

THE COMPOSITION OF HEAVY METALS AND THE CONTENT OF ESTERIFIED FATTY ACIDS IN BEE TISSUES DEPENDING ON THE ENVIRONMENTAL CONDITION

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Abstract

The work aimed to record the content of heavy metals, including toxic and esterified forms of fatty acids in tissues, and the honey productivity of bees in different natural zones of the Carpathian region. The level of dangerous elements of the first class of toxicity - Lead and Cadmium - increases significantly in the tissues of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment. In the Carpathian region, all heavy metals accumulate in more significant quantities in the abdominal tissues of honey bees than in the tissues of the chest and head. In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, a decrease in the content of esterified forms of fatty acids is observed in the tissues of honey bees. The intensity of conversion of the esterified form of linolenic acid into its longer-chain and unsaturated fatty acids of the omega-3 family in the tissues of the head of honeybees in the foothills and especially in the forest-steppe zones is sharply reduced.

Key words: bioindicator, fatty acids, heavy metals, tissues.

INTRODUCTION

The sources of heavy metal emissions and the ways of their entry into the environment are diverse. Still, they are generally of artificial origin due to urbanization and industrialization (Slivinska et al., 2018; 2019; 2020; 2021). Urbanization and industrialization, in particular, the activities of industry, agriculture, energy, and transport, as well as intensive extraction of minerals – all led to the release of heavy, including highly toxic, lead, cadmium, and arsenic into the air, water, soil and plants (Gutyj et al., 2017; 2019; Kovka & Nedashkivskiy, 2019; Tikhonova et al., 2020; Briffa et al., 2020).

The migration of heavy metals in objects of the external environment caused their accumulation in soils and plants (Krainskiy et al., 2020; Tikhonova et al., 2020; Briffa et al., 2020; Bashchenko et al., 2020; Prychepa et al., 2021). As a result, some types of plants gave way to others, and the terms of their flowering changed, thus the conditions of honey collection by bees (Kryvyi et al., 2018; Kovka & Nedashkivskiy, 2019; Krainskiy et al., 2020; Kobysheva et al., 2021; Al-Kahtani et al., 2021). The above also led to the accumulation of heavy metals in the tissues of honey bees and beekeeping products (Didaras et al., 2020; Mărgăoan et al., 2021; Ricigliano et al., 2021).

As is known, heavy metals, including toxic ones, are involved in synthesizing, desaturation, and peroxide oxidation of long-chain fatty acids in plant and animal tissues and fluids. As a result, the supply of plant tissues, in particular pollen and bees, with energetic, structural, biologically active, and antimicrobial material changes (Desbois & Smith, 2010; Giri et al., 2018; Corby-Harris et al., 2021; Butsiak et al., 2021). All this affects bee colonies' productivity and their products' quality indicators (Matin et al., 2016; Kovalskyi et al., 2018; Vishchur et al., 2019; Gizaw et al., 2020; Monchanin et al., 2021).

Therefore, the issue of producing ecologically safe beekeeping products is urgent. Moreover, the production of honey bees occupies a prominent place in human life (Matin et al., 2016; Didaras et al., 2020; Costa et al., 2021; Ricigliano et al., 2021; El-Seedi et al., 2022). Very high requirements are placed on the quality indicators of honey bee products because Ukraine has become the leading exporter of honey to Europe.

In the literature, there needs to be more data on the content of heavy metals and different forms of fatty acids in bee pollen and tissues of honey bees kept in different natural zones of the Carpathian region (Klym & Stadnytska, 2019; Saranchuk et al., 2021).

Given the above, studies of the content of heavy metals, including toxic and esterified forms of fatty acids in tissues, and honey productivity of worker bees in different natural zones of the Carpathian region are of significant scientific and practical interest.

The goal of the work. To record the content of heavy metals, including toxic and esterified forms of fatty acids in tissues and honey productivity of bees in different natural zones of the Carpathian region.

MATERIALS AND METHODS

Experimental apiaries of clinically healthy honey bees of the Carpathian breed (*Apis mellifera* (L.) *carpatica*) were selected based on the private mountain (Slavsko village, Stryi district), foothills (Nizhnya Stynava village, Stryi district) and forest-steppe (village Myklashiv, Lviv district) zones of Lviv region,

where natural and climatic conditions and ecological situation are different.

To assess the intensity of technogenic load on the environment where experimental honey bee apiaries are located, the content of heavy metals (Ferrum, Zinc, Copper, Chromium, Cobalt, Nickel, Lead, and Cadmium) in the topsoil, bee pollen, and tissues of honey bees was determined.

