Environmental Toxicology and Chemistry, 2026, **45**, 2340–2355
https://doi.org/10.1093/etojnl/vgag123
Published: May 5, 2026
Original Article | Environmental Toxicology

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Potentially toxic trace elements in bee pollen collected around a former municipal waste deposit site

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Abstract

Potentially toxic trace elements (PTTEs) pose a risk to living organisms. We used honey bees (*Apis mellifera*) to explore differences in PTTE contamination levels between two study sites. Two apiaries with five bee colonies each were studied: The experimental apiary was located at a former waste deposit site (Witzwil, Switzerland), and the control apiary was 4.5 km away in the neighboring area (Bellechasse, Switzerland). Pollen was collected from colonies from May to August 2022 and 2023, and we developed an analytical method to assess 22 PTTEs. We quantified 19 PTTEs in at least one of the pollen samples (*n* = 80), with maximal levels recorded for Mn (298.8 mg/kg), B (95.1 mg/kg), Zn (63.4 mg/kg), Cu (19.2 mg/kg), Rb (17.7 mg/kg), Ba (11.1 mg/kg), and Sr (3.2 mg/kg). Most notably, site-specific and seasonal variations were observed. For example, in June, pollen at the Witzwil site had statistically significant higher average concentrations of PTTEs—Mn (204.6 ± 53.1 mg/kg), Rb (13.9 ± 2.3 mg/kg), Ba (6.7 ± 2.2 mg/kg), and Ni (1.8 ± 0.4 mg/kg)—than the Bellechasse apiary, with Mn (74.2 ± 67.1 mg/kg), Rb (5.6 ± 2.0 mg/kg), Ba (4.4 ± 2.5 mg/kg), and Ni (1.1 ± 0.5 mg/kg). By contrast, the levels of several PTTEs (e.g., Mn, Ba, and Ni) were similar in July and August in both apiaries. For maximal Cu, Cd, and Cr levels in pollen, we expect no increased acute oral toxicity to adult honey bees above the expected mortality levels.

Keywords honey bees, pollen, waste deposit site, potentially toxic trace elements, inductively coupled plasma mass spectrometer

Introduction

Monitoring changes in ecosystems, food security, and human health has become increasingly important (Cunningham et al., 2022). Potentially toxic trace elements (PTTEs), including cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb), are of concern because of their impact on the environment and the health of living organisms. Globally, it is estimated that 14%–17% of croplands are affected by PTTE pollution, affecting 0.9–1.4 billion people (Hou et al., 2025). Potentially toxic trace elements in soils originate from natural sources, such as soil erosion, rock weathering, volcanic eruptions, and wildfires, but they mainly result from anthropogenic activities, such as waste disposal, agriculture, vehicular traffic, and industrial activities (Zhang & Wang, 2020). Such pollution sources have been recognized as an issue in many countries across the world and are addressed by agricultural policies (European Environment Agency [EEA], 2024; Nofriandi et al., 2024; United Nations Environment Programme [UNEP], 2013).

More than 38,000 contaminated sites (waste deposit, industrial, and accident sites) are registered in Switzerland (Federal Office for the Environment [FOEN], 2023a). One of the largest polluted sites in Switzerland is the "Scherbenland" (Shardland), Witzwil (856,156 m^2; Canton of Bern, Office for Geoinformation, 2025). From the beginning to mid-20th century (1914–1954), municipal waste from the city of Bern was deposited in the surrounding area of the Witzwil prison (Illi, 2016). The composting and burial of waste in peatland soils as part of land improvements for agricultural purposes resulted in the accumulation of several PTTEs in the soil in this area (Bopp et al., 2016; Evangelou et al., 2013; Fässler et al., 2010; Illi, 2016; Rytz, 2001). In Switzerland, PTTEs are surveyed by the National Surface Water Quality Monitoring (FOEN, 2023b), the National Air Pollution Monitoring Network (FOEN, 2024a, 2024b), and the National Soil Monitoring (Gross et al., 2024) programs.

In agriculture, PTTEs originate from various sources, including fertilizers (Schöneberg & Liebisch, 2023), liming (Holland et al., 2018), manure (Sager, 2007), and plant protection products (Defargé et al., 2018). For example, copper (Cu) is widely used as a fungicide for vegetables, fruits, berries, and vineyards (Federal Food Safety and Veterinary Office [FSVO], 2025). In addition, studies have shown that agricultural products such as

Received: November 20, 2025. **Revised:** March 31, 2026. **Accepted:** April 18, 2026

phosphate fertilizers, pesticides, and limestone may be contaminated by PTTEs: for example, Cd and uranium (U) in soils are related to phosphate fertilizers (Bigalke et al., 2017), while arsenic (As) and Pb were found as contaminants in plant protection products (Defargé et al., 2018; Séralini and Jungers, 2020).

In soils, PTTEs can persist for decades, as shown by Swiss soil monitoring efforts of 1985–2019 (Gross et al., 2024), and even for centuries, as reviewed by Asare and Afriyie (2021). The total PTTE concentration reveals total soil contamination, which is relevant, for example, for comparing the contamination levels of different geographical regions. However, for risk assessment of PTTEs on terrestrial organisms and their impact on the ecosystem, it is necessary to determine their bioavailable fractions. In soil, it's often resource-intensive to determine the available PTTE levels. Alternatives, such as conifer needles, mosses, and living organisms, including bees or snails, have gained popularity in pollution research due to their efficiency, specificity, and cost-effectiveness (Al-Alam et al., 2019). Furthermore, such procedures allow the assessment of acute exposure scenarios.

Honey bees are particularly interesting, as a single colony can comprise several thousand bees. During their foraging activities, they collect pollen, nectar, propolis, and water, bringing along pollutants contained within these matrices to their hives. From spring to summer, when food sources are abundant, honey bees typically forage within a 2 km radius of the hive, covering up to 15 km^2 around the apiary (Couvillon et al., 2015), interacting with diverse environmental compartments, such as vegetation, soil, air, and water (Gekière et al., 2023). Consequently, they can be exposed to many types of contaminants, such as pesticides (Schaad et al., 2023; Stalder et al., 2025), persistent organic pollutants, such as polychlorinated biphenyls (Villalba et al., 2020), and PTTEs (Bogdanov, 2006; Conti & Botrè, 2001). Hence, honey bees and their products have proven to be valuable bioindicators of pollutants (Bargańska et al., 2016; Chowdhury et al., 2023; Zavrtnik et al., 2024). Residues of PTTEs have been found in bee products, such as pollen, honey, and wax (Aldgini et al., 2019; Conti & Botrè, 2001; Flamminii et al., 2024).

Typically, bee pollen contains higher concentrations of PTTEs than honey (Conti & Botrè, 2001; Nowak & Nowak, 2023), which may be attributed to the honey bees acting as a filter and removing part of the PTTEs when processing nectar to honey (Fakhimzadeh & Lodenius, 2000). Furthermore, pollen reflects contamination directly from the environment outside of the hive, while honey and wax can be exposed to in-hive contaminants related to products used for the treatment of bees, such as those against *Varroa destructor*. Recent studies have shown that honey bees and their products can be used for assessing seasonal variations in the levels of contaminants, such as pesticides (Schaad et al., 2023; Stalder et al., 2025) and PTTEs (Topal et al., 2022).

The aim of this study was to use honey bees to explore differences in PTTE contamination levels and seasonal variations between two sites located in an agricultural area with crops and vegetables: a former municipal waste deposit site (Witzwil) with elevated PTTE levels in soil and a control site 4.5 km away (Bellechasse). We hypothesize that PTTE levels in pollen collected by bees at the waste deposit site are higher than in pollen from the control site. We initially developed a multi-element method for assessing PTTE in bee pollen. The PTTEs investigated were silver (Ag), As, boron (B), barium (Ba), beryllium (Be), Cd, cobalt (Co), Cr, cesium (Cs), Cu, gallium (Ga), manganese (Mn), Ni, Pb, rubidium (Rb), antimony (Sb), selenium (Se), strontium (Sr), thallium (Tl), U, vanadium (V) and zinc (Zn).

Materials and methods

Chemicals, consumables, and instruments

For the list of chemicals, reagents, and apparatus used in this study (see online supplementary material, Tables S1 and S2).

