TRANSFER OF SOME TOXIC METALS FROM SOIL TO HONEY DEPENDING ON BEE HABITAT CONDITIONS

– Research paper –

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Abstract: The transfer of toxic metals from soil to honey was studied based on two different areas of the Podkarpackie region located in the south-east part of Poland: U-urbanized and E-ecologically pure. The metal content was determined using the ICP-OES method with prior microwave mineralization of the soil, plant (goldenrod, dandelion, rapeseed, tilia and fir), bee bodies and honey samples collected from 10 sampling points (U-5 and E-5). The impact of soil pH on heavy metal mobility was also evaluated. It was found that Podkarpackie soils are less contaminated with heavy metals as compared to other regions of Poland and only in the case of cadmium an enhancement of the natural background level was observed. The migration of heavy metals, especially cadmium, in the soil-plant-bee-honey food chain was accelerated by soil acidity ($p < 0.05$). The influence of human activity (region development) on heavy metals concentrations was not significant ($p > 0.05$). Based on bioaccumulation factors, goldenrod and dandelion plants were confirmed as cadmium accumulators. It was also confirmed that the bodies of bees act as an effective barrier to the migration of heavy metals from the environment to honey, due to this, honey is free from these metals and safe for human consumption.

Key words: honey, heavy metals, soil, melliferous plant, food chain

INTRODUCTION

The accumulation of toxic metals in agricultural soils results from industrialization and other anthropogenic sources, mainly the metallurgy industry, energy and fuel production, traffic activities, intensive agriculture and sludge usage (Mirecki et al., 2015; Yan et al., 2018; Ali et al., 2019). The solubility of trace elements depends on various factors such as the presence of minerals and organic matter in the soil, soil pH, redox potential, physical and mechanical properties, soil temperature and humidity (Violante et al., 2010; Draszawka-Bolzan, 2015). Moreover, different processes, such as adsorption, ionic exchange, redox reaction or precipitation affect the uptake of soil pollutants by plants (Smical et al., 2008; Jin et al., 2018). The level of absorption of heavy metals by plants depends upon the plant species and its growth stages as well as soil type. Regardless of the mechanism of the transfer of

toxic metals from soil to plants, their presence induces morphological and structural changes in plant organs. Poorly mobile elements (as Cr, Cu, Pb) mainly damage the roots and indirectly the leaves, meanwhile elements with greater mobility (Cd, Ni, Zn) affect foliage directly causing more visible symptoms (Gostin, 2010).

Plants contaminated with heavy metals may be introduced directly into the human food chain, but they can also cause an additional indirect threat through pollinators being exposed to contaminated plants. (Afzal et al., 2013; Ali et al., 2019). Even when the environmental concentration of heavy metals is lower than the minimal risk levels for human health it may still be a relevant danger to pollinator activity and survival (Burden et al., 2019). Thus, the determination of heavy metal concentrations in honey bee (*Apis mellifera*) bodies, could serve as an intermediate parameter in the environmental biomonitoring of toxic metal pollution (Skorbiłowicz et al., 2018; Dżugan et al.,

Received: 20.12.2019.

Accepted in revised form: 22.04.2020

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2018). The ability to monitor the environment purity with bees is possible for the following reasons: a large flying area (2–3 km in diameter of the apiary), the penetration of areas inaccessible to humans, a high reproductive rate and sensitivity to toxic substances (Dżugan et al., 2018).

Bee products are the next link in the food chain and the level of their contamination with heavy metals is directly related to both: the exposure of bees and plants to pollution by these elements (Naggar et al., 2013). Bees collect heavy metals with plant nectar and pollen, and accumulate them in their bodies. However, during intensive contamination this biological barrier can be

ineffective and does not protect honey from contamination (Dżugan et al., 2018). For this reason, many authors have attempted to use honey to monitor environmental pollution (Naggar et al., 2013; Oroian et al., 2016; Skorbilowicz et al., 2018; Dżugan et al., 2018). However, the most important reason for controlling the presence of heavy metals in honey, is to protect the health of consumers (Aghamirlou et al., 2015).

For this reason, the aim of the research was to monitor the intensity of toxic metal transfer from the soil to honey *via* plant and bees with regard to the ecological quality of the bee habitat.

