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Assessment of heavy metals in soil, oilseed rape (*Brassica napus* L.) and honey

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Abstract: The aim of this study was to determine the heavy metals concentrations in soil, inflorescences of *Brassica napus* L. and rapeseed honey sampled from some regions of north-eastern Bulgaria. Thirteen locations were selected for experimental studies. The soils, plants and honey samples were taken from conventional beekeeping areas away from major industrial pollutants. The median amounts of elements in the soil samples (mg/kg dry weight (DW)) are 1.1246 for $Fe_{(s)}$, 0.7048 for $Al_{(s)}$, 0.5636 for $Pb_{(s)}$, 0.1658 for $Cu_{(s)}$ and 0.0148 for $Zn_{(s)}$. The median amounts of heavy metals measured in the inflorescences of *Brassica napus* (mg/kg DW) are 5.5430 for $Fe_{(p)}$, 2.9095 for $Zn_{(p)}$, 1.3225 for $Pb_{(p)}$, 0.2593 for $Cu_{(p)}$ and 0.2105 for $Al_{(p)}$. The median heavy metals concentrations in tested honey (mg/kg DW) are 1.0026 for $Fe_{(h)}$, 0.1849 for $Al_{(h)}$, 0.1832 for $Pb_{(h)}$, 0.1250 for $Zn_{(h)}$, and 0.0702 for $Cu_{(h)}$. The relationship between the heavy metal in soil, plants and honey was investigated using Spearman's rank correlation coefficient. Significant differences in the concentrations of $Fe_{(s)}$, $Al_{(s)}$ and $Pb_{(s)}$ in soils, $Fe_{(p)}$ and $Zn_{(p)}$ in the plant samples and $Fe_{(h)}$ in honey samples from the different locations were found. The heavy metal content tested in honey did not pose a risk to human health.

Keywords: estimation; canola; blossom; accumulation; metal ions

The accelerated pace of industrialisation in the modern world has led to permanent pollution of the environment. Agriculture, as part of modern production, is no exception. In recent years, an increase in the concentration of trace elements in the soil has been observed as a result of the industrial activity of modern society (Steinnes and Friedland 2006, Avci and Devici 2013, Sudhakaran et al. 2018). As is known, plants are at the base of the food chain and are the only living organisms that manage to synthesise sunlight and nutrients from the soil in an accessible form for animals and humans.

Environmental pollution has a direct impact on plants, leading to the accumulation of harmful trace elements in plant biomass. Some of these elements, such as Fe, Cu, Zn and Mg, are useful for the functioning of the human body, but only if they are in the necessary amounts (Stern 2010, Rout and Sahoo

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2015). Other elements, such as As, Cd, Pb, etc., have a harmful effect on human health, causing a number of diseases (Bartha et al. 2020). Also, plants contaminated with heavy metals can cause an additional indirect threat through their impact on some pollinators, such as honey bees *Apis mellifera* L. and bee products obtained from them (Afzal et al. 2013, Ali et al. 2019).

Honey is a food product that is in direct relationship with plants, which is obtained as a result of the activity of bees (Can et al. 2018). At the same time, bees are the second intermediate link after plants, where heavy metals accumulate before they reach honey. The bioaccumulation of heavy metals in honey bees, *Apis mellifera ligustica* and honey allows one to determine the level of pollution in the territory of a certain region and depends on many local conditions (Goretti et al. 2019). Bees' bodies are an effective barrier to the migration of heavy metals from the environment to honey; because of this, the honey is free from pollution and safe for human consumption (Tomczyk et al. 2020).

According to Angelova et al. (2017) and Tomczyk et al. (2020), individual plant species and different botanical parts of the plants exhibit varying degrees of tendency to accumulate heavy metals. Therefore, it is extremely important to study the dynamics of the transfer of heavy metals from soil to plants and from there to honey. In Atanasov et al. (2022), a model of four first-order ordinary differential equations is considered to describe the binding of heavy metal ions to hydrogen ions and their movement in plant biomass. For beekeeping, the tendency to accumulate heavy metals from the inflorescences of flowering plants and the contamination of nectar and pollen collected by bees is of great importance. In addition to the direct influence of the food environment on bees and the accumulation of heavy metals and toxic elements in their bodies (Costa et al. 2019, Sadowska et al. 2019, Borsuk et al. 2021), the bee organisms are also affected by the environment, through their until now contaminated water sources, polluted air, etc. The content of heavy metals and toxic trace elements in honey depends on the bee colonies' geographical origin and location (Bilandžić et al. 2019, Ghidotti et al. 2021, Ligor et al. 2022). The study of regional environmental pollution is of great importance for the bee products produced in a given region.

