Capacity of honeybees to remove heavy metals from nectar and excrete the contaminants from their bodies

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Abstract – We examined the residues of 13 elements in soil, plant parts, nectar, bee heads, thorax, and abdomens, feces from bee guts, and bee products sampled from two Polish cities (Lublin and Poznań). Our findings indicated that bees have an extraordinary ability to remove metals from nectar when converting nectar into honey. Compared to nectar, honey contained 40-fold lower Fe, 26-fold lower Zn, and eightfold lower Cu and Cd levels, indicating removal of these elements via nectar processing, during which water is evaporated and complex sugars are decomposed into simple ones. The amount of Pb remained unchanged; however, it can also be regarded as a fourfold decrease due to water evaporation from honey, compared to nectar. Some portion of the ingested Fe, Cu, and Zn was used by bees, and the excess amounts were excreted in feces. All analyzed elements were present as biocomplexes transported from the alimentary tract through the abdomen to the thorax and head. Elements transferred in the alimentary tract were partially immobilized/metabolized in the bee fat body, and their residues were excreted with feces from the gut. We postulate that honey is not a good indicator of environmental pollution, as a high amount of elements is removed by bees from their bodies.

Nectar / Honey / Heavy metals / Contamination products / Residues / Urban beekeeping / *Apis mellifera*

1. INTRODUCTION

Urban buildings, streets, pavements, and direct heat generation related to human activities contribute to a faster temperature increase in cities compared to surrounding rural areas (Oke 1973; Banaszak-Cibicka 2014). The average annual temperature in cities inhabited by 1 million residents is reportedly 1–3 °C higher than in non-urban areas (Oke 1982; Aniello et al.

1995). Increased anthropogenic heat emission promotes emergence of the so-called urban heat island (Landsberg 1981), which accelerates plant vegetation (Masierowska 2020).

Urban green areas are an attractive source of bee reward. Notably, urban agglomerations are favorable for beekeeping due to the reduced amounts of pesticides that are commonly used in arable areas (Goulson et al. 2015; Seibold et al. 2019; Sadowska et al. 2019). However, compared to rural areas, the soil and plants in large urban agglomerations may be contaminated with higher levels of metals associated with heavy

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car traffic, which may get into nectar and subsequently into honey (Freedman and Hutchinson 1981).

Honey, a well-known natural product that is frequently consumed by humans, is produced by honeybees from floral nectar or plant sap. After water evaporation, bees combine nectar with their body enzymes and leave the product in combs for maturation (Krell 1996; Solayman et al. 2016). There are two general types of honey: nectar and honeydew (Bogdanov 2016). Since it is widely used for both nutritional and medicinal purposes (Abeshu and Geleta 2016), honey should be free from any contamination (Jakšić et al. 2018). Honey contamination is promoted by collection of nectar from a polluted environment and management of honeybee colonies during honey production and after honey collection (Bogdanov et al. 2003; Bogdanov 2006).

The raw material for honey production, i.e., nectar or honeydew, may contain pollutants that are characteristic of the environment (Roman et al. 2011), including minerals of natural or anthropogenic origin (van der Steen et al. 2018; Skorbiłowicz et al. 2018; Goretti et al. 2020). Natural sources of mineral contamination include rock weathering and volcanic eruptions. Anthropogenic sources of metals include municipal and industrial wastewaters, mining and metallurgy industrial activities (Bojakowska 1995), road transport, dust from car traffic (Niedźwiecki et al. 2000; Kaniuczak et al. 2003), and agriculture (Klavins et al. 2000).

In addition to nectar and honeydew, honeybees collect water, pollen, and balsamic substances. It has been postulated that various raw materials that are collected from the environment and processed into bee products, as well as honeybees that have direct contact with these raw materials and products, can be useful bioindicators of environmental pollution (Bratu and Georgescu 2005; Bogdanov 2006; van der Steen 2016; Skorbiłowicz et al. 2018). However, there is some controversy regarding the suitability of honey for this purpose. While most authors claim that honey can be a good indicator of environmental contamination with elements/minerals

(Crane 1984; Leita et al. 1996; Čelechovská and Vorlová 2001; Przybyłowski and Wilczyńska 2001; Nalda et al. 2005; Bogdanov et al. 2007; Almeida-Silva et al. 2011; Ruschioni et al. 2013; Bartha et al. 2020), others argue that only pollen, propolis, wax, and bees themselves are useful for the assessment of environmental heavy metal content, but not honey (Conti and Botrè 2001).