Study site and honey bee colonies

The studied apiaries were located in the Seeland (central Swiss Plateau): Witzwil: 46° 58′ 37.08″ N, 7° 05′ 12.65″ E; and Bellechasse: 46° 58′ 58.02″ N, 7° 08′ 43.27″ E (registered apiaries). The apiary in Witzwil borders the former waste deposit site, which was contaminated by PTTEs due to the deposition of municipal waste from the City of Berne over decades. The site was used for waste disposal between 1914 and 1954 (Illi, 2016). The flight distance of the honey bees encompassed the waste deposit site (Figure 1). The apiary in Bellechasse was located 4.5 km from the apiary in Witzwil. Honey bees fly within a distance of 2 km (Couvillon et al., 2015), especially in springtime, when a lot of forage plants are available. Therefore, the waste deposit site was not included in the flight distance of this apiary (Figure 1). Both apiaries were situated in agricultural environments (Stalder et al., 2025; see online supplementary material, Figure S1). Cultivations grown within 2 km of the apiaries included cereals, maize, vegetables, oilseed rape, sunflower, and fruit trees. See online supplementary material (Figure S2) for the proportions of the cultivations. Changes in cultivations between the years 2022 and 2023 are shown in Figure 1.

The bee colonies were overwintered in 12-frame Dadant Blatt hives, occupying 7–8 frames. All colonies were treated for *V. destructor* infestation using formic and oxalic acids. In 2022, treatment for *V. destructor* in the summer was conducted using a Nassenheider Pro evaporator (290 mL of 60% formic acid, wick setting of 2). Nine colonies underwent treatment between August 19 and August 31, 2022, while treatment was not required for one colony due to low infestation. In 2023, *V. destructor* summer treatment was performed in two stages. From August 18 to September 1, 290 mL of Formivar 70% was applied to six colonies. A second round of treatment took place from September 15 to September 28, during which 290 mL of Formivar 70% was applied to four colonies. For the winter treatment, all colonies were sprayed with Oxuvar 5.7 (oxalic acid) during the month of December in 2022 and 2023. In addition, the colonies were regularly checked to prevent natural colony movements through swarming. The queen cells were regularly removed, and if necessary, the colonies were split into two separate colonies to avoid swarming. This management practice ensured that colonies did not swarm and relocate on their own.

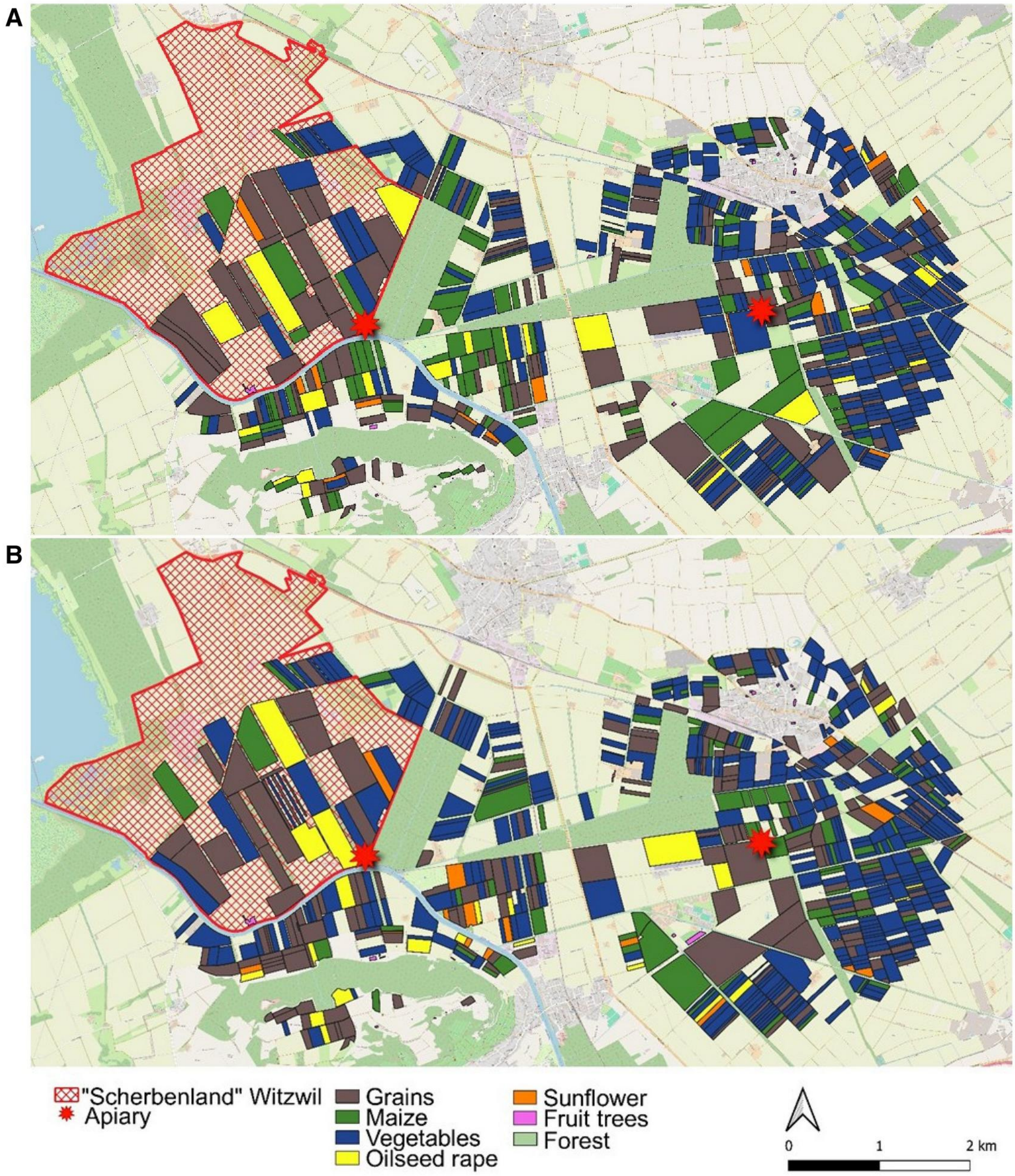

Figure 1 Cultivations around the apiaries of Witzwil (on the left) and Bellechasse (on the right) in 2022 (A) and 2023 (B). The "Scherbenland," with the former municipal solid waste of the city of Bern, is displayed as a red grid area close to the apiary of Witzwil. Agricultural data were provided by the Office of Geoinformation of the Cantons of Bern and Fribourg and displayed in QGIS (QGIS Development Team, 2024). The different colors for each field represent different crops for the corresponding year.

Pollen sampling

Pollen traps (made in a local workshop) were installed at the entrance of the bee hives. They consisted of a grid large enough (Ø 5 mm) to let honey bees pass but retain pollen pellets they carried in the pollen basket of the hind leg. Pollen was collected from five colonies during a single day each month in May, June, July, and August in 2022 and 2023, resulting in 40 samples per apiary (a total of 80 samples). An overview of the sampling design with the number of apiaries, hives, and pollen traps used in this study is shown in Figure 2. The pollen was collected for 24 hr. Pollen traps were closed in the late afternoon, and the pollen collected from the drawers 24 hr later (sampling day). The exact sampling dates and the total amount of pollen collected by honey bees on each sampling day are listed in the online supplementary material, Table S3.

Sample preparation

The pollen samples were homogenized by hand using a petri dish and an empty 50-mL Falcon tube as a pestle to grind the pollen. Next, the pollen was dried in a lyophilizer (Christ alpha 1–4, Kühner AG, Birsfelden, Switzerland) for 15 hr at −50°C and 0.5 mbar and stored at −20°C until further use.

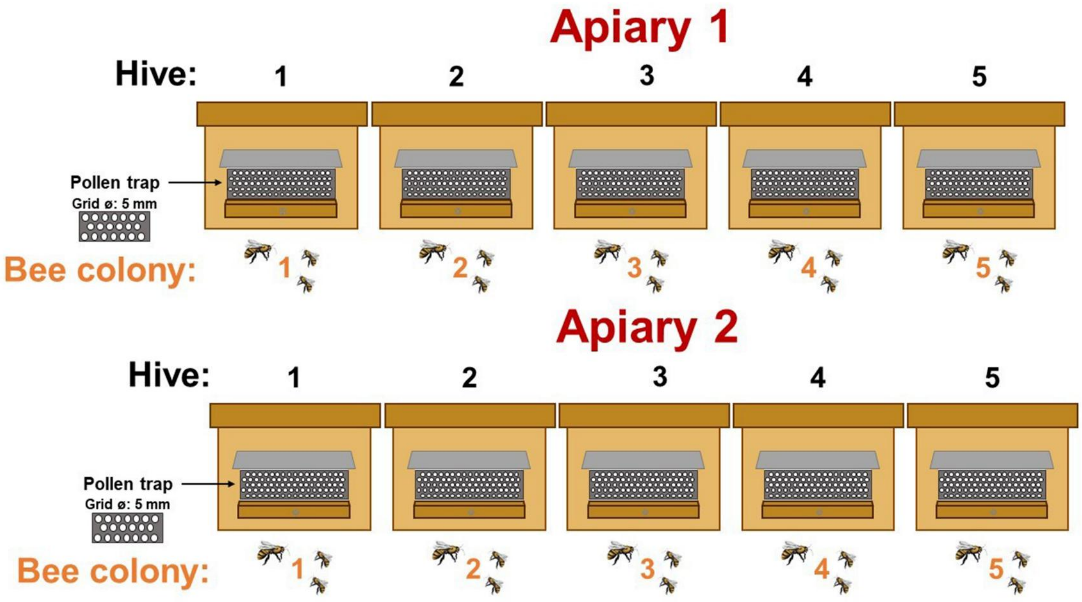

Figure 2 Experimental setup of apiary 1 (Witzwil) and 2 (Bellechasse), each containing five hives with colonies. Pollen traps (grid size: 5 mm) were installed at the entrance of the bee hive. The pollen traps were closed in the evening, and the pollen was collected from the drawer 24 hr later (sampling day).