The aim of our study was to determine the content of heavy metals in the soil, rapeseed inflorescences and in rapeseed honey depending on the local geographical features of different beekeeping regions of north-eastern Bulgaria.

MATERIAL AND METHODS

Locations of collected samples

The survey was conducted in 2021 in 13 beekeeping regions of north-eastern Bulgaria. The geographical location of the experimental regions are Volovo (A1) – 43°31'36.48"N, 25°47'49.72"E; Brestovica (A2) – 43°32'1.95"N, 25°45'28.70"E; Basarbovo (A3) – 43°46'36.36"N, 25°57'3.39"E; Yudelnik (A4) – 43°51'25.44"N, 26°15'14.85"E; Krivnia (A5) – 43°39'3.16"N, 26°20'3.38"E; Yuper (A6) – 43°54'40.33"N, 26°23'47.41"E; V. Tarnovo (A7) – 43°4'32.43"N, 25°37'1.75"E; Tetovo (A8) – 43°49'9.77"N, 26°17'11.03"E; Shtraklevo (A9) – 43°43'16.63"N, 26°2'34.24"E; Gorsko Ablanovo (A10) – 43°29'47.81"N, 26°4'28.20"E; Bazan (A11) – 43°44'13.92"N, 26°6'21.79"E; Slivo pole (A12) – 43°56'37.52"N, 26°12'16.26"E; Glavinica (A13) – 43°54'40.28"N, 26°50'3.19"E. In total, wear collected 13 rapeseed honey samples, 13 soil samples and 13 rapeseed flower samples. Sampling was carried out within each district, and specific apiaries and oilseed rape fields were chosen with the help of beekeepers and farmers. Three sampling apiaries were selected in each district, and a beekeeper was assigned to aid in the honey collection. The sampling of soil, flowers and honey were collected simultaneously. The distance between individual apiaries in each region was about 3.0 km. For the purpose of the experiment, the honey samples were taken from apiaries located 2.5 km from the oilseed rape fields. Honey samples of 300 g wear were collected directly from the hives by cutting the honeycomb and squeezing the honey into glass containers. After collection, the samples were stored in the dark at room temperature until analysis. The soil samples were collected at a depth of 20 cm by cross-diagonal traversal of rapeseed fields. Soil samples for each region were collected from three canola fields. After collecting the soil samples, they were mixed into one common sample for each studied region. After collection, the samples were stored in paper bags in a dry room. In all regions, the soil type studied was leached chernozem. Plant samples were taken from the same fields by cross-diagonal traversal of rapeseed fields. One of the rapeseed fields in region A4 and two in region A8 were located 15 m apart from the main road Ruse-Tetovo and Ruse-

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Yudelnik. After collection, the flowers were dried in the shade for 30 days, then ground and stored in paper bags at room temperature until analysis.

According to a report by the Executive Environment Agency (EEA) in 2021, in the north-eastern region of Bulgaria, no maximum allowable concentrations of toxic elements such as As, Cd and Hg in the soil have been detected. The locations from which the soil samples were taken are far from major industrial pollutants, which could be sources of contamination with toxic trace elements. Keeping this feature in mind, we focused on investigation concentrations of only some elements, such as iron (Fe), copper (Cu), lead (Pb), aluminium (Al) and zinc (Zn) in tested soil, plants and honey.

Metal analyses

Honey digestion method. 1 g of honey samples were weighed and placed in a conical flask. Honey samples were mixed and homogenised. 8 mL of concentrated nitric acid and 4 mL of hydrogen peroxide were added. The samples were heated for 4 h to dry in the water bath. To the cooled samples, de-ionised water was added to dissolve the dried mass, and the content was filtered. Subsequently, the solution was made up to volume using de-ionised water (Tuzen et al. 2007, Adugna 2020).