Some studies confirm that honey produced from nectar collected in areas exhibiting high environmental contamination with heavy metals meets the residue limits for contamination with elements/minerals (Erbilir and Erdoğan 2005). As reported by Bogdanov (2006), metal contamination levels are lower in honey compared to those in the bodies of bees, indicating that bees can filter and purify nectar to remove these contaminants. Similarly, Roman et al. (2011), Ruschioni et al. (2013), Saunier et al. (2013), Losfeld et al. (2014), and Conti et al. (2018) observed that bees could partially purify nectar by removal of heavy metals during honey production. Džugan et al. (2018) defined bees as “biofilters” that prevent elements from penetrating bee products, especially honey. However, the mechanism of nectar purification by bees has not been studied and described to date.

In the present study, we aimed to investigate the potential mechanism of nectar purification by bees and the pathways of element removal from bee bodies.

2. MATERIALS AND METHODS

This study was conducted at the apiary of the University of Life Sciences in Lublin (Poland; 51.224039 N—22.634649 E) and at the Institute of Plant Genetics, Polish Academy of Sciences (PAS) in Poznań (Poland; 52.44619 N—16.90464 E). The apiary in Lublin is situated on the outskirts of the city (ca. 340,000 inhabitants in 2019) near a dual carriageway with heavy car traffic (road S17), close to a special economic/industrial zone, and directly under the air corridor of the Lublin Airport runway (IATA code: LUZ). At the Institute of Plant Genetics PAS in Poznań, the

bee colonies were also located on the outskirts of the city (ca. 536,000 inhabitants in 2019) along the main road to the surrounding suburbs and residential areas. The sampling point was situated 5 km from the main city airport Ławica. From each city, four Dadant bee colonies of similar strength and structure were selected for the experiment. It lasted 6 weeks from August 10, 2019, to September 20, 2019. During this period, in both urban agglomerations, the plants yielding nectar comprised mainly wastelands covered with late goldenrod (*Solidago gigantea* Aiton).

2.1. Sampling and preparation of materials for analyses

The collected material and the number of analyzed samples are shown in Table I. From each colony, every 2 weeks (3 collections over 6 weeks), 50 forager bees were collected from the landing board at the hive entrance and frozen. Subsequently, the bees were defrosted, and

each body was divided into three parts: the head, thorax, and abdomen. The alimentary tract was dissected from the abdomen, and feces were collected from the hindgut. We also collected bee pollen with the use of pollen traps placed on the hive outlet, and pollen pellets (approx. 50 g per colony) were collected directly from the pollen foragers from each colony three times at 2-week intervals. At the end of the experiment, beebread (1000 cells per colony) and honey (0.5 L per colony) were collected. The material was subjected to the mineralization process.

Plants were collected simultaneously with honeybee collection every 2 weeks (3 collections over 6 weeks). We collected 54 entire late goldenrod plants (*Solidago gigantea* Aiton). In both cities (Lublin and Poznań), three plots with growing late goldenrod were established at a distance of ca. 200 m away from the apiary. Three plants growing at a ca. 20-m distance from each other were taken from each plot. The plants were tied in bunches of three and divided into roots, leaves, and flowers. All parts were weighed and

