balance, is involved in photosynthesis and the formation and transport of metabolic products and reserve substances, among other things [41]. Therefore, it is not surprising that we found both elements in pollen samples throughout the entire season at all study sites. Additionally, potassium phosphonate (K_3PO_4) is authorised as a fungicide against downy mildew in grapes and vegetables, and against root, leather and rhizome rot in berries [40]. Potassium hydrogen carbonate ($KHCO_3$) serves as a fungicide, insecticide, or plant growth regulator in vegetable cultivation and for fruit growing in vineyards and berry cultivation, for example, against powdery mildew or scab on pome fruit [40].

Fe levels in the studied pollen ranged from 21–164 mg/kg, which falls well within the overall reported international ranges in some countries in Europe (22–161 mg/kg) and South America (28–202 mg/kg) [24]. Zn ranged from 24–80 mg/kg, similar to levels reported for countries in Europe (19–94 mg/kg; e.g. Spain, 19–81 mg/kg), South America (Argentina, 27–72 mg/kg) and Asia (Jordan, 25–77 mg/kg) [24].

4.4. Toxicity of elements to adult honey bees

In general, the acute toxicity of PTEs to honey bees, especially the exposure route through pollen, is less frequently investigated compared to other potentially hazardous substances, such as pesticides [9,38]. To our knowledge, no acute LD_{50} values of B and Mo are available or published. As Cu-based products are frequently used for plant protection, LD_{50} values for active substances containing Cu are available from the EFSA and the Pesticide Properties Database [35,36]. LD_{50} values are also available for active substances containing Cd and Cr, respectively [37,38].

As the TER values for the Cu levels measured in the pollen samples were above 39, we expect no increased acute oral toxicity to adult honey bees above the expected mortality levels. Our observations at the apiaries are in accordance with these calculations. We did not find any unexpectedly high numbers of dead bees in front of the flight entrance at any of our studied sites, so no acute toxicity of the measured PTE levels is expected. Nevertheless, the sublethal, cumulative, or synergistic effects of PTEs cannot be excluded [9]. Furthermore, our study did not include interannual variations, which ideally should be addressed in subsequent studies.

The discussion of the effects of B on honey bees is controversial. Dağlıoğlu et al. [57] found a reduced survival rate of honey bees under exposure to nano boron particles in the laboratory, but Siede et al. [58]

concluded no hazards for honey bee colonies using foliar boron fertiliser in blooming OSR in field trials. Søvik et al. [59] showed that Mn exposure (50 mM) negatively affects the foraging behaviour of honey bees.

4.5. Bee pollen as a food supplement for humans

Bee pollen is also used as a human food supplement due to the high flavonoid and phenolic content of pollen and its antioxidant properties [52]. Knowledge about PTE contamination in pollen can therefore provide important information for beekeepers in selecting locations that are suitable for the production of pollen that is safe for human consumption. However, our study was not designed to address PTE levels in pollen for use as food supplement. We determine PTE values in pollen collected on a single day. For commercially available bee pollen, beekeepers in Switzerland usually combine pollen collected over a period of several months, usually April to June. This may result in lower PTE values, as the peak values are diluted. In this respect, PTE levels reported in this study should be interpreted with caution, since they do not reflect bee pollen sold on the market, and PTE values might be overestimated in our study.

Across the time period of our study, the levels of Cd, Cr, Pb and As were low in pollen from most of the tested study sites, often below the quantification limits. Occasional higher values were found for Cd (maximal value of 774 µg/kg) in CL, for Cr (maximal value of 370 µg/kg) in FU, both locations in the southern part of Switzerland and for Pb (maximal value of 284 µg/kg) in BU, and for As (maximal value of 56 µg/kg) in SA, both locations north of the Alps. Years ago, maximum limits for Pb (500 µg/kg) and Cd (30 µg/kg) were proposed as quality criteria for commercial bee pollen [10,33]. While Pb levels were below the limit of 500 µg/kg in all our samples, 57% of our samples contained Cd levels above 30 µg/kg (Table 2). However, our samples do not reflect the bee pollen available on the market. Further studies, ideally conducted over several years on commercially available bee pollen, are required to reliably interpret the PTE values. Mean values exceeding a Cd limit of 30 µg/kg have previously been reported for pollen from several countries [10]. In a study from Turkey, for example, the levels of Cd (range of 39–1390 µg/kg) in pollen [11] were comparable to those in our study. However, the pollen in Turkey contained distinctly higher levels of Cr (range of 800–6900 µg/kg) and As (range of 1300–2200 µg/kg). Despite these elevated PTE levels, the authors consider this pollen as safe for human consumption, although a moderate carcinogenic risk was calculated for these levels [11].

5. Conclusion

Our study revealed seasonal and location-specific differences in PTE levels across the study sites. Given the pronounced seasonal variation in elemental concentrations, conducting multiple sampling points within the season is important. Pollen collected by honey bees provides a simple way for an overview of PTE levels across various agricultural landscapes, allowing the survey of the use of plant protection products, such as Cu and Al, and the monitoring of further mineral and potentially toxic elements in pollen. One of the advantages of using honey bees is the possibility to determine the botanical origin of the crop types containing PTEs by fractionating the pollen samples into pollen loads of the same colour. Furthermore, analysing seasonal variations of PTEs in pollen is useful information not only for evaluating the exposure risks to honey bees, but also for beekeepers, allowing the evaluation of apiary locations, as well as the determination of optimal time periods for minimal contamination levels for pollen production as a food supplement.

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Author contributions

CRedit: **Samira Stalder**: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing; **Marion Fracheboud**: Investigation, Methodology, Writing – review & editing; **Sophie Peiry**: Methodology, Validation; **Coline Python**: Methodology, Validation; **Benoît Droz**: Conceptualization; **Marilyse Raemy**: Methodology, Resources, Validation; **Christina Kast**: Conceptualization, Project administration, Resources, Supervision, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

From the corresponding author, data are available upon reasonable request.

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