Extraction of the PTTEs by open vessel microwave-assisted acid digestion

To extract the PTTEs, 0.2 g of each pollen sample was weighed into a 50 mL Corning tube using a plastic spatula, before adding 2.0 mL nitric acid (HNO_3; 65%) and 6.0 mL hydrochloric acid (HCl; 34%). The tubes were mixed gently by hand and left slightly open, allowing the gases to escape. The tubes were then placed in the microwave for 1 hr and heated in three stages with a ramp of 10 min to 55°C (held for 10 min) to 75°C (held for 10 min), and finally to 95°C (held for 30 min). The microwave settings are summarized in the online supplementary material, Table S4. A fiber optic probe (MTS 300 Fiber, Kamp-Lindtfort, Germany) was placed into one of the samples to control the temperature steps. After cooling down the samples to 30°C, they were filled to 40 mL with ultrapure water, vortexed for 5 s, and centrifuged (5 min, 2,500 rpm) to separate the solid from the liquid components. Next, 10 mL of the samples were pipetted into 15 mL Corning tubes. Alternatively, the samples were diluted (1:1) by adding 5-mL aliquots of the samples to 5 mL of ultrapure water in 15 mL Corning tubes. The samples were stored at 5°C. Before analysis, the samples were vortexed once more for 5 s.

Certified reference material and spiking of a pollen sample

Two certified reference materials (CRM)—namely NIST 1573a (Tomato Leaves) and ERM-CD281 (Rye Grass)—were measured in triplicate for each microwave batch (total $n = 12$). No reference material was available for the matrix pollen. Therefore, commercial pollen (Bienen Roth GmbH, Wila, Switzerland) was spiked in duplicates at four different levels by adding 3, 30, 100, or 300 μg/L of the commercially available standard multi-element solution IV-ICPMS-71A, resulting in the following spiking levels: 0.6, 5.6, 19.3, and 58.6 mg/kg. Similarly, CRM (ERM-CD281) was spiked at four levels (0.6, 5.8, 20.3, and 59.5 mg/kg). We also spiked the commercial pollen (Bienen Roth GmbH, Wila, Switzerland) and CRM (ERM-CD281) with the Sb ICP standard at two different levels (1.5 and 15 μg/L), resulting in spiking levels of 0.3 and 2.8 mg/kg in commercial pollen and 0.3 and 2.9 mg/kg in CRM, respectively. Recoveries were calculated for spiking levels that exceeded the background concentrations of the pollen and CRM. For calculations, the background concentrations, when detected, were subtracted from the measured values prior to calculating the recovery levels of the spiked pollen and the CRM.

For the analysis of the pollen samples, the following references were included in each extraction round: ERM-CD281, NIST 1573a, and commercial pollen from Bienen Roth GmbH, with and without spiking. Additionally, one of the tested pollen samples was measured in triplicate in each microwave batch to verify proper grinding procedure (homogeneity of the sample) as well as the analytical performance. In addition, a method blank (acid and ultrapure water alone) was included. To check instrument performance and calibrations, an additional reference standard of groundwater (NIST 1643f) was analyzed with every batch of samples measured on an Agilent 8900 triple quadrupole inductively coupled plasma tandem mass spectrometer (ICP-MS/MS; Agilent Technologies, Santa Clara, California, USA).

PTTE analysis by ICP-MS/MS

Element analysis was performed using ICP-MS/MS. Sample introduction was done via an SPS 4 autosampler (Agilent Technologies, Santa Clara, California, USA), and a nebulizer (Glass Expansion, Melbourne, Victoria, Australia) was attached to a quartz cyclonic spray chamber. A quartz torch, a nickel sampler, and skimmer cones (Glass Expansion, Melbourne, Victoria, Australia) were used. For tuning and optimization purposes of the device, we used a tuning solution (250 mL) containing 1 μg/L cerium (Ce), Co, lithium (Li), Tl, and yttrium (Y) in 1% HNO_3.

A T-junction was used in-line to continuously inject internal standards rhodium (Rh) and indium (In). Internal standard solutions at the final concentrations of 1 μg/L Rh and 10 μg/L In were prepared from 1 mg/L Rh standard and 10 mg/L In standard, respectively, in 1% HNO_3 (dilution, 1:1000). A rinsing solution was used as described in the online supplementary material in S1. Several calibration levels (as described in the online supplementary material S2) were re-measured every 20 samples to check for instrumental drift.

The following isotopes were analyzed using the helium mode: ^{9}Be, ^{11}B, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{71}Ga, ^{85}Rb, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{121}Sb, ^{123}Sb, ^{133}Cs, ^{137}Ba, ^{205}Tl, ^{208}Pb, and ^{238}U. In addition, ^{51}V, ^{75}As, and ^{78}Se were measured using oxygen mode. Operating parameters and sample acquisition of the ICP-MS/MS are summarized in the online supplementary material, Tables S5 and S6. The PTTEs were quantified using external calibration, as described in the online supplementary material in S2.

The instrumental background equivalent concentrations (BEC) were used as the limit of detection (LOD) values, except for Be, Rb, Sr, Cd, Cs, and Pb. For those PTTEs, the method blank values were above the instrumental LODs; thus, these LOD values were defined as two times the maximal method blanks. The limit of quantification (LOQ) was defined as 10 times the BEC values. The instrumental LOD values of the PTTEs, as well as the corresponding LOD and LOQ values in pollen, are listed in the online supplementary material, Table S7.

The median and mean PTTE concentrations in the pollen samples collected from the five bee colonies on the same day (n = 5) were calculated as follows: values above the LOQ were used as reported, values between LOD and LOQ were treated as LOD values, and values below the LOD were considered to be 0.

Statistical analysis

RStudio (R Core Team, 2024; Version 4.2.3) was used for the statistical analysis of the results. We performed a generalized linear model (GLM) using the function "stats" to identify study site and seasonal differences of PTTE levels in pollen of the two studied apiaries, whereby the years, months, and study sites were treated as fixed factors. The Gamma link family was chosen to account for the right-skewed distribution of the data. A DHARMa residual diagnosis (Hartig, 2024) was performed to test overdispersion and uniformity to ensure model quality.

Results

Sensitivities and recoveries

In this study, we developed a multi-elemental method that allowed for the quantitation of 22 PTTEs in bee pollen. High sensitivity was achieved for 18 of the tested PTTEs, with LOQ levels ranging from 0.002 to 0.2 mg/kg in the pollen samples. For four of the PTTEs, the method was less sensitive, resulting in LOQ levels of 0.3–0.4 mg/kg (Cu, V, and Zn) or 3.6 mg/kg (B). The LOD and LOQ levels of the PTTEs are shown in the online supplementary material, Table S7.

Plant-based CRMs—ERM-CD281 (rye grass; 11 PTTEs) and NIST 1573a (tomato leaves; 13 PTTEs)—were used, as no certified pollen reference material was commercially available. The measured mean values and the corresponding recoveries are listed in the online supplementary material, Table S8, together with the certified values of the CRMs. Good or acceptable recovery rates were obtained for all PTTEs in both CRMs, except for V (Figure 3). For ERM-CD281, the values obtained for 9 of the 11 PTTEs analyzed were within 10% deviation of the certified values (B, Cd, Cr, Cu, Mn, Ni, Pb, Se, Zn). The recoveries of As and Sb were 112% and 78%, respectively. For NIST 1573a, 8 of 13 PTTEs analyzed B, Cd, Co, Cu, Mn, Ni, Rb, and Zn were within 10% deviation of the reference values. The recovery rates for the additional PTTEs were 132% for As, 81% for Cr, 87% for Sb, 86% for Se, and 42% for V.