Table I
Material and number of analyzed samples

Collection number	Four bee colonies in Lublin	Four bee colonies in Poznań	Total
Bees	3 50 Dissected bees 600 heads, 600 thoraxes, 600 abdomens, 600 feces	50 600 heads, 600 thoraxes, 600 abdomens, 600 feces	300 1200 heads 1200 abdomens 1200 thoraxes 1200 feces
Pollen pellets	3 Approx. 50 g per colony	approx. 50 g per colony	900 g
Beebread	1 1000 cells per colony	1000 cells per colony	8000 cells
Honey	1 0.5 L per colony	0.5 L per colony	4 L
Plants	3 Plants from Lublin 3 goldenrod plants from 3 plots Dissected plants 27 roots, 27 leaves, 27 flowers	Plants from Poznań 3 goldenrod plants from 3 plots 27 roots, 27 leaves, 27 flowers	Total 54 54 roots 54 leaves 54 flowers
Nectar	4 2 g	2 g	16 g
Soil	3 20 g	20 g	120 g

left to dry at room temperature. The plants prepared and fixed in this way were mineralized. From the plots where the plants were growing, we collected an approx. 100-g soil sample (on average 20 g) from the top organic layer. The soil samples were averaged by thorough mixing, and on average, 20 g of the soil from both locations were collected for the mineralization process. We also collected 2 g of nectar four times from fresh plants using the pipetting method (Jabłoński 2002).

2.2. Microscopic analysis

Microscopic analysis was performed to determine the contribution of the dominant pollen in the honey (Table II). This research was conducted in accordance with the Polish Standard PN-88/A-77626 “Honey” (1988), the Regulation of the Minister of Agriculture and Rural Development of 2003 and 2009 (Journal of Laws 2003; No. 181, items 1772 and 1773; Journal of Laws 2009, No. 17, item 94), and international guidelines (von der Ohe et al. 2004).

From each sample, 10 g of honey was weighed, supplemented with 20 ml of distilled water, and then completely dissolved in a WSL-LWT 4/100 water bath at 40 °C. The suspension was centrifuged twice in a MPW 341 centrifuge for 10 min at 3000 rpm, and the supernatant was decanted each time. We used an automatic pipette to collect 50 µl from the 2-ml precipitate sample and transferred this aliquot onto glass slides. Finally, glycerol gelatine was added yielding permanent microscopic preparations.

We analyzed the pollen spectrum in the honey samples using the Nikon Eclipse E600 light microscope at a magnification of 40×15. In each preparation, we counted at least 300 pollen grains in successive lines of the field of view (Moar 1985). These pollen grains were assigned to the relevant taxon (species, genus, structure, or family) through identification using reference slides and keys (Ricciardelli d’Albore 1998; Bucher et al. 2004). In each honey sample, we distinguished pollen grains from nectariferous and non-nectariferous plants (entomophilous and

anemophilous). Then, considering the sum of pollen grains from nectariferous plants as 100%, we calculated the percentages of pollen from individual taxa. The pollen grains from the nectariferous plants were classified into four groups: dominant pollen (>45% in the sample), companion pollen (16–45%), single pollen (3–16%), and sporadic pollen (<3%).

Each sample of collected bee pollen was carefully mixed, weighed, and subsequently 3.5-g portions were used for microscopic analysis. The weighed portions of bee pollen were dissolved in 10 ml of a 1:1 mixture of distilled water and glycerine. Then, permanent microscope slides were prepared in glycerol gelatine ($n=6$ of each sample). Microscopic analysis was performed using a Nikon Eclipse E600 light microscope (magnification×600) and consisted in counting at least 300 pollen grains in each slide (Moar 1985). Among the identified pollen grains, we calculated the percentage proportions of individual taxa and distinguished dominant pollen (>45% in the sample), companion pollen (16–45%), single pollen (3–16%), and sporadic pollen (<3%). Based on the pollen spectrum in each sample, we calculated the frequency and share of individual taxa in the total material.

From 35 comb cells per colony, beebread was collected with a spiral move. The beebread was then dissolved in a 1:1 ratio of distilled water and glycerine and used to make microscopic preparations ($n=6$ of each sample). The subsequent procedure was identical to the methodology used to analyze pollen.