Soil extraction method. Mehlich-1 solution (10:1) was used. Soil extracts (10:1 extractant/soil ratio) were obtained with Mehlich-1 (0.05 mol/L HCl + 0.025 mol/L H_2SO_4) solution and were shaken for 5 min (Abreu et al. 1998, Fonseca et al. 2010). We chose an extraction solution that does not contain helating agents, such as DTPA-TEA or EDTA, because we applied spectrophotometric methods that may be incompatible with helating agents, and we used the same solution for all the investigated elements. In addition, the Mehlich solution application gives high correlation coefficients with the change of soil pH in the extraction of some metals (Fonseca et al. 2010). Mehlich is indicated as a suitable solution for the extraction of trace elements in alkaline and neutral soils (Wang et al. 2021), such as the calcium, leached and typical chernozems, spread in the region of Northern Bulgaria, Danube Plain.

Plant materials digestion method. Dried rapeseed flower samples were ground. 5 mL of HNO_3 was added to 0.5 g of sample in a 250 mL dry flask and stirred. In a well-ventilated hood, 4 mL of 33% H_2O_2 were carefully added to flasks and were stirred intensively. These solutions were heated on a hot plate and produced a strong effervescence. When the brown fumes were less dense, heating was stopped and the samples were allowed to cool. The solutions were filtered, washed with 5 mL of (1:1) HCl (density 1.18 g/mL) and diluted with de-ionised H_2O (Pequerul et al. 1993).

The obtained solutions of honey, flowers and soil were brought to the required pH for spectrophotometric measurements and up to the volume of 50 mL with de-ionised H_2O. CECIL 2000 series uv-vis spectrophotometer (Cecil Instruments Limited, Cambridge, UK) and Merck spectrophotometric tests for Pb, Lovibond for Cu, Fe, Spectroquantum for Al, and Hach for Zn were used.

Statistical analysis

For the statistical processing of the data obtained, the software Statistica 10 (StataCorp LP®, Texas, USA) was used. The concentrations of elements in tested soil, inflorescences of *Brassica napus* and honey of *Brassica napus* were expressed as mean ± standard deviation (SD). The link between the heavy metal levels in soil, inflorescences of *Brassica napus* and honey of *Brassica napus* samples was investigated using the Spearman's rank correlaon $P < 0.05$.

RESULTS AND DISCUSSION

According to the change of the median of the heavy metals, a decrease in the concentration of heavy metals in the soil is observed in the sequence $Fe_{(s)}$ > $Al_{(s)}$ > $Pb_{(s)}$ > $Cu_{(s)}$ > $Zn_{(s)}$ (Table 1).

From the data analysis of the soil samples taken, it was found that the concentration of metal ions was the highest for $Fe_{(s)}$ and the lowest for $Zn_{(s)}$. Significant differences in the concentrations of $Fe_{(s)}$, $Al_{(s)}$ and $Pb_{(s)}$ were found in soils from different locations. The highest levels of $Fe_{(s)}$ (3.6669 mg/kg dry weight (DW)) were determined in region A2, located in Brestovica, and $Fe_{(s)}$ (3.6618 mg/kg DW), for region A7, located in Veliko Tarnovo. The distance between A2 and A7 is 53 km. Soil collected from region A1, located near the Volovo, showed the highest $Al_{(s)}$, (1.0486 mg/kg DW) content. In soil from the Veliko Tarnovo location (A7), the highest $Pb_{(s)}$ levels of 0.6962 mg/kg were measured. Due to the absence of industrial pollutants in regions A2 and A1, the higher $Fe_{(s)}$ and $Al_{(s)}$ concentration in the soil samples is most likely due to geogenic factors. The possible reason for the increased concentration of $Pb_{(s)}$ in the soil samples

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Table 1. Heavy metals concentrations in tested soil collected from some regions in north-eastern Bulgaria (mg/kg dry weight ± standard deviation)