MATERIAL AND METHODS

Sampling area

Two different sampling areas were designated on the map of the Podkarpacie region located in south-eastern Poland based on the intensification of its development: U-urbanized and E-ecologically pure. In each area five local apiaries were selected as sampling points. The exact location of each apiary was recorded using a Garmin eTrex10 satellite receiver (Figure 1).

Soil

During beekeeping season, soil samples (n=3) were collected from each sampling point precisely from the fields where melliferous plants were grown (not more than 1.5 km from the apiary). Then, representative soil samples from a depth of 0-10 cm were taken. Before analysis the soil samples were dried at 60°C in a laboratory dryer until a constant weight was achieved. Then the non-humified parts (roots) were removed, the soil was ground in a mortar, sieved through a 2 mm sieve and preserved in polyethylene bags in a desiccator pending further analysis.

Plants

Bee forage plants (goldenrod 10, dandelion 10, rapeseed 10, tilia 10 and fir needle 10) were collected within an area of 0-1.5 km radius around the apiary at the same points where the soil was sampled. Fully developed inflorescences (5 per each plant species) were collected in the middle of the flowering season based on the advice of beekeepers. Fir needles (50g) were collected during honeydew secretion. The plant material was dried at 60°C in a laboratory dryer until a constant weight was achieved and then ground in a laboratory grinder. The powder obtained was

stored in polyethylene bags in a desiccator pending further analysis.

Honey bees (*Apis mellifera* L.)

Honey bee worker samples (n=30) were collected three times during the June-July period in regular two-week intervals from every sampling point. After fumigation, bees were carefully brushed into disposable polyethylene bags. On each occasion at least 100 individuals were sampled from the honeycombs placed on the farthest sidewalls of the hive. Next, bee samples were transported to laboratory and frozen at -4°C. Before analysis, the bee samples were dried until a constant weight was achieved (moisture analyser at 105°C) and ground with a manual laboratory grinder.

Honey samples

Fifty honey samples were taken in total, including five honey varieties collected from each sampling point in the E and U areas. The tested materials contained 10 dandelion, 10 rapeseed, 10 goldenrod, 10 tilia and 10 honeydew honeys. The honey variety was determined by a beekeeper according to the nectar flow availability. The honey samples were stored at 21°C±2 in tightly closed glass jars until the time of analysis.

Elements determination

The concentration of the 5 chosen metals (Cd, Pb, Hg, Al, Ni) were determined by optical emission spectrometry with inductively induced plasma (ICP-OES) using a Thermo iCAP 6500 spectrophotometer (Thermo Fisher Scientific Inc., Bridgewater, USA) regardless of the sample type. Prior to spectrometry analysis, the samples were mineralized in a microwave mineralizer Milestone Ethos Ultrawave-One (Milestone SRL, Sorisole,

Italy). For this purpose, 1 g of sample (honey, plants and bees) was weighed in Teflon vessels, and then 8 ml of concentrated HNO₃ (65% pure-basis, POCH Gliwice) was added. In the case of soil samples, two different metal fractions were examined. In the case of the total metal content, the soil sample was mineralized with 8 ml of aqua regia (2 ml concentrated HNO₃ and 6 ml concentrated HCl), whereas for the absorbable forms of the metals in the soil, an extract in 1M NH₄NO₃ solution was used. The process of mineralization lasted about 30 minutes. Cooled samples were transferred to sterile flasks (50 ml volume) and made up to the mark with distilled water. The detection threshold for each element was at least 0.01 mg kg⁻¹ based on the assumed detection capacity of the device used at a level above 1 ppb). The curve fit factor for the elements studied was above 0.99. Analyses were made independently in triplicate. The targeted repeatability (RSD) and recovery were 20 and 97 to 102%, respectively. For method validation a certified reference material (NIST –1515) was used. The method of internal standard addition was applied to identify the analytical line and to avoid interferences. Yttrium (2 mg dm⁻³) and ytterbium (5 mg dm⁻³) were used as internal standards. The results were expressed as mg per kg dry weight (d.w.) of soil, plants and bees and mg per kg for honey.

Soil pH determination

Soil pH was measured using an Elmetron C-410 pH meter. Briefly, 25 ml of 1 M KCl solution was

added to 10 g of dried soil and shaken thoroughly. After 15 minutes, the pH of the suspension was measured.