To exclude possible matrix effects, commercial pollen obtained from Bienen Roth GmbH was spiked with all PTTEs of interest at various concentration levels. The PTTE levels of the blank pollen and recoveries in spiked pollen are listed in the online supplementary material, Tables S9 and S10, respectively. Recoveries were between 78% and 125%. For comparison, we also spiked ERM-CD281 (online supplementary material, Table S10). The recoveries obtained were similar to the results of the spiked pollen, suggesting that there was no significant matrix effect when analyzing PTTEs in pollen.

Quantified and detected PTTEs

The temporal concentration profiles of various PTTEs were investigated using pollen samples collected from five colonies located in Witzwil and Bellechasse from May to August in 2022 and 2023. We quantitated the levels of 19 PTTEs of the 22 tested PTTEs in at least one sample of the two investigated sites. Among those, 10 of the PTTEs (B, Ba, Cd, Co, Cu, Mn, Ni, Rb, Sr, and Zn) were quantitated in all (100%, n = 80) and Pb in 69 (86%, n = 80) samples. The highest PTTE levels in the analyzed pollen samples of both apiaries across both years were found for Mn (298.8 mg/kg), B (95.1 mg/kg), Zn (63.4 mg/kg), Cu (19.2 mg/kg), Rb (17.7 mg/kg), and Ba (11.1 mg/kg; Table 1). The levels of other PTTEs showed a decreasing order of Sr (3.2 mg/kg) $>$ Ni (3.1 mg/kg) $>$ Cr (1.2 mg/kg) $>$ Ag (0.8 mg/kg) $>$ Cd (0.5 mg/kg) $>$ V (0.4 mg/kg) $>$ Pb (0.3 mg/kg) $>$ Se (0.3 mg/kg) $>$ Co (0.2 mg/kg) $>$ As (0.1 mg/kg) $>$ Cs (0.08 mg/kg) $>$ Tl (0.08 mg/kg) $>$ U (0.06 mg/kg; Table 1). Three PTTEs (Be, Ga, and Sb) remained below the LOQ ($<$LOQ) but were detected ($>$LOD) in at least one pollen sample. Monthly median and mean concentrations of the PTTEs in pollen samples are listed in the online supplementary material, Tables S11 and S12. Median and mean values were comparable for most PTTEs (except for Mn in June at the control site, with a median of 43.0 mg/kg and a mean concentration of 74.2 mg/kg).

Comparison of the study sites Witzwil and Bellechasse

Data evaluation could be performed with a GLM model and showed that the results of both study years could be combined for

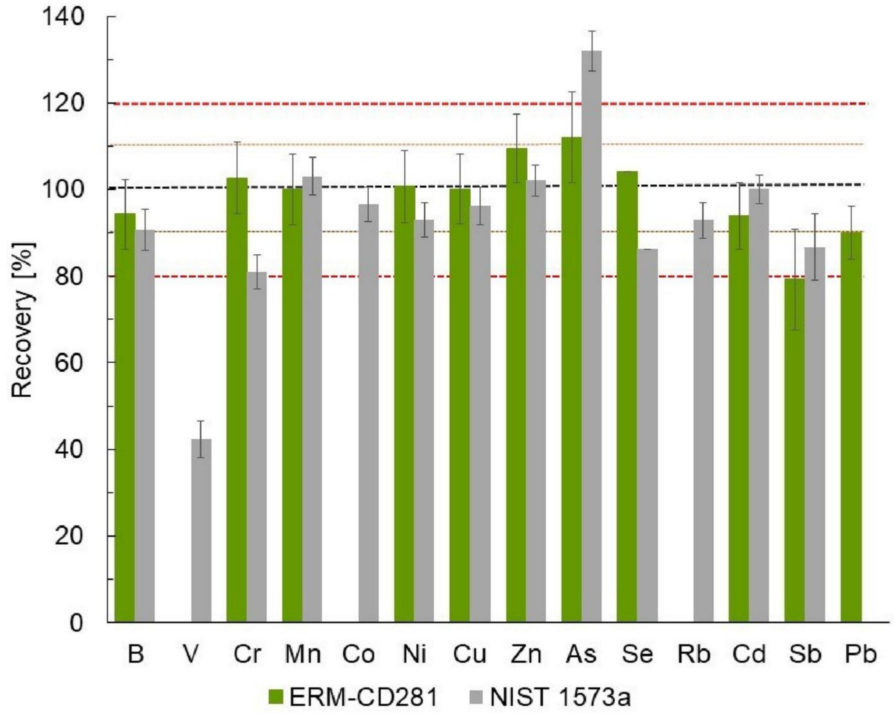

Figure 3 Recovery rates [%] and standard deviations ($n = 16$; except Sb $n = 12$) of elements from the measured certified reference materials ERM-CD281 (green) and NIST 1573a (grey; $n = 12$). The red dotted line shows the range for recoveries between 80% and 120%, the orange dotted line shows the range for recoveries between 90% and 110%, and the black dotted line shows the 100% recovery line.

the PTTEs Co, Mn, Rb, Ba, Ni, Cu, Sr, and Pb, since no significant differences were observed between the years 2022 and 2023 (see online supplementary material, Tables S13 and S14). On the other hand, the results could not be combined for B, Zn, and Cd, since significant differences were observed for the two study years (see online supplementary material, Table S14). The model was not applied to Cr and Cs as most values were below LOQ.

Multiple PTTEs showed higher levels in pollen at the former municipal waste deposit site Witzwil compared to the levels found in pollen at the neighboring control area (Bellechasse). Statistically significant differences were observed for Co ($p < 0.001$), Mn ($p < 0.001$), Rb ($p < 0.001$), Ba ($p < 0.05$), Ni ($p < 0.001$), and Cu ($p < 0.01$; see online supplementary material, Table S15). No statistically significant site-specific differences were observed for Sr and Pb.

Temporal profiles of the PTTEs

The temporal patterns of PTTE levels revealed distinct seasonal behaviors. Among these, Co and Cd (year 2023) were enriched in May, with median concentrations of 0.12 and 0.24 mg/kg at the former waste deposit site, respectively, and B (year 2023) with median concentrations of 89.8 mg/kg at the control site (Figures 4A and 5). Peak median concentrations were observed in June for Mn (206.7 mg/kg), Rb (14.0 mg/kg), Ba (6.2 mg/kg), Ni (1.8 mg/kg), and Sr (2.0 mg/kg) at the former waste deposit site (Figure 4A,B). In August, Pb exhibited the highest median concentration, reaching 0.2 mg/kg at the former waste deposit site (Figure 4B). By contrast, Cu displayed relatively similar median concentrations throughout the season (Figure 4A).

Higher PTTE levels in pollen of the study versus the control site were observed at a significance level of $p < 0.001$ for Co in May and July, and for Mn and Rb in June, and at a significance level of $p < 0.01$ for Rb in May and Ni in June (Figure 4A; see online supplementary material, Table S16). Levels of Mn, Ni, and Cu in pollen of the study site were also above the levels of the control site in May, levels of Ba in June and July, and levels of Co in August at a significance level of $p < 0.05$. No statistically significant time-specific differences were observed for Sr and Pb (Figure 4B; see online supplementary material, Table S16). In addition, low levels of Cr (below or barely above the LOQ level) were detected at both sites.

Levels of Zn and Cd in pollen of the study site were above the levels measured in pollen of the control site at various time points throughout the seasons 2022 and/or 2023 ($p < 0.05$ or below; Figure 5; see online supplementary material, Tables S17 and S18). On the other hand, the levels of B were higher in August 2022 ($p < 0.05$) and May and June 2023 in pollen from the control site ($p < 0.001$). Furthermore, Cs was detected throughout both seasons in 2022 and 2023 in Witzwil, whereas Cs was detected (<LOQ) in Bellechasse only in 2023.

Comparison of PTTE levels in pollen vs. cultivations at the study site Witzwil

Rytz (2001) measured levels of Cd, Pb, and Zn in various vegetables and crops grown on the former municipal waste deposit site Witzwil (Table 2; Rytz, 2001). The study revealed similar Cd, Pb, and Zn levels in wheat, spinach, oilseed rape, and rhubarb

Table 1 Maximal potentially toxic trace element levels in pollen samples.