2.3. Analysis of element composition

To determine the element composition in each pooled worker bee sample, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, iCAP Series 6500, Thermo Scientific, USA) was used. In total, 96 sets of pooled bees were analyzed, including 2 sites×4 colonies×3 samples×4 parts of bees; each bee part sample comprised 50 heads, 50 thoraxes, 50 abdomens, and 50 feces. These sets were mineralized in a Microwave Digestion System (Speedwave,

Table II

Microscopic analysis of bee products obtained in the experiment

Product	Pollen grains	Sampling site			
		Lublin		Poznań	
		Range of type pollen grains (%)	Mean (%)	Range of type pollen grains (%)	Mean (%)
Honey	Dominant pollen > 45%				
	<i>Solidago</i> type	52.9–92.9	66.7	45.9–51.9	49.9
	Secondary pollen 16–45%				
	<i>Calluna</i>	–	27.2	–	–
	Brassicaceae	16.2–36.7	26.4	16.1–41.3	23.2
	<i>Lamium</i> type	–	–	–	19.7
	<i>Trifolium repens</i>	–	–	–	16.5
	<i>Taraxacum</i> type	–	–	–	16.4
Pollen loads	Dominant pollen > 45%				
	<i>Solidago</i> type	64.5–100.0	81.4	45.7–98.1	72.1
	Secondary pollen 16–45%				
	<i>Taraxacum</i> type	17.4–35.5	24.1	16.4–44.5	24.4
	<i>Artemisia</i>	16.1–22.1	19.1	–	–
	Brassicaceae	–	18.1	18.1–18.6	18.3
	<i>Calluna</i>	–	16.8	–	–
	<i>Geranium</i>	–	–	16.1–16.3	16.2
Beebread	<i>Aster</i> type	–	–	–	19.4
	Dominant pollen > 45%				
	<i>Solidago</i> type	45.3–47.9	46.6	–	–
	Secondary pollen 16–45%				
	<i>Solidago</i>	–	–	32.8–36.2	34.4
	Brassicaceae	18.3–19.0	18.6	–	20.1
	Chenopodiaceae	–	20.7	–	–
	<i>Artemisia</i>	–	19.2	–	–
	<i>Trifolium repens</i>	–	16.8	–	–
	<i>Calluna</i>	–	16.4	–	–
	<i>Taraxacum</i> type	–	–	19.2–26.2	22.7
	<i>Helianthus</i> type	–	–	–	18.1
	<i>Achillea</i> type	–	–	17.4–25.3	20.6

Berghof, Eningen, Germany) with optical, temperature, and pressure monitoring during acid digestion in Teflon vials (type DAP 100). The mineralized parts of the worker bees' bodies (50 heads, 50 thoraxes, 50 abdomens, and 50 feces samples) were then digested with 7 ml HNO₃ (65% v/v) and 3 ml H₂O₂ (30% v/v). Each bee

part sample was analyzed in triplicate. For each experimental colony, 12 measurements were taken, yielding a total of 12 measurements for each element (3 samples × 4 pooled samples of bee parts).

We analyzed 8 nectar samples (4 samplings), 8 honey samples (1 sample × 8 colonies), 24

pollen samples (3 samplings $\times$ 8 colonies), and 8 beebread samples (1 sample $\times$ 8 colonies) collected in the experimental sites. The mineralization of each 0.5-g sample of nectar, honey, pollen, or beebread sample was conducted in a Microwave Digestion System (Speedwave) with optical, temperature, and pressure monitoring during acid digestion in Teflon vials (type DAP 100). The mineralized honey samples were then digested with 7 ml HNO_3 (65% v/v) and 3 ml H_2O_2 (30% v/v). Each sample was analyzed in triplicate.

The mineralization of honeybee nectar, honey, pollen, and beebread was conducted as follows: 15 min from room temperature to 140 °C, 5 min at 140 °C, 15 min from 140 to 185 °C, 10 min at 185 °C, and then cooling down to room temperature. During this process, the pressure did not exceed 20 bars. After mineralization, the clear solution was cooled to room temperature and transferred to 50-ml graduated flasks, which were then filled with deionized water (ELGA Pure Lab Classic).

We obtained 54 plant part samples, 27 per each sampling site (Lublin and Poznań). The pooled plant sample comprised 3 roots, 3 leaves, and 3 flowers. Each sample was analyzed in triplicate. From each pooled sample, 0.5 g of plant parts was digested in 8 ml aqua regia (Sigma-Aldrich) with 2 ml hydrofluoric acid in a high-pressure microwave digestion system (Speedwave). We also obtained six soil samples (three per sampling site). From each sample, 0.5 g of soil was digested in 8 ml aqua regia with 2 ml hydrofluoric acid in a high-pressure microwave digestion system (Speedwave).