Data analysis

Bioaccumulation factor

The metal concentration in the soils and plants were calculated per 100 g d.w. The plant bioaccumulation factor (BF) was calculated using the formula:

$$BF = C_{\text{plant}} / C_{\text{soil}}$$

where C_{plant}- metal concentration in 100 g d.w. of plants; C_{soil}- metal concentration in 100 g d.w. of soil.

Metal concentration visualization

Based on the calculated bioaccumulation factors, the areas of increased migration of toxic elements from soil to plants have been designated on the map of the Podkarpackie Voivodeship. The projection was made using Surfer v12 software.

Statistical analysis

The significant differences (p<0.05) for the average concentration of the tested metals, in particular, links in the food chain between U and E regions as well as between the mean values of the tested materials were determined using one-way analysis of variance (ANOVA) combined with Tukey's multiple range test. The relationships between tested element concentrations in analysed biological materials were estimated based on Pearson's correlation coefficients. All data were analysed using Statistica 13.1 software.

RESULTS AND DISCUSSION

Concentration of toxic metals in the individual links of the food chain

The study was focused on the examination of the impact of the anthropogenic modification of bee habitat on honey contamination with heavy metals. The two tested areas: U-urbanized and E-ecologically pure were selected based on earlier findings (Dżugan et al., 2018), they are located in the Podkarpackie region (south-eastern Poland) adjacent to Ukraine and Slovakia. The chosen region is considered to be the most environmentally unspoiled regions in Poland having the highest purity water. However, an elevated geochemical background of cadmium in the Podkarpackie soil resulting from its origin from the natural bedrock (Carpathian flysch) was reported (Reszel et al., 2003). Moreover, in recent times some parts of the Podkarpackie region have been

characterized by intensive economic and urban development which has resulted in increasing air contamination.

Soil contamination by toxic metals

The pH of the tested soils showed that they ranged from slightly acidic to very acidic and the more acidic soil originated from the U-region (pH 4.64) in comparison with the E-region (pH 5.20; Table 1). As the sampling points in the U area were located close to urban agglomerations whereas E was in a rather rural area, the observed differences in soil pH seem to be the effect of anthropogenic activity. However, despite the huge variation in the content of the tested toxic metals in soil significant differences between the designated areas (E and U) were not observed (p>0.05). The total concentration of toxic metals in the tested soil was

significantly higher as compared to their available fraction (Table 1). The share of the available fraction of Cd and Ni was higher for the E-region and accounted for between 36 and 24% of the total as compared to the U-region which reached 26 and 14%, respectively. The opposite observation was made for Pb, Al and Mn where the available fraction made up a slightly higher share of the aforementioned metals found in the U-region (4, 3 and 7%) in comparison with the E-region (1, 2 and 5%).

The observed variability in the content of Al, considered to be a neurotoxin for humans with a tolerable weekly intake set by the European Food Safety Authority (EFSA) as 1 mg aluminium/kg body weight (Klotz et al., 2017), seems to indicate the presence of man-made local pollution.

However, as determined in the present study, the concentrations of Pb and Cd in most of the samples were below the limits established for soil set by the Polish regulations of the Minister of the Environment (2002), where the maximum permissible levels of heavy metals in soil were set as 4, 100, 100 mg/kg d.w. for Cd, Pb and Ni

respectively. When comparing the presented results to the findings of other authors concerning toxic metals in soil samples from different regions in Poland, it may be stated that Podkarpackie soil is moderately contaminated with Cd and less polluted with Pb.

Dabkowska-Naskret et al. (2016) sampled soil from the park areas in Bydgoszcz city located in the unspoilt north-west region of Poland and found a comparable level of Cd (0.3-1.2 mg/kg d.w.) but higher levels of Pb (5.4-148.9 mg/kg d.w.). In turn, for Silesia, the region in Poland most polluted by the non-ferrous metal industry, significantly higher levels of Cd (up to 12.32 mg/kg d.w.) and Pb (up to 781.91 mg/kg d.w.) were reported in soils (Diatta et al., 2008; Miśkowiec et al., 2015). Thus, this study confirmed the high ecological quality of Podkarpackie soils, naturally rich in cadmium. However, the Cd level found in Podkarpackie soils is far from the limit of 3 mg/kg d.w. reported by Buczkowski et al. (2002) as a toxic concentration to plants.