In each of the above-described natural zones of the Carpathian region, the honey productivity of worker bees was studied in 3 apiaries and each of 5 hives (Kovalskyi & Kyryliv, 2011). In particular, the honey productivity of bees was reviewed in early June, mid-July, and mid-August by pumping out honey frames. These studies were conducted based on one bee colony of average strength and the same queen age. In addition, samples of bee pollen and honey bees were taken for laboratory research at each apiary from 3 hives in the spring-summer period. At the same time, examples of the arable layer of the soil were born in the radius of the available flight of honey bees.

The content of heavy metals, including toxic ones, was determined in selected samples of the topsoil, bee pollen, and tissues of the abdomen, chest, and head of honeybees and the pieces of the tissues above of honeybees – esterified forms of fatty acids. At the same time, the value of the level of heavy metals, including toxic and esterified forms of fatty acids, in tissues for the body of honey bees in different natural zones of the Carpathian region was analyzed.

The content of heavy metals (Ferrum, Zinc, Copper, Cobalt, Chromium, Nicol, Lead, and Cadmium) in the selected samples of the arable layer of the soil, bee comb, and tissues of the abdomen, chest, and head of honey bees was determined according to the currently valid state standard (DSTU 4405: 2005) on an atomic absorption spectrophotometer - Selma-115 (Vlizlo, 2012). The content of esterified forms of fatty acids in the tissues mentioned above of honey bees was determined by gas-liquid chromatography (Rivis et al., 2022).

The obtained digital material was processed by the method of variational statistics using the Student's criterion (Ibatullin & Zhukorskyi, 2017).

Arithmetic mean values (M) and errors ($\pm m$) were calculated. Differences were considered probable at $p < 0.05$. The computer program Origin 6.0 and Microsoft Excel were used for calculations.

RESULTS AND DISCUSSIONS

It was established that in the arable soil layer of the foothills and forest-steppe zones of the Carpathian region, compared to the

conditionally clean mountain zone, there is probably a higher content of the studied heavy metals (Table 1). At the same time, the arable layer of the soil of the forest-steppe zone of the Carpathian region contains the highest level of the studied heavy metals. The content of hazardous elements of the first toxicity class - Lead and Cadmium - in the arable layer of the soil in the above zone is 1.1 times higher than the maximum permissible concentration (Yatsuk & Balyuk, 2019).

Table 1. Gross content of heavy metals, including toxic ones, in the topsoil in different natural zones of the Carpathian region, g-10-3/kg of air-dry mass ($M \pm m$, $n = 3$)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	14325.00 \pm 294.214	15184.29 \pm 454.862*	16573.04 \pm 294.429**
Zinc, Zn	47.58 \pm 4.488	78.52 \pm 3.722**	96.13 \pm 4.890***
Cuprum, Cu	21.60 \pm 1.391	34.56 \pm 1.828**	45.64 \pm 2.264***
Cobalt, Co	11.76 \pm 0.375	13.63 \pm 0.560**	17.20 \pm 1.830***
Chrome, Cr	41.69 \pm 2.283	63.65 \pm 3.584**	87.53 \pm 4.163***
Nicole, Ni	21.24 \pm 1.625	41.33 \pm 2.512**	59.42 \pm 3.214***
Lead, Pb	19.37 \pm 0.784	25.83 \pm 1.442*	33.30 \pm 2.870***
Cadmium, Cd	2.03 \pm 0.088	2.60 \pm 0.115*	3.20 \pm 0.271***

Note. Here and further, differences are likely compared to the mountainous zone: * - $p < 0.05-0.02$; ** - $p < 0.01$; *** - $p < 0.001$

It is believed that the increase in the content of lead in the arable layer of the soil is associated with the intensive movement of motor vehicles (Loretta et al., 2015) and cadmium - with the introduction of meliorants and mineral fertilizers, primarily phosphogypsum and superphosphate, respectively (Razanov et al., 2015; Vozhehova et al., 2021). It can be seen that phosphoric acid residues in phosphogypsum and superphosphate bind solid elements and carriers for cadmium.

The obtained data characterize artificial environmental pollution in the experimental territories. The high level of heavy metals, including toxic ones, in the soil, is the reason for the increase in their concentration in bee pollen (plant pollen) obtained in the foothills and forest-steppe zones of the Carpathian region (Table 2). All this is a consequence of greater urbanization and industrialization of the above territories.