PTTE[a]	Max. conc.[b] [mg/kg]
Mn	298.8
B	95.1
Zn	63.4
Cu	19.2
Rb	17.7
Ba	11.1
Sr	3.2
Ni	3.1
Cr	1.2
Ag	0.8
Cd	0.5
V	0.4
Pb	0.3
Se	0.3
Co	0.2
As	0.1
Cs	0.08
Tl	0.08
U	0.06
Be	$<$ LOQ
Ga	$<$ LOQ
Sb	$<$ LOQ

Note: Ag, silver; As, arsenic; B, boron; Ba, barium; Be, beryllium; Cd, cadmium; Co, cobalt; Cr, chromium; Cs, cesium; Cu, copper; Ga, gallium; max.conc., maximal concentration; Mn, manganese; Ni, nickel; Pb, lead; PTTE, potentially toxic trace element; Rb, rubidium; Sb, antimony; Se, selenium; Sr, strontium; Tl, thallium; U, uranium; V, vanadium; Zn, zinc.

[a]PTTE, potentially toxic trace element

[b]Maximal concentration levels of pollen samples of Witzwil or Bellechasse in 2022 or 2023 (*n* = 80).

at concentrations that were comparable to those measured in our pollen samples collected in Witzwil (Table 2).

Discussion

Our study revealed site-specific differences in PTTE contamination and seasonal variations using pollen collected from honey bees. Overall, PTTE concentrations in pollen from the apiary at the waste deposit site in Witzwil were higher during both investigated seasons compared to levels in pollen from the control apiary in Bellechasse. Higher levels were observed for Co, Mn, Rb, Ba, Ni, Cu, Zn, Cd, and Cs. However, seasonal variations in PTTE levels were almost more pronounced than the differences found between the two investigated locations. Finally, analyzing PTTE levels in pollen allowed to evaluate exposure risk for honey bees during the agricultural season.

Method development using ICP-MS/MS for the detection of PTTEs in pollen

We developed a method for a multi-element analysis of bee pollen by ICP-MS/MS and analyzed 22 PTTEs. Ideally, CRMs of the same matrices would be included. As there was no pollen-based CRM available, previous studies used plant-based CRMs, such as apple leaves (SRM 1515), or non-plant-based CRMs, such as oyster tissue (SRM 1566a), bovine muscle powder (RM 8415), and chicken (NCS ZC 73016; for a review, see Pohl et al., 2020). In this study, two plant-based types of CRMs were chosen to ensure broad coverage of PTTEs. For instance, Pb is present only in ERM-CD281, whereas Co is found only in NIST 1573a. Furthermore, the two types of CRM cover a wider concentration range. For example, B is present at 33.13 ± 0.42 mg/kg in NIST 1573a, and Cu is present at 10.2 ± 0.5 mg/kg in ERM-CD281, which are similar concentration levels as in our pollen samples. In our study, recoveries of the PTTEs from the ERM-CD281 were all good or suitable for our purpose (78%–112%). Similarly, recoveries of all PTTEs (81%–103%; As, 132%) from NIST 1573a were acceptable, except for V (42%). The low recoveries observed for V in NIST 1573a might be attributed to insufficient digestion of the sample matrix. Trimmel et al. (2023) suggested that the low recoveries of certain PTTEs in plant matrices could be related to their silicate content. The authors demonstrated that the additional use of hydrofluoroboric acid (HBF_4) improved the recoveries of PTTEs such as V in certified plant materials, including bush twigs and leaves (GBW07603). In our study, we quantified V at a low level in just one pollen sample. In our spiked pollen, we achieved 98%–101% recoveries (see online supplementary material, Table S10), suggesting no need for further method improvements regarding V.

To test the potential matrix effect, a commercial batch of Swiss pollen was used to spike the PTTEs at various concentration levels. We observed no significant matrix effect, which is in line with the findings of other studies reviewed by Pohl et al. (2020). However, spiking pollen allowed us to test additional PTTEs that were not included in the CRMs, such as Ba, as well as additional PTTE levels relevant for the analyzed samples, such as Pb. Thus, our method was validated with two types of CRMs, and spiking PTTEs in pollen and CRMs at various levels allowed the covering of all types and levels of PTTEs that were relevant to our study.

Comparing PTTE levels and seasonality to previous studies

Of the 22 PTTEs analyzed, 19 PTTEs were measured in at least one sample collected in 2022 and 2023 at the two studied apiaries, namely As, Ag, B, Ba, Cd, Co, Cr, Cu, Cs, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, and Zn. The highest concentrations were found for Mn (299 mg/kg), B (95 mg/kg), Zn (63 mg/kg), Cu (19 mg/kg), Rb (18 mg/kg), Ba (11 mg/kg), and Sr (3.2 mg/kg). Three other PTTEs (Be, Ga, and Sb) were detected but remained below the LOQ.

Comparing our current results regarding PTTE levels in pollen from the two studied apiaries with the levels reported in studies conducted across Europe and overseas is challenging due to the large seasonal differences in PTTE levels. Only a few studies have focused on temporal PTTE contamination levels in pollen over several months. Seasonal PTTE levels in bee pollen from Brazil were investigated by Silva et al. (2012) throughout 2006. Maximal Cu levels (9 mg/kg) were found in January, whereas concentrations of Mn (86 mg/kg), Pb (18 mg/kg), and Zn (90 mg/kg) were maximal between March and May. The levels of Cu, Mn, and Zn were in the same order of magnitude, whereas the levels of Pb were significantly above the levels in our study. Another study that collected pollen from five different regions in Brazil found Zn at