Table 1. Concentration of toxic metals (mg/kg d.w.) in tested soil (total and available) and plant samples collected from ecological (E) and urbanized (U) regions of Podkarpackie. The results are expressed as mean±SD and (min-max) values

Sample		Area	Cd	Pb	Al	Mn	Ni
Soil	total	E pH 5.2±1.1 (4.2-7.3)	0.39±0.29 (0.08-1.50)	19.25±7.21 (9.23-39.83)	2706.27±685.00 (2447.35-3789.73)	531.80±263.29 (80.29-1030.00)	1.03±2.73 (0.00-9.01)
		UpH 4.6±1.0 (3.4-6.8)	0.35±0.26 (0.04-1.15)	23.35±11.22 (9.89-58.07)	2392.78±448.10 (1816.73-3444.41)	525.94±253.00 (56.44-956.47)	2.25±5.00 (0.00-16.18)
	available	E	0.14±0.12 (0.02-0.41)	0.20±0.27 ^a (0.00-0.93)	53.95±71.86 (0.00-289.50)	25.97±24.24 (0.85-70.23)	0.25±0.37 (0.00-1.50)
		U	0.09±0.07 (0.01-0.27)	0.93±1.83 ^b (0.00-7.63)	77.55±96.27 (0.00-348.25)	34.26±48.01 (0.33-221.35)	0.32±0.52 (0.00-1.67)
Plants	Fir	E	0.29±0.20 (0.03-0.63)	1.05±0.49 (0.42-2.02)	505.62±167.39 (142.60-803.19)	681.16±406.18 (95.77-1394.39)	1.74±2.11 (0.00-5.65)
		U	0.50±0.32 (0.07-1.21)	0.66±0.52 (0.05-1.75)	401.52±249.55 (97.81-792.39)	542.01±502.64 (51.16-1425.68)	3.13±2.21 (0.78-8.99)
	Goldenrod	E	0.48±0.31 (0.07-0.99)	0.57±0.17 (0.41-0.89)	128.29±90.33 (31.86-284.64)	127.23±111.67 ^a (24.18-325.00)	0.01±0.03 (0.00-0.10)
		U	0.77±0.17 (0.59-1.04)	0.62±0.36 (0.07-1.14)	108.58±43.75 (56.16-152.54)	537.47±305.23 ^b (136.78-1058.26)	0.15±0.33 (0.00-0.82)
	Dandelion	E	0.64±0.64 (0.13-1.74)	0.46±0.37 (0.00-0.85)	274.91±183.26 (22.75-511.71)	53.46±21.98 (29.17-84.82)	1.58±2.23 (0.00-5.37)
		U	0.44±0.34 (0.06-1.13)	0.48±0.56 (0.03-1.89)	452.87±642.39 (80.17-2104.41)	47.97±27.43 (19.92-95.95)	2.25±3.20 (0.00-7.48)
	Rapeseed	E	0.15±0.04 (0.12-0.20)	0.06±0.09 (0.00-0.18)	36.55±17.50 (21.38-55.70)	28.90±7.27 (23.39-39.61)	0.25±0.67 (0.00-1.58)
		U	0.20±0.20 (0.04-0.70)	0.04±0.06 (0.00-0.17)	45.69±28.54 (8.39-105.21)	41.21±19.13 (20.69-71.82)	0.46±0.90 (0.00-2.63)
	Tilia	E	0.18±0.20 (0.06-0.47)	0.19±0.16 (0.01-0.36)	64.32±21.60 ^a (43.85-91.14)	108.65±49.07 (73.95-143.34)	n.d.
		U	0.16±0.08 (0.05-0.24)	0.32±0.33 (0.00-0.77)	28.00±6.41 ^b (20.60-31.72)	78.95±84.72 (19.98-176.04)	n.d.