For all analyses, we prepared standards using the following multi-element stock solutions from Inorganic Ventures: Analitik-46 for Cu and Fe in 5% HNO_3 (1000 $\mu\text{g/mL}$), and Analitik-47 for Co, Cd, Cr, Mo, Pb, Mn, Hg, Ni, Sn, Se, and Zn in 10% HNO_3 (100 $\mu\text{g/mL}$) (The LOD and LOQ values are presented in Table III in Supplementary Material). The element symbols are compliant with the standards of the International Union of Pure and Applied Chemistry (IUPAC).

2.4. Statistical analyses

The results are given on the fresh weight basis. Statistical analyses were performed using Statistica software version 13.3 (2017) for Windows (StatSoft Inc., USA). The data distribution was analyzed using the Shapiro–Wilk test. Statistical analyses included Kruskal–Wallis ANOVA and multiple comparisons of mean ranks. A p value of 0.05 was used to indicate statistical significance. All results are reported as the mean and standard deviation ($\pm$ SD). Spearman's rank order correlations were also calculated.

3. RESULTS

3.1. Microscopic analysis

The pollen analysis of the honey, bee pollen, and beebread revealed predominance of golden-rod *Solidago* type pollen grains (Table II).

3.2. Analysis of element composition

Thirteen elements, i.e., Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Pb, Cd, Sn, and Hg, were determined in the samples collected at the experimental sites in two Polish cities (Lublin and Poznań). All elements were detected in the soil, golden-rod parts (roots, leaves, flowers, and nectar), bee body parts (head, thorax, abdomen, and feces), and bee products (honey, pollen, and beebread). In the analyzed materials, we detected significant differences in the content of five metals, namely, Fe, Cu, Zn, Pb, and Cd. The other elements showed similar tendencies to those exhibited by the five metals; however, their contents were very low (Supplementary Material Table IV). Hence, the presented data focus on five elements whose content showed significant differences in the bee products, especially in the honey.

Regardless of the sampling site, the Fe, Cu, and Zn contents were significantly lower in the honey than in the nectar samples (Figs. 1, 2, and 3). The differences in the Fe, Cu, and Zn contents were in the range of 92–99%. All

Fig. 1 Iron contents in soil, plants, bees, and bee products from two cities in Poland (Lublin and Poznań). Lowercase letters (a–d) indicate differences among the soil, plants, bees, and products ($p < 0.05$; Kruskal–Wallis test). Error bars represent the standard deviation of the data

analyzed material samples (soil, plants, bees, and bee products) from both cities exhibited similar contents of these metals, except that the Cu and Zn contents in bee feces were substantially higher in the samples from Poznań (Figs. 2 and 3). In the samples from Lublin, the Fe and Cu contents in the honey were significantly negatively correlated with their contents in the nectar (Fe, $r = -0.934$; Cu, $r = -0.934$). In the samples from Poznań, these negative correlations were observed, but they were not significant (Fe, $r = -0.444$; Cu, $r = -0.626$). The Cd content was significantly lower in honey compared to that in the nectar, i.e., by 75% in the samples from Poznań and 88% in the samples from Lublin (Fig. 5). In the samples from both cities, the Pb content was similar between the honey and the nectar (Lublin: 0.312 ppm in the nectar, 0.367 ppm in the honey; Poznań: 0.692 ppm in the nectar, 0.400 ppm in the honey) (Fig. 4). In both cities, the Pb content was similar in all analyzed materials.