Region	$Fe_{(s)}$	$Cu_{(s)}$	$Pb_{(s)}$	$Al_{(s)}$	$Zn_{(s)}$
A1	1.8029 ± 0.17	0.1250 ± 0.04	0.5049 ± 0.16	1.0486 ± 0.07	0.0020 ± 0.000
A2	3.6669 ± 0.94	0.0995 ± 0.04	0.4769 ± 0.04	0.8349 ± 0.09	0.0148 ± 0.007
A3	1.9380 ± 0.34	0.2703 ± 0.09	0.2066 ± 0.02	0.1372 ± 0.03	0.0296 ± 0.015
A4	1.1246 ± 0.03	0.1938 ± 0.08	0.5789 ± 0.03	0.7048 ± 0.20	0.0048 ± 0.005
A5	1.7595 ± 0.34	0.3366 ± 0.08	0.5865 ± 0.28	0.9231 ± 0.24	0.0041 ± 0.002
A6	0.2117 ± 0.12	0.3213 ± 0.03	0.2142 ± 0.12	0.1754 ± 0.04	0.0250 ± 0.014
A7	3.6618 ± 0.77	0.2270 ± 0.05	0.6962 ± 0.15	0.5845 ± 0.11	0.0135 ± 0.002
A8	0.8721 ± 0.04	0.0816 ± 0.03	0.1250 ± 0.04	0.1010 ± 0.02	0.1984 ± 0.040
A9	0.1964 ± 0.13	0.1658 ± 0.09	0.6630 ± 0.15	0.6860 ± 0.18	0.0107 ± 0.001
A10	1.9074 ± 0.19	0.0306 ± 0.01	0.5636 ± 0.16	0.7217 ± 0.19	0.0094 ± 0.003
A11	0.6324 ± 0.10	0.4157 ± 0.16	0.1148 ± 0.09	0.1540 ± 0.03	0.0365 ± 0.019
A12	1.1118 ± 0.12	0.0944 ± 0.04	0.6146 ± 0.12	0.8129 ± 0.03	0.0148 ± 0.007
A13	0.3392 ± 0.07	0.0153 ± 0.01	0.6630 ± 0.02	0.7752 ± 0.18	0.0222 ± 0.011
Average	1.4788	0.1828	0.4621	0.5892	0.0297
Median	1.1246	0.1658	0.5636	0.7048	0.0148
Max	3.6669	0.4157	0.6962	1.0486	0.1984
Min	0.1964	0.0153	0.1148	0.1010	0.0020

A1 – Volovo; A2 – Brestovica; A3 – Basarbovo; A4 – Yudelnik; A5 – Krivnia; A6 – Yuper; A7 – V. Tarnovo; A8 – Tetovo; A9 – Shtraklevo; A10 – Gorsko Ablanovo; A11 – Bazan; A12 – Slivo pole; A13 – Glavinica

for region A7 is the field's proximity to a main road with high vehicular traffic. Despite the increased levels of the two toxic elements $Al_{(s)}$ and $Pb_{(s)}$ in the soil samples in two of the studied regions, they remain close to or below a tolerable weekly intake set by the European Food Safety Authority (EFSA).

When comparing the presented results to the findings of other authors concerning toxic metals in soil samples from different regions in different countries (Tomczyk et al. 2020, Sur et al. 2022), our study establishes low contamination levels with trace elements.

The change of the median in the inflorescences of *Brassica napus* (Table 2) is in sequence $Fe_{(p)} > Zn_{(p)} > Pb_{(p)} > Cu_{(p)} > Al_{(p)}$.