^{a, b} – means with various letters are significantly different (p<0.05) between E and U region in particular biological material

Plants contamination by toxic metals

Fir needles were found to be the most susceptible to contamination by the tested metals, while the most uncontaminated plant parts were the aerial parts of rapeseed (Table 1). Moreover, the contamination of plants by toxic metals was not dependent on the development intensity of the region, the differences between the E and U regions were insignificant ($p>0.05$). The high concentration of Pb, Al, Mn and Ni in the fir needles could be a result of their effective transfer from soil as well as atmospheric deposition (Gandois & Probst, 2012). Moreover, the presence of sticky wax on the surface of the needles could act as a glue for pollutants (Gostin, 2010). However, the exact cause of such high fir needle contamination is difficult to assess. Taking into consideration the comparable Cd content in soil samples from the E and U regions indicates that the lower Cd content in fir needles in the E region is not associated with the purity of the soil but more likely with air pollution. The specific susceptibility to heavy metal contamination by *Abies alba* needles has been confirmed in the studies of Gandois and Probst, (2012), who tested levels of Al and Ni similar to those of the present study, and slightly lower levels of Cd, Pb and Mn. Among the melliferous plants, for goldenrod (*Solidago*) a particularly high accumulation of cadmium was found. However, the differences between samples collected from the E and U regions were not significant ($p>0.05$). It was confirmed that the common dandelion (*Taraxacum officinale*) is a lead concentrator ($p<0.05$). Despite the high Cd content found in the examined samples of goldenrod, the results of the present research are much lower than those found by Tóth et al. (2012), who tested the level of Cd in goldenrod leaves from Hungary and found it to be about 1.4 mg/kg d.w. In turn, common dandelion is considered to be a useful reference point in heavy metal environmental monitoring, due to the fact that it is a widespread species in all temperate regions of the world, as a ubiquitous wild plant of gardens, meadows, lawns, road-sides, and ruderal habitats, often growing on heavily polluted sites, such as railway tracks or in the dusty surroundings of industrial plants (Ligocki et al., 2011). Studies have shown that among the examined plants, the dandelion is characterized by an especially high content of Cd, Al and Ni, which indicates the susceptibility of this plant to the accumulation of the aforementioned elements. Kajka and Rutkowska (2018) tested for toxic metals in the above ground parts of dandelion which grow near roads with a high traffic intensity in central Poland

and found significantly higher levels of Ni (4-7 mg/kg d.w.) and concentrations of Cd and Pb comparable to the present study (0.3 – 1 and 0.15 to 0.7 mg/kg d.w., respectively). On the other hand, Ligocki et al. (2011) tested the metal content in the leaves of dandelion from the urbanized and village regions in the north part of Poland and found levels significantly lower than the present study of Cd, Pb, and Ni and comparable to the present study concentrations of Mn. However, similarly to the present study, authors did not find any significant differences between the samples collected from differently polluted regions.

In the literature *Brassica napus* is known to exhibit a tendency to accumulate metals, especially toxic ones (Angelova et al., 2017). The authors assert that rapeseed roots are specific accumulators of toxic metals, whereas the aboveground parts do not exhibit such strong properties. Yu et al. (2012) reported of *Brassica napus* that the aboveground parts had a comparable level of Cd to the present study (0.168 mg/kg d.m.) while higher levels of Pb and Ni were present (0.416 and 0.76 mg/kg d.m., respectively) On the other hand, Niedźwiecka and Zamorska-Wojdyla (2017) did not detect Pb in *Brassica napus* growing near power plants. *Tilia*, as the only leafy tree tested was characterized by a relatively low content of the analysed elements, and was the only plant where Ni was not detected. Similar observations have been made by Tomašević et al. (2004). In turn, Šijacic-Nikolic et al. (2012) found in the leaves of *tilia* trees growing along the highway significantly higher levels of Ni and Pb (0.52-2.91 and 1.14-6.27 mg/kg d.m., respectively) and lower levels of Mn (12.12-74.87 mg/kg d.m.) as compared to the present study. The same authors stated that the contents of trace elements in *tilia* leaves are primarily influenced by the genotype when they grow in the same soil conditions.

The variable concentrations of heavy metals in different plant species indicate the different absorption abilities of pollutants from the environment (Kajka and Rutkowska, 2018). It was confirmed by Filipović-Trajković et al. (2012) that based on metal distribution in plant organs, it is possible to assess heavy metal migration from soil to plant. Moreover, Petryk (2016) indicated that the intensity of element uptake and release by plants is significantly affected by soil composition.