The Fe, Pb, and Cd contents were significantly higher in the soil than in the other materials (Figs. 1, 4, and 5). In terms of the parts of the plants, the Fe content in the roots was significantly higher than in the other plant parts. This was observed in the samples from both cities (Fig. 1). In the samples from Lublin, the

Cu content in the roots was significantly higher than in the leaves, flowers, and nectar (Fig. 2). In the samples from Poznań, significant differences in the Cu content were determined between the roots and the leaves and nectar and between the flowers and the leaves and nectar (Fig. 2). The Zn and Pb contents were significantly higher in the bee feces compared to that in the nectar, honey, and pollen (Figs. 3 and 4). The same relationship was also identified for Cu only in the samples from Poznań and for Cd only in the samples from Lublin (Figs. 2 and 5). Zn was accumulated in all parts of the bee body, i.e., in the head, thorax, and abdomen (Fig. 3).

Similar content of Fe, Cu, Zn, and Pb was found in the flowers, bee pollen, and beebread collected at both sampling sites (Figs. 1–4). In the samples from Poznań, the Cd contents in the bee pollen and beebread were slightly lower compared to those in the flowers, but this difference was not significant (Fig. 5).

The soil contained the highest levels of Fe, Pb, and Cd (Figs. 1, 4, and 5). In contrast, the plants were less severely contaminated, as their root filter partially prevented soil-contained elements from penetrating their tissues. In Figs. 1–5, we present the translocation of Fe, Cu, Zn, Pb, and Cd from the soil through the plant parts and into

Fig. 2 Copper content in soil, plants, bees, and bee products from two cities in Poland (Lublin and Poznań). Lowercase letters (a–d) indicate differences among the soil, plants, bees, and products ($p < 0.05$; Kruskal–Wallis test). Error bars represent the standard deviation of the data

the nectar, followed by the distribution of these elements in the bees' bodies.

4. DISCUSSION

Our investigations clearly confirmed that bees purify nectar through removal of metals, as proposed by Bogdanov (2006), Džugan et al.

(2018), and Tomczyk et al. (2020). We found that the metal content was significantly lower in the honey than in the nectar, except for Pb in Lublin and Poznań and Cd only in Lublin, which did not exhibit statistically significant differences between their content in the nectar and in the honey. During processing and ripening into honey, nectar loses approximately 80% of water, i.e., it is fourfold

Fig. 3 Zinc content in soil, plants, bees, and bee products from two cities in Poland (Lublin and Poznań). Lowercase letters (a–d) indicate differences among the soil, plants, bees, and products ($p < 0.05$; Kruskal–Wallis test). Error bars represent the standard deviation of the data

Fig. 4 Lead content in soil, plants, bees, and bee products from two cities in Poland (Lublin and Poznań). Lowercase letters (a–c) indicate differences among the soil, plants, bees, and products ($p < 0.05$; Kruskal–Wallis test). Error bars represent the standard deviation of the data

concentrated (Ball 2007); thus, the contents of elements in honey should be increased; however, no such increase was observed in the present study. Honeybees are able to evaporate water but have no possibility of evaporation of volatiles with these elements. Surprisingly, compared to the nectar samples, the honey contained up to 40-fold lower Fe levels, 26-fold lower Zn levels, and eightfold lower Cu and Cd levels, indicating loss of these elements during nectar processing. The removal of

Pb during processing was the least effective, with no apparent reduction in its content. However, given the fourfold concentration of the nectar during honey production and ripening, the similar Pb contents between the honey and the nectar indicate that a portion of Pb was eliminated with feces, in which we detected a high level of this element.

The conversion of nectar into honey is accompanied by the release of enzymes from the salivary and hypopharyngeal glands (Ball

Fig. 5 Cadmium content in soil, plants, bees, and bee products from two cities in Poland (Lublin and Poznań). Lowercase letters (a–c) indicate differences among the soil, plants, bees, and products ($p < 0.05$; Kruskal–Wallis test). Error bars represent the standard deviation of the data

2007), which decompose complex saccharides, especially sucrose, into monosaccharides. This breakdown of sugars is performed by saccharase. Thus, the chemical transformation of nectar into honey begins almost immediately (White 1992). The removal of metals from honey may involve the actions of enzymes responsible for chelation of elements.