From the analysis of the plant samples taken, it was found that for the individual regions from A1 to A13, an increase in the concentration of metal ions was observed in the inflorescences of *Brassica napus* compared to the soil samples from the same regions. The statement of Angelova et al. (2017) that *Brassica napus* tend to accumulate heavy metals in different botanical parts of the plant is confirmed. The results Table 2 show the high ability of inflorescences of rape to accumulate $Fe_{(p)}$ (5.5430 mg/kg DW), $Zn_{(p)}$ (2.9095 mg/kg DW) and $Pb_{(p)}$ (1.3225 mg/kg DW). In the case of the other elements $Cu_{(p)}$ and $Al_{(p)}$, the accumulation capacity is insignificant. The highest levels of $Fe_{(p)}$ 16.0770 mg/kg DW) were determined in region A13 located in Glavinica, $Fe_{(p)}$ (13.5700 mg/kg DW) for region A9 located in Shtraklevo, $Fe_{(p)}$ (10.4176 mg/kg DW) for region A4 located in Yudelnik and $Fe_{(p)}$ (10.0456 mg/kg DW) for region A8 located in Tetovo. In inflorescences of *Brassica napus* from the Yuper location (A6), the highest $Zn_{(p)}$ levels of 4.6725 mg/kg DW were measured. For location (A4), the highest $Pb_{(p)}$ levels of 2.4691 mg/kg DW were measured. The variable concentrations of heavy metals in different oilseed rape cultivars (Angelova et al. 2017, Tomczyk et al. 2020) indicate the different absorption abilities of each cultivar. The intensity of element uptake and release by plants is significantly affected by the mechanical and chemical composition of the soil (Petryk 2016). The difference in the concentration of heavy metals in the plant samples taken from the different geographical regions A1 to A13 is determined by the difference in the accumulative capacity of the sown rapeseed cultivars and soil composition.

In the honey samples (Table 3), the variation of the concentration of heavy metals according to the

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Table 2. Heavy metals concentrations in tested inflorescences of *Brassica napus* collected from some regions in north-eastern Bulgaria (mg/kg dry weight ± standard deviation)

Region	$Fe_{(p)}$	$Cu_{(p)}$	$Pb_{(p)}$	$Al_{(p)}$	$Zn_{(p)}$
A1	4.1490 ± 0.59	0.1466 ± 0.00	0.9020 ± 0.03	0.1872 ± 0.12	2.2775 ± 0.43
A2	3.2545 ± 0.36	0.1150 ± 0.02	1.6445 ± 0.63	0.2427 ± 0.08	2.9095 ± 0.70
A3	2.3920 ± 0.98	0.1265 ± 0.05	1.4145 ± 0.42	0.1909 ± 0.02	2.6910 ± 0.66
A4	10.4176 ± 1.86	0.1691 ± 0.03	2.4691 ± 0.38	0.3833 ± 0.12	2.2436 ± 0.06
A5	6.8425 ± 1.35	0.6095 ± 0.06	1.3225 ± 0.25	0.2576 ± 2.89	3.5294 ± 0.93
A6	5.5430 ± 0.91	0.4255 ± 0.18	1.4145 ± 0.26	0.2024 ± 0.03	4.6725 ± 0.28
A7	6.4170 ± 0.73	0.5405 ± 0.16	1.1845 ± 0.23	0.2427 ± 0.06	2.5346 ± 0.56
A8	10.0456 ± 1.21	0.2480 ± 0.04	2.4127 ± 0.30	0.1184 ± 0.02	3.2211 ± 0.19
A9	13.5700 ± 2.53	0.5405 ± 0.08	1.5755 ± 0.03	0.2105 ± 0.07	2.6220 ± 0.26
A10	4.3240 ± 0.60	0.4830 ± 0.05	1.0235 ± 0.39	0.3611 ± 0.06	3.3040 ± 0.66
A11	1.8170 ± 0.15	0.2593 ± 0.06	1.0147 ± 0.05	0.3867 ± 0.03	3.9427 ± 0.35
A12	5.4294 ± 0.76	0.1548 ± 0.05	1.1500 ± 0.04	0.1902 ± 0.06	3.6269 ± 0.12
A13	16.0770 ± 0.67	1.6790 ± 0.17	0.9315 ± 0.14	0.1737 ± 0.01	1.9412 ± 0.52
Average	6.9445	0.4229	1.4200	0.2421	3.0397
Median	5.5430	0.2593	1.3225	0.2105	2.9095
Max	16.0770	1.6790	2.4691	0.3867	4.6725
Min	1.8170	0.1150	0.9020	0.1184	1.9412

A1 – Volovo; A2 – Brestovica; A3 – Basarbovo; A4 – Yudelnik; A5 – Krivnia; A6 – Yuper; A7 – V. Tarnovo; A8 – Tetovo; A9 – Shtraklevo; A10 – Gorsko Ablanovo; A11 – Bazan; A12 – Slivo pole; A13 – Glavinica