Bee body contamination with toxic metals

Bees can come into contact with toxic metals not only through contaminated water, but also during the collection of contaminated nectar and pollen from plants (Van der Steen et al. 2012; Costa et al.,

2019). A similar concentration of tested metals was found in bee bodies collected from urbanized (U) and ecological (E) regions ($p>0.05$; Table 2). In the opposite case, Sadowska et al. (2019) found that in contrast to Pb, Cd concentration in bee bodies was dependent on environmental contamination. The concentration of Cd found in bees from the Podkarpackie region was comparable to the results obtained by Fakhimzadeh and Lodenius (2000) for Finnish bees (0.05–1.2 mg/kg) but significantly higher as compared to Costa et al. (2019; 0.008–0.195 mg/kg), or Perugini et al. (2011; 0.05–0.06 mg/kg) for Italian bees. The content of Pb in tested bee bodies was comparable to the findings of Porrini et al. (2002) for bees inhabiting the Italian national park (0.15–0.55 mg/kg d.m.), and other non-contaminated sites of Italy (0.25–1.40 mg/kg d.m.; Costa et al., 2019; Conti and Botre, 2001) as well as in the urban and industrial sites of Belgium (0.33–0.41 mg/kg d.m.; Van Der Steen et al., 2015). Roman (2010) measured cadmium in bee bodies from the industrialized areas of Poland in the range of 1.46 to 2.32 mg/kg d.m. Thus, compared to the cited authors, the results obtained for Pb in the present study are in compliance with the data for environmentally pure bees habitats. The Mn content in the tested bees was very variable.

As compared to the literature, the average content in the tested bees was found to be higher than in the study conducted in Poland by Skorbiłowicz et al. (2018) who found a level of 49.4 mg/kg d.m. in bees collected in June, and 26.6 mg/kg d.m. in bees collected in March. In the study carried out by Zhelyazkova (2012) in Bulgaria (in the vicinity of Stara Zagora), the concentration of Mn in the analysed bees varied between 43–114 mg/kg d.m. while in the study of Zarić et al. (2016) in Serbia the Mn concentrations ranged from 34 to 90 mg/kg d.m. The results obtained are comparable with our previous study focused on the transfer of heavy metals from bees to honey (Dżugan et al., 2018).

Honey contamination by toxic metals

As the last link in the food chain, honey was tested for the concentration of toxic metals. As expected, our results indicated different levels of toxic metals depending on the honey variety, plant species and region of collection (E and U; Table 2). Moreover, significant differences in the content of particular elements, which were measured in the same honey variety but with different origins were observed. A similar tendency during the testing of heavy metals in different types of Polish honey was reported (Sitarz-Palczak et al., 2015; Dżugan et al., 2017; 2018).

Table 2. Concentration of toxic metals (mg/kg d.w.) in tested bee body and honey samples collected from ecological (E) and urbanized (U) regions of Podkarpacie. The results are expressed as mean±SD and (min-max) values

Sample		Area	Cd	Pb	Al	Mn	Ni
Bees		E	0.51±0.54 (0.00-2.41)	0.12±0.17 (0.00-0.72)	12.93±11.95 (0.00-50.17)	109.72±161.53 (0.01-734.53)	0.36±0.41 (0.00-1.45)
		U	0.71±0.84 (0.02-3.19)	0.13±0.19 (0.00-0.77)	11.78±12.93 (1.06-50.70)	159.22±173.19 (5.09-449.86)	0.32±0.31 (0.00-1.06)
Honey	Honeydew	E	0.03±0.01 (0.02-0.04)	0.08±0.12 (0.00-0.31)	47.00±32.78 (2.30-86.29)	4.03±1.606 (1.580-5.875)	0.53±0.47 (0.00-1.49)
		U	0.04±0.01 (0.02-0.05)	0.09±0.19 (0.00-0.77)	54.37±27.37 (0.36-91.33)	4.55±1.97 (0.16-6.82)	0.54±0.54 (0.00-1.52)
	Goldenrod	E	0.02±0.01 (0.01-0.03)	0.03±0.04 (0.00-0.08)	7.97±3.93 (0.97-10.06)	2.44±2.72 (0.15-7.14)	0.08±0.11 ^a (0.00-0.23)
		U	0.02±0.01 (0.01-0.04)	0.02±0.02 (0.00-0.05)	8.63±7.47 (1.79-16.66)	1.29±0.76 (0.18-1.88)	0.55±0.37 ^b (0.00-0.79)
	Dandelion	E	0.03±0.01 (0.02-0.05)	0.09±0.09 (0.01-0.40)	8.13±6.57 ^a (0.00-46.16)	3.59±2.43 (0.28-7.42)	0.35±0.46 (0.00-1.44)
		U	0.02±0.01 (0.01-0.05)	0.04±0.05 (0.00-0.17)	1.72±2.45 ^b (0.00-10.29)	4.31±3.83 (0.29-11.34)	0.17±0.27 (0.00-1.02)
	Rapeseed	E	0.02±0.00 (0.02-0.02)	n.d.	1.81±1.73 (0.47-4.65)	0.26±0.08 (0.18-0.36)	0.72±0.34 (0.14-0.97)
		U	0.01±0.01 (0.01-0.03)	0.04±0.05 (0.00-0.12)	2.85±2.36 (0.48-5.61)	0.46±0.12 (0.34-0.63)	0.70±0.56 (0.00-1.12)
	Tilia	E	0.04±0.03 (0.02-0.07)	0.04±0.01 (0.02-0.05)	2.35±0.85 (1.37-2.89)	2.22±1.39 ^a (0.71-3.43)	0.17±0.07 ^a (0.10-0.24)
		U	0.02±0.00 (0.02-0.02)	0.02±0.02 (0.01-0.05)	1.97±0.94 (1.19-3.01)	0.90±0.47 ^b (0.39-1.33)	0.63±0.85 ^b (0.05-1.66)