The predominant acid in honey is gluconic acid (2,3,4,5,6-pentahydroxyhexanoic acid), which is produced by glucose oxidase through the action of bee secretions on glucose, and which exerts strong chelating activity (Ball 2007). Honey also contains a small amount of ascorbic acid, approximately 22 ppm (Ball 2007), and nectar contains approximately 300 ppm of proline originating from the bee (White 1992). All of these compounds act together during the conversion of nectar into honey, which is accompanied by formation of biocomplexes, in which elements are already chelated in the crop. The proventriculus captures biocomplexes with chelated elements that penetrate subsequent parts of the alimentary tract. This promotes formation of a temporary biocomplex, in which the chemical element is bound via a covalent (chelated) bond with an organic molecule, and their interaction produces a new biological effect, facilitating transport in a living organism (Skalny 2011, 2014; Markert et al. 2015;). Thus, we conclude that all of the presently analyzed elements formed biocomplexes, as they were transported from the alimentary tract to the bee hemocoel and subsequently to the fat body, abdomen, thorax, and head. Non-utilized Fe, Cu, and Zn elements (Skalny 2011, 2014) were excreted in feces. The Cd and Pb elements (Skalny 2011, 2014) present in the alimentary tract were partially immobilized in the fat body, and their residues were excreted with feces.

Some amounts of the Fe, Cu, and Zn ingested with nectar and pollen are utilized by bees for their own needs, as also reported by Kuterbach and Walcott (1986). Our present study demonstrated that Fe, Cu, and Zn were deposited in the head, thorax, and abdomen. Upon sufficient accumulation for proper function of the organism, the excess elements are excreted by the bee

in feces. As reported by Shaw et al. (2018), some iron grain deposition in the abdominal cavity begins on day 5 post-emergence and stabilizes around day 9 of bee life. Our study has revealed that deficient iron can potentially be replenished with age from nectar, as older bees do not feed on beebread (Shaw et al. 2018). Since it is accumulated in the form of grains, Fe must be transported in the bee body as a chelate, as is the case of Cu and Zn. Bees utilize Fe, Cu, and Zn for flight- and walk-related muscle movement and for enzyme production (Skalny 2014). Additionally, iron is required for sensing earth radiation and is stored as ferritin or hemosiderin grains in the abdomen (Nichol et al. 1995). Wang et al. (2013) suggest that elements ingested by bees enter the alimentary tract. Our study revealed large amounts of elements present in the bee feces. This clearly indicates that the organism can excrete excess of elements to prevent intoxication and premature death.

Zinc is an antagonist of Pb and Cd (Puzanowska-Tarasiewicz et al. 2009; Wołonciej et al. 2016). We detected Zn accumulation in the head, thorax, and abdomen of the bees. This element can reportedly be used for detoxification of the organism (Skalny 2011, 2014; Markert et al. 2015), which was confirmed by the methodology applied in the present study. After the dissection of the alimentary tract from the abdomen, only chitin rings and the fat body layer remained. We found that the fat body accumulated only part of the Zn ingested with nectar and pollen (Raes et al. 1989; Locke and Nichol 1992), and the excess Zn was excreted in feces. Based on the amount of Zn excreted by bees in the Poznań and Lublin samples, we can assume that when nectar contains greater levels of Pb and Cd contaminants, bees use Zn to block their absorption and these elements are then excreted. Our assumptions were confirmed by the highly significant correlation between the Pb contents in the abdomen and feces in both experiment sites, i.e., in the Lublin samples ($r=0.831$) and the Poznań samples ($r=0.942$). When Pb and Cd are not removed with feces but rather penetrate the hemocoel, a detoxification pathway is triggered in the hemolymph, and these elements are eventually trapped

in fat droplets in the fat body, as reported by Raes et al. (1989) and Locke and Nichol (1992).

Bees are extraordinary insects (Tautz 2008), as they can purify nectar (especially that of goldenrod and sunflower) by removal of balsamic substances, which are regurgitated from the crop and used for the production of propolis (Kadhim et al. 2018). In this manner, some amounts of metals present in nectar can be excreted with balms and deposited in propolis (Conti and Botrè 2001) due to the antagonistic effect of Zn against Pb and Cd (Puzanowska-Tarasiewicz et al. 2009; Wołonciej et al. 2016). The present study has demonstrated that bees can also purify nectar by removal of elements, including heavy metals, thus making honey a safe product for both bees and humans.