Table 3. Heavy metals concentrations in tested honey of *Brassica napus* collected from some regions in north-eastern Bulgaria (mg/kg dry weight ± standard deviation)

Region	$Fe_{(h)}$	$Cu_{(h)}$	$Pb_{(h)}$	$Al_{(h)}$	$Zn_{(h)}$
A1	0.829 ± 0.07	0.0399 ± 0.01	0.2730 ± 0.04	0.1942 ± 0.02	0.3465 ± 0.12
A2	0.439 ± 0.62	0.0585 ± 0.02	0.1287 ± 0.02	0.1696 ± 0.03	0.1871 ± 0.04
A3	1.126 ± 0.06	0.1088 ± 0.01	0.2103 ± 0.04	0.1769 ± 0.00	0.2350 ± 0.03
A4	0.793 ± 0.20	0.0662 ± 0.02	0.0099 ± 0.01	0.1434 ± 0.02	0.1169 ± 0.02
A5	1.003 ± 0.03	0.1722 ± 0.02	0.4954 ± 0.13	0.1569 ± 0.01	0.1250 ± 0.02
A6	2.944 ± 0.22	0.1860 ± 0.02	0.2893 ± 0.06	0.2004 ± 0.03	0.1095 ± 0.01
A7	1.033 ± 0.14	0.0148 ± 0.00	0.0682 ± 0.02	0.1874 ± 0.02	0.0931 ± 0.01
A8	0.977 ± 0.17	0.3542 ± 0.14	0.1832 ± 0.05	0.2443 ± 0.02	0.1417 ± 0.02
A9	3.249 ± 0.14	0.0149 ± 0.00	0.1602 ± 0.03	0.2162 ± 0.04	0.1579 ± 0.02
A10	0.763 ± 0.05	0.0000 ± 0.00	0.0596 ± 0.01	0.2014 ± 0.02	0.1168 ± 0.01
A11	0.7618 ± 0.01	0.2691 ± 0.01	0.3042 ± 0.02	0.1849 ± 0.01	0.1954 ± 0.01
A12	2.241 ± 0.08	0.0702 ± 0.01	0.3233 ± 0.01	0.1695 ± 0.03	0.0858 ± 0.01
A13	1.153 ± 0.21	0.2940 ± 0.06	0.0341 ± 0.01	0.1529 ± 0.04	0.0223 ± 0.00
Average	1.3315	0.1268	0.1953	0.1845	0.1487
Median	1.0026	0.0702	0.1832	0.1849	0.1250
Max	3.2494	0.3542	0.4954	0.2443	0.3465
Min	0.4389	0.0000	0.0099	0.1434	0.0223

A1 – Volovo; A2 – Brestovica; A3 – Basarbovo; A4 – Yudelnik; A5 – Krivnia; A6 – Yuper; A7 – V. Tarnovo; A8 – Tetovo; A9 – Shtraklevo; A10 – Gorsko Ablanovo; A11 – Bazan; A12 – Slivo pole; A13 – Glavinica

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Table 4. Spearman's rank correlation coefficients between heavy metals in soil ($Fe_{(s)}$, $Cu_{(s)}$, $Pb_{(s)}$, $Al_{(s)}$, $Zn_{(s)}$) and inflorescences of *Brassica napus* ($Fe_{(p)}$, $Cu_{(p)}$, $Pb_{(p)}$, $Al_{(p)}$, $Zn_{(p)}$). Bold correlations are at $P < 0.05$

	$Fe_{(s)}$	$Cu_{(s)}$	$Pb_{(s)}$	$Al_{(s)}$	$Zn_{(s)}$	$Fe_{(p)}$	$Cu_{(p)}$	$Pb_{(p)}$	$Al_{(p)}$
$Fe_{(s)}$	1.000								
$Cu_{(s)}$	−0.027	1.000							
$Pb_{(s)}$	−0.017	−0.264	1.000						
$Al_{(s)}$	0.264	−0.253	0.443	1.000					
$Zn_{(s)}$	−0.278	0.030	−0.543	**−0.757**	1.000				
$Fe_{(p)}$	−0.495	−0.324	**0.619**	0.038	−0.195	1.000			
$Cu_{(p)}$	−0.443	0.011	**0.555**	−0.003	−0.138	**0.638**	1.000		
$Pb_{(p)}$	0.014	0.107	−0.157	−0.363	0.152	0.272	−0.253	1.000	
$Al_{(p)}$	0.206	0.512	−0.030	0.022	−0.288	−0.250	0.106	0.105	1.000
$Zn_{(p)}$	−0.181	0.379	−0.473	−0.209	0.358	−0.445	−0.058	−0.003	0.228

median decreases in the order $Fe_{(h)} > Al_{(h)} > Pb_{(h)} > Zn_{(h)} > Cu_{(h)}$.