^{a, b} – means with various letters are significantly different ($p<0.05$) between the E and U region in particular biological materials

The Cd content in the tested honeys varied from 0.01 to 0.07 mg/kg whereas for Pb it varied from 0.00 to 0.77 mg/kg. The observed Cd values were generally within the allowable limits set out in the Polish Regulations of the Minister of Health (2003), which permits a level of 0.03 and 0.30 mg/kg for Cd and Pb, respectively. An excess of cadmium was noted in 5% of the samples (2 honeydew, 2 dandelion and 1 tilia) whereas only 2 honeydew honey samples contained too much lead. In general, the metal content in honey that originated from the E and U areas were comparable. More differences with regard to the honey variety were observed, i.e. honeydew honey contained higher levels of Al and Mn, whereas rapeseed honey was abundant in nickel. As compared to the literature, significantly higher concentrations of Pb than in the present study were described by Sitarz-Palczak et al. (2015) for Polish honeydew honey and multifloral honey (1.95-39.0 and 0.97-4.97 mg/kg, respectively). Aghamirlou et al. (2015) tested multifloral honey from Iran and found comparable results to the present study for levels of Pb (0.45 mg/kg) but lower levels of Cd (0.013 mg/kg), and higher levels of Ni (0.87 mg/kg). Oroian et al. (2016) tested for heavy metals in multifloral Romanian honey and found lower levels of Cd and Mn (0.003 and 1.001 mg/kg) but comparable levels of Ni and Pb (0.183 and 0.04 mg/kg). Moreover, the same authors observed different concentrations of the tested elements depending on the honey type.

Heavy metals transfer in the studied food chain

An analysis of the average content of the tested elements in individual links in the food chain showed the occurrence of the migration of these elements from the soil to the bodies of bees (Table 3). In the case of Pb and Mn, a significant decrease was observed in the individual subsequent parts of

the food chain. The comparable levels of Al and Ni in bees and honey indicate that these metals are not accumulated by bees and that they do not constitute a biological barrier limiting the migration of these metals in the food chain. Meanwhile, based on the detected levels of Cd and Pb in the individual links of the tested food chain it was proven that bees are susceptible to the bioaccumulation of Cd and Pb and simultaneously that they function as an effective biological barrier preventing the transference of such elements to the honey.

In order to check for the potential of bioaccumulation of toxic metals by individual plant species, the bioaccumulation factor was calculated (Table 4). The results obtained, despite the high degree of variability, indicate that the phenomenon of bioaccumulation is particularly relevant to cadmium ($BF \geq 1$), and the plants that particularly intensively absorb this element from the soil are: dandelion and goldenrod, while the least absorbing one is rapeseed plant. The ratio of the concentration of pollutants in the plant and soil can be used as an approximate indicator of the level of element transfer from soil to plant (Kabata-Pendias & Pendias, 2001; Ehlken and Kirchner, 2002). The soil to plant transfer intensity depends primarily on the concentration of trace metals in the soil, but also on soil pH, specific metals availability in the soil and on the interaction with other cations (Kabata-Pendias & Pendias, 2001; Ehlken & Kirchner, 2002; Probst et al., 2009).