After conversion of the nectar into honey, the honey samples from Lublin were characterized by 0.495 ppm Cu, 0.367 ppm Pb, 0.056 ppm Cd, 1.035 ppm Zn, and 1.040 ppm Fe. In turn, the samples from Poznań exhibited the following contents of the elements: 0.617 ppm Cu, 0.400 ppm Pb, 0.046 ppm Cd, 1.089 ppm Zn, and 2.130 ppm Zn.

Among the honey contaminations, the only element that has a maximum residue limit established by European Union law is Pb, for which the maximum threshold in honey is 0.10 mg/kg wet weight, i.e., approx. 2 µg/kg dry weight, as specified by the Commission Regulation EU 1005 (2015).

Regarding Pb, based on relevant epidemiological studies, EFSA (2012) has established a range of benchmark dose level (BMDL) confidence limits to set the 95th BMD of 1% extra risk (BMDL01) as a reference point (RP) or point of departure (POD) for Pb risk characterization (Contia et al. 2020). For adults, BMDLs01 of 1.50 µg/kg body weight per day (with exerts effects on systolic blood pressure as the end-point) and 0.63 µg/kg body weight per day (with chronic kidney disease as the endpoint) were estimated (EFSA 2012; Contia et al. 2020).

Therefore, our research has shown for the first time that the Pb content in goldenrod honey from urban areas (Lublin and Poznań) exceeds the strict Commission Regulation EU 1005 (2015)

standards. This may be related to the presence of lead residue from leaded gasoline left by cars and the long time required by soil to be remedied. Concurrently, this indicates that consumption of honey produced in urban areas may pose some risk to human health, especially in the case of occasional consumption of high honey doses. Therefore, there is a need for examination of the Pb content in “urban honeys” produced from the nectar of other plants or honeydew to check whether it exceeds the Commission Regulation EU 1005 (2015).

Our present findings support the conclusions formulated by Conti and Botrè (2001), Ruschioni et al. (2013), Saunier et al. (2013), Losfeld et al. (2014), and Conti et al. (2018) that honey is not a good indicator of environmental pollution. We demonstrated that it is difficult to determine the amount of elements that may have been removed by bees. The use of honey to assess environmental contamination with heavy metals would require the development of conversion factors to calculate the concentration and purification of metals in nectar. This may depend on the element, type of nectar, and the characteristics of bees themselves. Bee pollen and bee bread may be better indicators of the actual environmental contamination, as they exhibited similar element contents to those found in flowers and were not purified by bees.

The novelty of this study is the determination of the contents of elements in individual parts of plants, including nectar, in the parts of the bee body (the head, thorax, and abdomen), and in feces. Previous studies that compared the contents of elements in bee bodies and in the final product, i.e., honey (Bogdanov 2006; Roman et al. 2011; Džugan et al. 2018; Tomczyk et al. 2020), have concluded that bees purify honey by removal of minerals, including heavy metals. Our present findings have revealed that bees use the elements present in pollen and nectar to meet their own needs. Moreover, our present research indicated the potential pathways involved in the purification of nectar, which yields the production of honey. Our findings confirmed that, in terms of the heavy metal content, honey from cities is safe for consumption.

The study also raises an issue whether honeybees flying between the nectar collection site and the colony begin the process of elimination of elements from the nectar. This can be determined by dissection of the crop of foragers and comparison of the transported nectar with that collected directly from flowers.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTIONS

GB, AS, and MJ conceived this research and designed the experiments; GB, AS, ES, NR, and MJ participated in the design and interpretation of the data; ES, DW, and AN performed the experiments and analysis; GB, KO, AS, and MJ wrote the paper and participated in the revisions thereof; all authors have read and approved the final manuscript.

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DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

DECLARATIONS

Conflict of interest The authors declare no competing interests.

Ethical approval No approval of the Research Ethics Committee was required to achieve the goals of this study, as

the experimental work involved an unregulated invertebrate species (*Apis mellifera*).

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