In contrast to soil and plants, the concentration of heavy metals in rapeseed honey is significantly lower. According to Costa et al. (2019) and Sadowska et al. (2019) bees' bodies accumulate heavy metals. There are differences in the concentration of individual elements in the honey samples taken in different geographical regions. As with the soil and plant samples, as with the rapeseed honey, the highest levels of metal ions were for $Fe_{(h)}$ and the lowest for $Cu_{(h)}$. The highest levels of $Fe_{(h)}$ (3.249 mg/kg DW) were determined in region A9 located in Shtraklevo, $Fe_{(h)}$ (2.944 mg/kg DW) for region A6 located in Yuper and $Fe_{(h)}$ (2.241 mg/kg DW) for region A6 located in Slivo pole. Honey collected from region A8, located in Tetovo, showed the highest $Al_{(h)}$, (0.2443 mg/kg DW) content. In honey from the Krivnia location (A5), the highest $Pb_{(h)}$ levels of 0.4954 mg/kg DW were measured. The $Pb_{(h)}$ content in honey samples for all regions from A1 to A13 is within the limits allowed by EU Commission Regulation No. 1881/2006. Comparison of our study with other literature data shows differences with respect to the influence of geographical locations and the botanical origin of honey on the degree of concentration of heavy metals in honey. For example, Tomczyk et al. (2020) found that the content of heavy metals in bee honey from honeydew, goldenrod, dandelion, rapeseed and tilia is different, as with rapeseed honey, there are elevated levels of nickel (Ni) and Al. Other authors (Bilandžić et al. 2019, Ghidotti et al. 2021, Ligor et al. 2022, Ševevičienė et al. 2022) prove that with the same type of honey but of different geographical origin, there are differences in the concentration of individual elements.

Table 5. Spearman's rank correlation coefficients between heavy metals in soil ($Fe_{(s)}$, $Cu_{(s)}$, $Pb_{(s)}$, $Al_{(s)}$, $Zn_{(s)}$) and honey ($Fe_{(h)}$, $Cu_{(h)}$, $Pb_{(h)}$, $Al_{(h)}$, $Zn_{(h)}$). Bold correlations are at $P < 0.05$

	$Fe_{(s)}$	$Cu_{(s)}$	$Pb_{(s)}$	$Al_{(s)}$	$Zn_{(s)}$	$Fe_{(h)}$	$Cu_{(h)}$	$Pb_{(h)}$	$Al_{(h)}$
$Fe_{(s)}$	1.000								
$Cu_{(s)}$	−0.027	1.000							
$Pb_{(s)}$	−0.017	−0.264	1.000						
$Al_{(s)}$	0.264	−0.253	0.443	1.000					
$Zn_{(s)}$	−0.278	0.030	−0.543	**−0.757**	1.000				
$Fe_{(h)}$	−0.544	0.016	0.448	−0.159	0.110	1.000			
$Cu_{(h)}$	−0.465	0.121	−0.450	−0.355	**0.669**	0.107	1.000		
$Pb_{(h)}$	−0.170	0.516	−0.325	0.066	0.129	0.192	0.325	1.000	
$Al_{(h)}$	−0.181	−0.099	−0.275	−0.429	0.206	0.115	−0.198	0.077	1.000
$Zn_{(h)}$	0.239	0.330	**−0.609**	−0.069	−0.008	−0.415	−0.091	0.264	0.286