Based on the calculated coefficients of heavy metals migration from soil to plants, with the help of special software, maps of the Podkarpackie Voivodeship designating areas which are most exposed to heavy metal contamination have been created (Figure 1). Such maps can be useful for the local beekeeping sector.

Table 3. Migration of toxic metals in the soil-plant-bee-honey chain

Element	Soil	Plants	Bees	Honey
Cd	0.37±0.27 ^b (0.04-1.25)	0.40±0.34 ^b (0.03-1.74)	0.63±0.72 ^b (0.02-3.19)	0.02±0.01 ^a (0.00-0.07)
Pb	21.30±9.57 ^a (9.23-58.07)	0.52±0.49 ^b (0.00-2.02)	0.13±0.18 ^c (0.00-0.77)	0.05±0.10 ^d (0.00-0.77)
Al	2552.60±596.69 ^c (1598.73-3789.73)	244.13±225.94 ^b (20.60-803.19)	12.59±12.36 ^a (1.06-50.70)	11.64±19.88 ^a (0.00-91.33)
Mn	528.93±255.72 ^a (56.44-1063.72)	291.13±378.38 ^b (19.92-1425.68)	138.56±168.35 ^c (5.09-734.53)	3.39±2.89 ^d (0.15-16.72)
Ni	1.63±4.02 (0.00-16.18)	1.28-2.09 (0.00-8.99)	0.34±0.36 (0.00-1.45)	0.45±0.54 (0.00-2.43)

^{a,b,c,d} – different letters indicate significant differences ($p < 0.05$) in the table row

Table 4. Bioaccumulation factor (BF) of selected toxic metals in the analysed plant species with regard to the pollution area (E-ecological, U-urbanized)

Plant	Area	Cd	Pb	Al	Mn	Ni
Fir (<i>Abies alba</i> Mill)	E	0.74±0.69	0.05±0.07	0.19±0.24	1.28±1.54	1.70±0.77
	U	1.43±1.23	0.03±0.05	0.17±0.56	1.03±1.99	1.39±0.44
Goldenrod (<i>Solidago</i>)	E	1.23±1.07	0.03±0.02	0.05±0.13	0.24±0.42 ^a	0.01±0.01
	U	2.20±0.65	0.03±0.03	0.05±0.10	1.02±1.01 ^b	0.07±0.06
Dandelion (<i>Taraxacum officinale</i>)	E	1.64±2.20	0.02±0.05	0.10±0.27	0.10±0.08	1.53±0.82
	U	1.26±1.31	0.02±0.05	0.19±1.43	0.09±0.11	1.00±0.64
Rapeseed (<i>Brassica napus</i>)	E	0.38±0.14	0.00±0.01	0.01±0.03	0.05±0.03	0.24±0.22
	U	0.57±0.77	0.00±0.01	0.02±0.06	0.08±0.07	0.20±0.18
Tilia (<i>Tilia</i>)	E	0.42±0.69	0.01±0.02	0.02±0.03	0.20±0.18	n.t.
	U	0.46±0.31	0.01±0.03	0.01±0.01	0.15±0.26	n.t.

^{a,b} – different letters indicate significant differences ($p<0.05$) between particular plants collected from the E and U region

Figure 1. Map of the Podkarpackie Voivodeship with the marked areas of increased migration of Cd (left) and Pb (right) from soil to plants. The sampling points (apiaries) were indicated for E (▲) and U (●) areas.

CONCLUSIONS

Studies have shown the occurrence of heavy metal migration in the soil-plant-bee-honey food chain, with particular reference to cadmium. Melliferous plants accumulate heavy metals to a small extent, with the exception of cadmium. From all of the tested varieties of plants, goldenrod and dandelion

were confirmed as cadmium accumulators. The bodies of bees 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.

ACKNOWLEDGMENTS

The work was partially supported by the program of the Minister of Science and Higher Education entitled “Regional Initiative of Excellence” in 2019-2022 (Project no. 026/RID/2018/19).

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