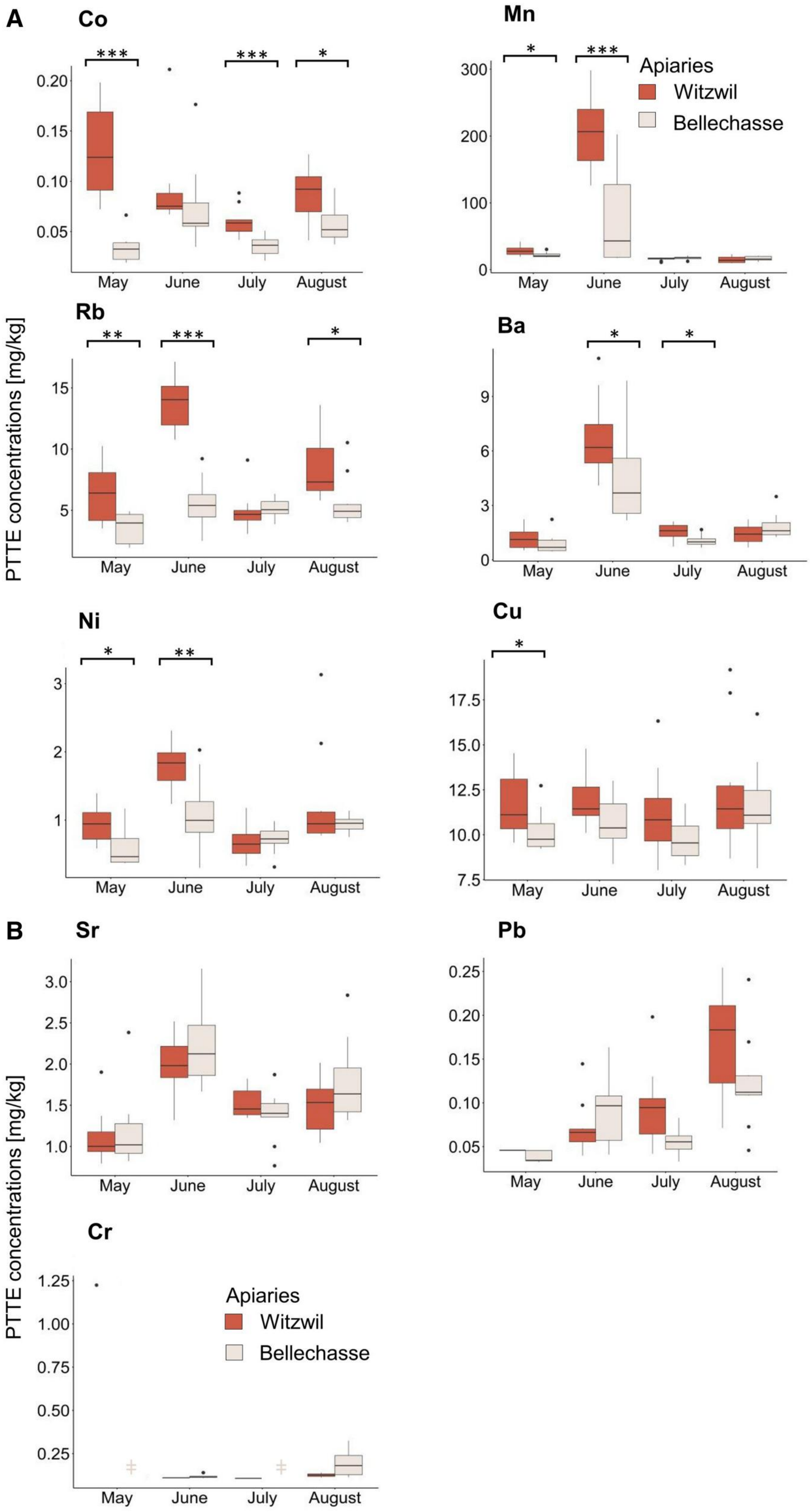

Figure 4 Seasonal potentially toxic trace element (PTTE) concentration levels in pollen of Witzwil and Bellechasse. The boxplots (10 values per plot) show the distribution of the PTTE concentrations throughout the seasons, with the median represented by the line within each box and the interquartile range (IQR) indicated by the box itself. Whiskers extend 1.5 times the IQR; outliers are represented by dots. Values above the limit of detection and below the limit of quantification are displayed with ǂ. Data of both years were combined, as no significant interannual differences were observed according to the generalized linear model ($p > 0.05$). PTTE levels of cobalt (Co), manganese (Mn), rubidium (Rb), barium (Ba), nickel (Ni), and copper (Cu) were significantly higher ($p < 0.05$) at the waste deposit site in some months compared to the control apiary (A). No significant differences ($p > 0.05$) between the two study sites were observed for strontium (Sr) and lead (Pb) (B). Within the group, the PTTEs were ordered according to their seasonal concentration levels.

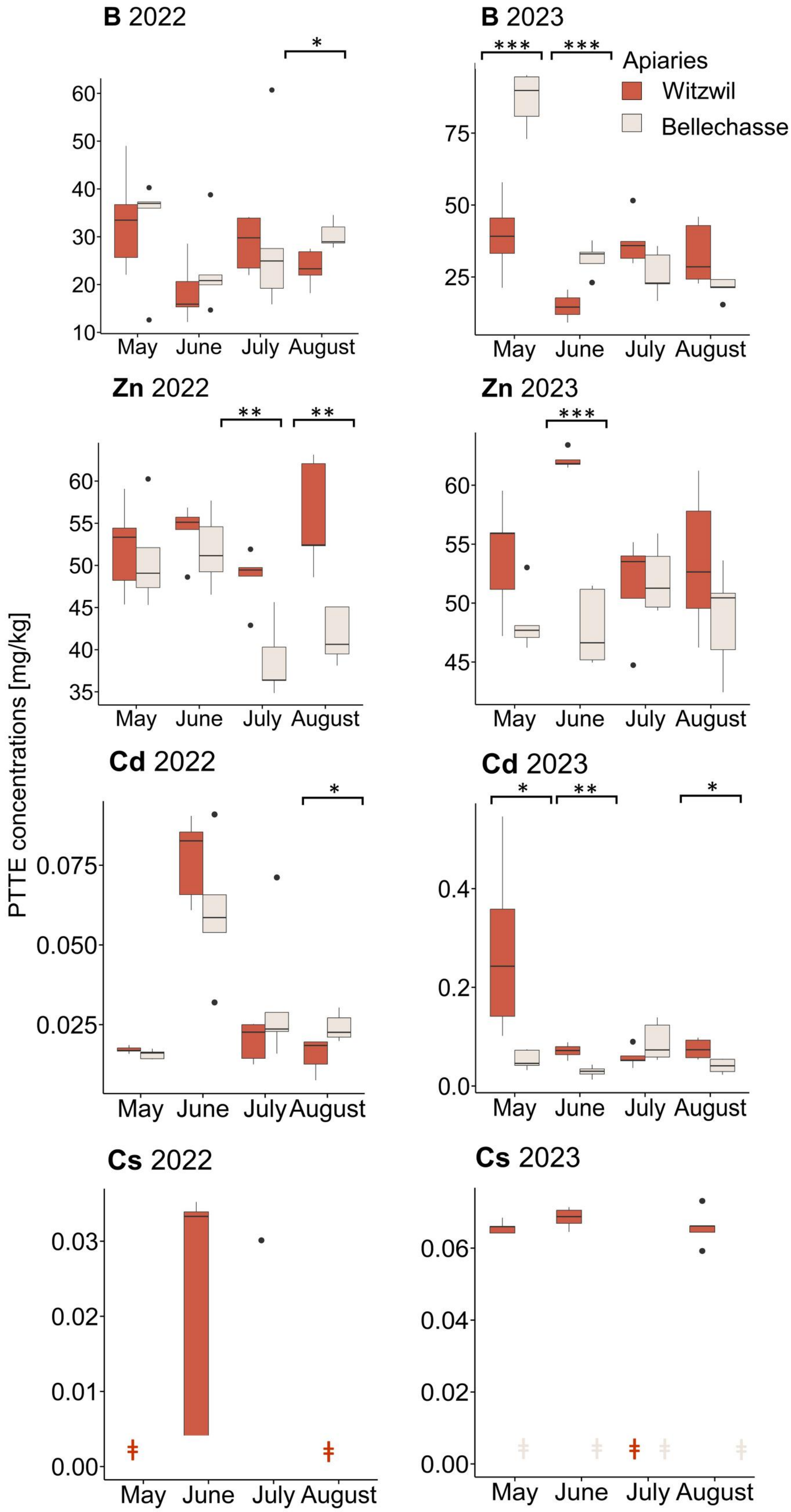

Figure 5 Seasonal potentially toxic trace element (PTTE) concentration levels in pollen of Witzwil and Bellechasse in 2022 and 2023. The boxplots (5 values per plot) show the distribution of the PTTE concentrations throughout the seasons, with the median represented by the line within each box and the interquartile range (IQR) indicated by the box itself. Whiskers extend 1.5 times the IQR; outliers are represented by dots. Values above the limit of detection, but below the limit of quantification (LOQ), are displayed with ǂ. Data from both years were not combined as significant interannual differences were observed according to the generalized linear model (GLM) ($p < 0.05$). For cesium (Cs), the GLM could not be applied as several PTTE levels were below LOQ. PTTEs were ordered according to their seasonal concentration levels.

Table 2 Pb, Cd, and Zn levels in bee pollen and in cultivations in Witzwil.

	PTTE		
	Pb [mg/kg]	Cd [mg/kg]	Zn [mg/kg]
Conc. range in bee pollen in Witzwil[a]	0–0.25	0.008–0.5	43–63
Leek[b]	n.d.-0.07	n.d.-0.01	n.m.-2.3
Barley[b]	n.d.-0.54	n.d.-0.015	24–41
Beans[b]	0.07	n.d.	2.8
Carrots[b]	n.d.-0.08	n.d.-0.061	1–3
Cauliflower[b]	n.d.	n.d.	2
Celery[b]	0.14	0.041	6.9
Cherries[b]	n.d.	n.d.	n.m.
Fennel[b]	0.09	n.d.	n.m.
Kale[b]	n.d.	n.d.	n.m.
Lettuce[b]	n.d.-5.2	0.01–0.029	2–3
Maize[b]	n.d.	n.d.	19
Oilseed rape[b]	n.d.	0.054	43.9
Onions[b]	n.d.	n.d.	1.7–2
Parsley[b]	n.d.	0.007	6
Potatoes[b]	n.d.-0.13	n.d.-0.016	2–3
Rhubarb[b]	0.07–0.11	0.005–0.007	1–2
Spinach[b]	0.05–0.06	0.043–0.089	18–29
Strawberries[b]	n.d.	n.d.	1
Turnip cabbage[b]	n.d.	n.d.	n.m.
Wheat[b]	n.d.	0.024–0.067	25–47

Note: conc. range, concentration range; n.d., not detected; n.m., not measured; Pb, lead; Cd, cadmium; PTTE, potentially toxic trace element; Zn, zinc.
[a]Range of concentrations ($n = 40$) measured in bee pollen of the apiary in Witzwil (Scherbenland) from May to August (2022 and 2023).
[b]Measured concentration ranges of vegetables and crops, as reported by Rytz (2001).

46.7 mg/kg, Cu at 9.6 mg/kg, and Mn at 64 mg/kg (Morgano et al., 2012), which were comparable levels found in our study.