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Table 6. Correlation coefficients between heavy metals in inflorescences of *Brassica napus* ($Fe_{(p)}$, $Cu_{(p)}$, $Pb_{(p)}$, $Al_{(p)}$, $Zn_{(p)}$) and honey ($Fe_{(h)}$, $Cu_{(h)}$, $Pb_{(h)}$, $Al_{(h)}$, $Zn_{(h)}$). Bold correlations are at $P < 0.05$

	$Fe_{(p)}$	$Cu_{(p)}$	$Pb_{(p)}$	$Al_{(p)}$	$Zn_{(p)}$	$Fe_{(h)}$	$Cu_{(h)}$	$Pb_{(h)}$	$Al_{(h)}$
$Fe_{(p)}$	1.000								
$Cu_{(p)}$	**0.638**	1.000							
$Pb_{(p)}$	0.272	–0.253	1.000						
$Al_{(p)}$	–0.250	0.106	0.105	1.000					
$Zn_{(p)}$	–0.445	–0.058	–0.003	0.228	1.000				
$Fe_{(h)}$	0.484	0.377	0.008	–0.498	0.000	1.000			
$Cu_{(h)}$	0.116	0.080	0.044	–0.346	0.256	0.107	1.000		
$Pb_{(h)}$	–0.407	–0.127	–0.215	–0.080	**0.714**	0.192	0.325	1.000	
$Al_{(h)}$	–0.088	0.033	0.055	–0.212	0.264	0.115	–0.198	0.077	1.000
$Zn_{(h)}$	**–0.572**	–0.545	0.074	0.094	0.014	–0.415	–0.091	0.264	0.286

The relationship between heavy metal levels in soil, inflorescences of *Brassica napus* and honey of *Brassica napus* samples was analysed by Spearman's rank correlation coefficient (Tables 4–6). The Spearman's correlation relationship between the different heavy metals analysed in the soil and inflorescences of *Brassica napus* is summarised in Table 4. A strong correlation was found between $Pb_{(s)}$ and $Fe_{(p)}$ (r = 0.619) and also between $Pb_{(s)}$ and $Cu_{(p)}$ (r = 0.555). A positive correlation between $Fe_{(p)}$ and $Cu_{(p)}$ (r = 0.638) is also observed. The negative relationship is between $Al_{(s)}$ and $Zn_{(s)}$. No correlation was observed between the other elements.

A Table 5 summarised that there is a significant correlation between the $Zn_{(s)}$ and $Cu_{(h)}$ (r = 0.669). The negative relationship is between $Al_{(s)}$ and $Zn_{(s)}$ and also between $Pb_{(s)}$ and $Zn_{(h)}$.

A Table 6 summarised that there is a significant correlation between the $Zn_{(p)}$ and $Pb_{(h)}$ (r = 0.714). The negative relationship is between $Fe_{(p)}$ and $Zn_{(h)}$.

From the analysis of the obtained results in Tables 5 and 6, it can be seen that the amount of zinc in the soil $Zn_{(s)}$ affects the amount of copper $Cu_{(h)}$ in honey and the amount of zinc in plants $Zn_{(p)}$ affects the amount of lead $Pb_{(h)}$ in honey.

In conclusion, the differences in the content of the investigated elements in the soil, plant and honey samples taken depend on the geographical area, the composition of the soil and the cultivated cultivars and cultivars of rapeseed. A strong correlation was found between lead in soil $Pb_{(s)}$ and iron in plants $Fe_{(p)}$ (r = 0.619), between lead in soil $Pb_{(s)}$ and copper in plants $Cu_{(p)}$ (r = 0.555), between zinc in soil $Zn_{(s)}$ and copper in honey $Cu_{(h)}$ (r = 0.669), between zinc in plants $Zn_{(p)}$ and lead in honey $Pb_{(h)}$ (r = 0.714). The median concentration of metal ions in honey compared to those in inflorescences of *Brassica napus* decreased by 4.5404 mg/kg DW for iron Fe, by 0.1891 mg/kg DW for copper Cu, by 1.1393 mg/kg DW for lead Pb, with 0.0256 mg/kg DW for aluminium Al, with 2.7845 mg/kg DW for zinc Zn. The content of heavy metals in the honey from the thirteen investigated sites does not pose a risk to human health. The results offer valuable information to rapeseed honey consumers regarding the safety of consuming it in the examined areas.

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