Comparison of PTTE levels in pollen of different study sites is rare, especially when comparing a waste deposit site to a control site in an agricultural environment where cereals and vegetables are grown for human consumption. Roman (2009) revealed differences in PTTE levels in bee pollen between a former military airfield and an agricultural woodland area. Similar to our study, the authors reported higher levels of As, Cd, and Pb in bee pollen at the former military airfield compared to the agricultural woodland area in the months of July and August of 2005 and 2006.

Furthermore, PTTE levels may also differ among pollen of different plant types. High As and aluminum (Al) concentrations were found in monofloral pollen of watermelon (7 and 218 mg/kg, respectively), oilseed rape (5 and 100 mg/kg, respectively), and sunflower (12 and 111 mg/kg, respectively) from China's main pollen-producing regions (Yang et al., 2013). By contrast, Mn concentrations were much lower in their study, whereas Cd, Cu, Pb, or Zn concentrations were in a range similar to that of our study. The uptake of PTTEs by plants might be an important pathway leading to PTTEs in pollen, apart from external sources, such as PTTEs from pesticides and fertilizers in agriculture.

Possible sources of PTTEs in pollen and reasons for their variability

Accumulation of PTTEs in foraging plants

Sunflower, maize, and oilseed rape were cultivated around our study apiaries. They are very popular foraging plants for honey bees (Keller et al., 2005). Cadmium present in the soil can accumulate in oilseed rape plants (Cao et al., 2019). This is in line with our observations of maximal Cd levels in pollen collected from Witzwil at the beginning of May during the flowering season of oilseed rape (year 2023). Furthermore, sunflowers and maize bloom in the summer and could explain the higher PTTE levels we observed later in the season since sunflowers can accumulate As, Cd, Cu, Pb, or Zn from soil (Madejón et al., 2003), while As, Cd, Cu, and Zn can be enriched in maize (Molina et al., 2013).

Seasonal availability of flowering plants plays a crucial role in pollen collection by honey bees, and temporal variations in PTTE levels in pollen throughout the season may be related to the pollen types collected by honey bees. The importance of the types of cultivation during a season was shown by Al Naggar et al. (2013). The authors suggested that seasonal differences in Cd, Cu, iron (Fe), Pb, and Zn content in bee pollen samples collected in Egypt in the spring and summer might be attributed to variations in the botanical pollen composition. Silva et al. (2012) concluded that seasonal and plant dynamics could be explanatory factors for seasonal fluctuations in PTTE concentrations in bee pollen in Brazil. Their research showed that Mn and Zn were enriched in bee pollen, especially in the months of March and April. Additionally, Mn and Zn were present at the highest concentration levels in aerial parts of the studied native plant species, while Cu levels were higher in the roots. These results suggest that the uptake and translocation of PTTEs from soil to plant and within the plant itself depend on the respective plant species, which in turn affects the PTTE concentrations in the pollen available to honey bees.

Soil–plant transfer and soil mobility

The concentrations of most PTTEs were higher in pollen collected from the apiary situated at the former municipal waste deposit site of Witzwil compared to pollen from the control apiary in Bellechasse, suggesting that analyzing PTTE levels in bee pollen may allow differentiation of the contamination levels of different locations. However, many factors may influence the extent to which PTTEs in the soil are taken up by plants and subsequently mirrored in the levels of pollen, including the composition and properties of the soil and the plant types grown at the location. The behavior of PTTEs in soil is mainly determined by physicochemical properties of the soil, such as the amount of clay, the organic matter content, and the pH (Dube et al., 2001; Kicińska et al., 2022; Kwiatkowska-Malina, 2018).

A study on summer squash showed that the translocation of Cu, Ni, Pb, and Zn from the soil led to increased element concentrations in floral rewards, such as pollen and nectar (Xun et al., 2017). These findings suggest that soil contaminants can be transferred via the vascular system of plants to reproductive tissues that are directly accessible to pollinators.

Nevertheless, there are still relatively few studies investigating the direct relationship between soil contamination, uptake by plants, and the resulting concentrations in pollen. As Xun et al. (2017) point out, further research is needed to better understand these transfer pathways.

PTTEs in agricultural chemicals

Potentially toxic trace elements such as B, Cu, Mn, and Zn are widely used in agriculture. The temporal variability of PTTEs, such as Cd, Mn, or Ni, observed in pollen samples in our study may also be influenced by the use of agricultural chemicals. This source of PTTEs is most likely more relevant than soil concentrations since PTTEs in the agricultural chemicals applied are likely to be more available to plants. Copper is used as a plant protection product for vegetables, fruits, berries, or vineyards (FSVO, 2025). Zinc is used as a fertilizer for vegetables such as onions and spinach (Schöneberg & Liebisch, 2023). Fertilizers containing B are important for beet, oilseed rape, and sunflower (Flisch et al., 2009). Oilseed rape and sunflower were cultivated at both our study sites. However, the level of B was higher in May 2023 at our control site compared to the waste deposit site, which might be related to the use of fertilizers containing B at the control site this year. Fertilizers containing Mn are important for sugar beet, grains, fruit trees, maize, oilseed rape, and potatoes (Neuweiler, 2011). In addition to its use as a fertilizer, Mn is also used as a food supplement to livestock and ends up as manure in the fields (Sager, 2007). This could be an explanation for the higher Mn levels in June at both our study sites.

Some agricultural chemicals contain PTTEs as contaminants, which may be spread directly on crops or indirectly through soil and water. In herbicides, PTTEs such as As, Co, Cr, Ni, and Pb have been detected (Defargé et al., 2018; Séralini and Jungers, 2020). Limestone is used for pH regulation in acidic soils (Holland et al., 2018) and may contain Mn, Ti, or Zn (Mulwa et al., 2012). Furthermore, Cd and U contaminations may derive from phosphate fertilizer (Bigalke et al., 2017). Such fertilizer may lead to PTTE accumulation in grain crops, such as wheat (Sabiha-Javied et al., 2023).

Climatic conditions, eolian transport, and atmospheric deposition of PTTEs

Climatic conditions may also influence the atmospheric deposition of PTTE on pollen. Wind causing eolian transport and deposition of particulate matter is well known (e.g., Zobeck & Van Pelt, 2006). Potentially toxic trace elements can travel long distances as fine particles in the air before settling down on plants and soil (He et al., 2023). Costa et al. (2019) found a correlation between Cd, Ni, and Pb concentrations in the atmosphere and those detected in honey bees. Overseas, Morgano et al. (2010) reported that the concentrations of Cd, Co, and Pb were higher during the dry season from August to October, a period that is more prone to air pollution. Similarly, Lambert et al. (2012) showed that Pb levels in pollen collected during dry months (late June/early July and late September/early October) were significantly higher than those collected earlier in the season. These studies are in line with our observations of peak concentrations of some PTTEs in the dryer season, such as Mn, Rb, Ba, Ni, and Cd in June or Pb and Cr in August (see online supplementary material for climate data in the surrounding of the studied apiaries; Figure S3).

PTTE levels in pollen compared to soil levels

We compared our data on pollen with total PTTE concentrations measured in topsoil between 1997 and 2015 in the surroundings of our apiaries, as reported by several studies (Bopp et al., 2016; Evangelou et al., 2013; Fässler et al., 2010; Reusser et al., 2023; Rytz, 2001). At the former municipal waste deposit site in Witzwil, PTTE levels in the topsoil were substantially above the levels measured at the control apiary in Bellechasse (Bopp et al., 2016; Evangelou et al., 2013; Fässler et al., 2010; Reusser et al., 2023), as summarized in online supplementary material, Tables S19 and S20. Evangelou et al. (2013) reported high levels of Cu (750 mg/kg), Mn (1146 mg/kg), Ni (92 mg/kg), Pb (878 mg/kg), Sb (36 mg/kg), and Zn (971 mg/kg) in topsoil samples obtained from Witzwil, most likely related to the previous deposition of waste. By contrast, the analysis of total PTTE concentrations in topsoil close to the apiary in Bellechasse revealed substantially lower Cu (38 mg/kg), Mn (398 mg/kg), Ni (34.0 mg/kg), Pb (26 mg/kg), Sb (0.4 mg/kg), and Zn (58 mg/kg) levels, as listed in the online supplementary material, Table S19 (Reusser et al., 2023).

The PTTE levels in the pollen samples of both apiaries were clearly below the total PTTE values of the topsoil. At the waste deposit site in Witzwil, the levels of Mn and Zn in pollen were 5–10 times below the values of the soil around the apiary, while the Pb concentration was 1,000 times lower in pollen. At the control apiary in Bellechasse, Cd and Zn levels were comparable in pollen and soil, while Mn and Pb concentrations were 3 and 250 times lower, respectively, in pollen than in topsoil (see online supplementary material, Table S19). As described above, PTTEs in soil are often only partially available for plant uptake; thus, a direct correlation between the total PTTE levels in soil and the levels found in pollen seems not possible for most PTTEs. A weak correlation between PTTE levels in polluted soils and bee pollen was observed in Kosovo by Kastrati et al. (2021).

The authors attributed their findings to partial PTTE uptake into plants related to soil characteristics. Plants grown on contaminated soil probably accumulate a larger PTTE fraction compared to plants grown on non-contaminated soil.

PTTE levels in pollen compared to levels in cultivations

Various crops and vegetables are grown on the former municipal waste deposit site Witzwil. A previous study by Rytz showed that Cd, Pb, and Zn levels in some crops grown on the waste deposit site were comparable to those measured in our pollen samples collected at our study site in Witzwil (Table 2; Rytz, 2001). The author attributed these results to the reduced bioavailability of PTTEs in soil as a result of spreading waste materials together with slag from coal ovens on the fields over decades in the 20th century (Rytz, 2001). Over time, the solubility of PTTEs in soils may be further reduced (Tuin & Tels, 1990), which may also be the case for Witzwil (Fässler et al., 2010). The levels of PTTEs found in our pollen samples correspond relatively well with the levels of some food products grown in the same area, for example, barley, wheat, spinach, or oilseed rape (Rytz, 2001). Therefore, bee pollen might be an easy way to prescreen agricultural areas with respect to potential problematic PTTE levels in vegetables and crops, especially where crops are grown on soil with high PTTE levels.

Toxicological implications for honey bees and humans

Acute toxicity of PTTEs to honey bees

In ecotoxicology, the acute toxicity of PTTEs is less frequently investigated compared to other potentially hazardous substances, such as pesticides (Sgolastra et al., 2018; Gekière et al., 2023). In general, levels of PTTE in pollen are higher than in nectar (Murashova et al., 2020), thus exposure to PTTEs in pollen usually poses the main risk for honey bees. Based on our results, a worst-case scenario for acute oral toxicity was calculated for adult honey bees for Cu, Cd, and Cr (Table 3). The measured maximal Cu, Cd, and Cr concentrations in pollen were multiplied by the assumed maximal daily pollen consumption of 12 mg to obtain the maximal PTTE uptake per bee and day (Table 3; EFSA, 2013). The lethal dose required to kill half of the tested population (Lewis et al., 2016) was subsequently divided by the maximal daily uptake of the PTTEs to obtain the toxicity exposure ratio (TER). All TER values of Cu, Cd, and Cr were considered low risk for acute toxicity, since they were well above 10. The lowest TER value was 53, a value calculated for copper oxychloride ($Cu_2(OH)_3Cl$). Copper oxychloride is an active substance in products used as a fungicide for fruit trees, vegetables, and vineyards (FSVO, 2025).

We cannot exclude synergistic and sublethal effects: For example, the combination of Pb, Cu, and As slowed down learning and impaired memory retrieval in honey bees, or high Mn concentrations may lead to a precocious switch from nurse to foragers and impair the bees' sense of direction, their learning, and memory abilities (Gekière et al., 2023).

Pollen as a food supplement for human consumption

Pollen is also occasionally used as a food supplement for human consumption or a food additive based on its antioxidant properties related to its high flavonoid and phenolic content (Zafeiraki et al., 2022). There are currently no maximum levels for PTTEs in pollen according to the European legislation (Regulation—2023/915—EN—EUR-Lex). However, if pollen is a component of a food

Table 3 Acute oral toxicity to adult honey bees.

PTTE	Max. conc. in pollen (mg/kg)	Max. daily uptake (μg/bee)[a]	Active substance	LD50[b] (μg/bee)	TER[c]
Cu	19.2	0.23	Copper oxychloride ($Cu_2(OH)_3Cl$)	12.1[d]	53
			Bordeaux mixture ($CuSO_4$ and CaO)	23.3[d]	101
			Tribasic copper sulphate ($Cu_4(OH)_6(SO_4)$)	40[d]	174
			Copper hydroxide ($Cu(OH)_2$)	49[d]	213
			Copper oxide (CuO)	116[d]	504
Cd	0.55	0.007	Cadmium sulphate ($CdSO_4$)	2.34[e]	334
			Cadmium chloride ($CdCl_2$)	3.51[e]	501
Cr	1.22	0.01	Chromium nitrate $Cr(NO_3)_3$	25.5[f]	2,550

Note: Cd, cadmium; Cr, chromium; Cu, copper; LD50, lethal dose; max. conc., maximal concentration; max. daily uptake, maximal daily uptake; PTTE, potentially toxic trace element; TER, toxicity exposure ratio.

[a] Max. daily uptake per bee: the measured maximal Cu, Cd, or Cr concentrations in pollen were multiplied by the assumed maximal daily pollen consumption of 12 mg for an adult honey bee (EFSA, 2013).

[b] Acute (48 hr) oral lethal dose (LD50) required of Cu, Cd, or Cr to kill half of the tested adult honey bees.

[c] The TER was calculated by dividing the LD50 by the maximal uptake of Cu, Cd, or Cr.

[d] EFSA (2018).

[e] Cronn (1991).

[f] Sgolastra et al. (2018).

supplement, the corresponding maximum levels of 3 mg/kg for Pb apply to the end product. The maximum value for lead of 0.3 mg/kg in our samples is well below these specifications. Furthermore, pollen samples were collected in our study over a period of 24 hr only. These daily pollen samples, therefore, do not represent pollen as it is sold for human consumption. Pollen for this purpose is usually a mixture of pollen collected over several months, and as a result, contaminants are usually present in lower concentrations than in daily collected pollen samples. Thus, the assessment of a realistic consumption scenario is beyond the scope of this study.

Conclusion

An analytical method was validated that allowed for the quantitation of 22 PTTEs in pollen. Nineteen PTTEs were identified in bee pollen collected from an apiary bordering a former municipal waste deposit site in Witzwil or a control apiary, both of which were located in an agricultural area. The comparison of pollen from the former waste deposit site with pollen from a non-polluted site revealed a tendency toward higher PTTE levels, as shown, for example, by elevated levels of Ba, Cd, Co, Cs, Cu, Mn, Ni, Rb, and Zn in pollen from the former waste deposit site. The significant temporal variations in PTTE levels in pollen during the two analyzed seasons emphasize the importance of conducting multiple samplings throughout the year. Although many factors affect PTTE levels in pollen, bee pollen is relatively cost-effective and might be an easy way to obtain an overview of PTTE contamination in a broader area.

Finally, studying PTTEs in pollen allows the evaluation of exposure scenarios for honey bees. Based on our results, we expect no increased acute oral toxicity to adult honey bees above the expected mortality levels. Our study is limited to PTTE levels in pollen of the two study sites. However, measuring PTTE levels in pollen is a simple, low-cost, and time-efficient method for evaluating exposure risks on honey bees through variable landscapes. With a density of 4.4 bee colonies per km^2 in Switzerland (Charrière & Würgler, 2024), there is huge potential for future surveys that include other types of contaminants. The variations of PTTEs in pollen throughout the season may be related to the sequential blooming periods of various foraging plants and thus to plant type-related variations in PTTE uptake. To test this hypothesis, further studies are necessary to identify the botanical origins of the collected pollen types while simultaneously assessing the PTTE levels in each type of pollen. The knowledge of pollen types containing high PTTE levels could explain which plant types accumulate PTTEs.

Acknowledgments

We would like to thank the Construction and Transport Directorate of the Canton of Bern and the National Soil Monitoring Programme (NABO) for providing data. The soil data close to the Bellechasse apiary were collected as part of the nationwide grid sampling of topsoil in the Biodiversity Monitoring (BDM) programme of the National Soil Monitoring Programme (NABO).

Supplementary material

Supplementary material is available at *Environmental Toxicology and Chemistry* online.

Conflicts of 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.

Funding

Open access was provided by Agroscope.

Data availability

Data can be found in the Supporting information.

Author contributions

Samira Stalder (Data curation, Investigation, Methodology, Writing—original draft, Writing—review & editing), René Nussbaumer (Methodology, Supervision), Marion Fracheboud (Investigation, Methodology, Writing—review & editing), Benoît Droz (Investigation, Methodology, Writing—review & editing), Adrien Mestrot (Conceptualization, Methodology, Project administration, Supervision, Writing—review & editing), and Christina Kast (Conceptualization, Methodology, Project administration, Supervision, Writing—review & editing)

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Environmental Toxicology and Chemistry, 2026, 45, 2340–2355
https://doi.org/10.1093/etojnl/vgag123
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