Table 2. The content of heavy metals, including toxic ones, in bee pollen in different natural zones of the Carpathian region, g-10-3/kg air-dry mass ($M \pm m$, $n = 3$)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	33.52 \pm 0.830	37.11 \pm 0.781*	43.39 \pm 2.253**
Zinc, Zn	34.39 \pm 1.91	39.20 \pm 0.900*	42.72 \pm 0.872**
Cuprum, Cu	2.01 \pm 0.089	3.02 \pm 0.169*	4.20 \pm 0.170***
Cobalt, Co	1.01 \pm 0.029	1.14 \pm 0.050*	1.44 \pm 0.112***
Chrome, Cr	4.10 \pm 0.177	5.02 \pm 0.180*	6.68 \pm 0.149***
Nicole, Ni	0.58 \pm 0.015	0.65 \pm 0.015*	0.74 \pm 0.023**
Lead, Pb	0.12 \pm 0.007	0.18 \pm 0.009*	0.26 \pm 0.012**
Cadmium, Cd	0.04 \pm 0.003	0.07 \pm 0.007*	0.10 \pm 0.009**

It should be noted that in the forest-steppe zone of the Carpathian region, compared to the mountain zone, the arable layer of the soil and bee pollen has a relatively high content of probiotic heavy metals - Zinc, Copper, Cobalt, Chromium, and Nickel. The

above-mentioned heavy metals in acceptable quantities are essential for plant tissues and bees (Hsu et al., 2021). However, the increased level of toxic lead and cadmium in the arable layer of the soil and bee hives can neutralize the positive effect

of probiotic heavy metals on the mentioned tissues (Purać et al., 2019).

The high level of Ferrum, Zinc, Copper, Cobalt, Chromium, Nicol, Plumbum, and Cadmium in bee honey, in turn, is the reason for the increase in their content in the tissues of honey bees. In particular, it was established that in the tissues of the abdomen (respectively 163.76 g·10⁻³/kg of raw mass and 191.91 against 127.61), breast (80.10 and 97.24 against 65.27), and head (respectively 100.32 and 119.32 against 81.24 g·10⁻³/kg of raw weight) of honey bees of the foothill and forest-steppe

zones, compared to the mountain zone, there is a higher total content of the studied heavy metals (Tables 3, 4 and 5). The level of dangerous elements of the first class of toxicity - Lead (by 1.33-4.00 times) and Cadmium (by 1.78-4.00 times) - is especially significant in the tissues of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment). The concentration of the element of the second toxicity class - Chromium - also increases noticeably (by 1.18-1.60 times).

Table 3. The content of heavy metals, including toxic ones, in the abdominal tissues of honey bees in different natural zones of the Carpathian region, g·10⁻³/kg raw weight (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	46.48 ± 1.046	63.72 ± 1.220***	77.03 ± 1.630***
Zinc, Zn	77.08 ± 1.190	91.32 ± 1.536**	104.24 ± 2.060***
Cuprum, Cu	0.34 ± 0.012	0.47 ± 0.014**	0.59 ± 0.014***
Cobalt, Co	0.31 ± 0.009	0.36 ± 0.014*	0.43 ± 0.017**
Chrome, Cr	2.43 ± 0.070	3.12 ± 0.082*	3.78 ± 0.112**
Nicole, Ni	2.43 ± 0.035	3.40 ± 0.067***	4.13 ± 0.059***
Lead, Pb	0.88 ± 0.035	1.21 ± 0.038**	1.50 ± 0.046***
Cadmium, Cd	0.09 ± 0.009	0.16 ± 0.006**	0.21 ± 0.012**

Table 4. The content of heavy metals, including toxic ones, in the breast tissues of honey bees in different natural zones of the Carpathian region, g·10⁻³/kg of raw mass (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	36.10 ± 0.931	42.18 ± 0.812**	49.06 ± 0.555***
Zinc, Zn	17.51 ± 0.587	23.50 ± 0.625**	31.46 ± 0.507***
Cuprum, Cu	1.93 ± 0.041	2.82 ± 0.061***	3.17 ± 0.070***
Cobalt, Co	1.84 ± 0.035	2.19 ± 0.077*	2.40 ± 0.049***
Chrome, Cr	3.08 ± 0.046	3.51 ± 0.058**	4.34 ± 0.186**
Nicole, Ni	4.00 ± 0.049	4.80 ± 0.085**	5.47 ± 0.128***
Lead, Pb	0.78 ± 0.023	1.04 ± 0.068*	1.25 ± 0.038***
Cadmium, Cd	0.03 ± 0.003	0.06 ± 0.003*	0.09 ± 0.003**

Table 5. The content of heavy metals, including toxic ones, in the tissues of the head of honey bees in different natural zones of the Carpathian region, g·10⁻³/kg of raw mass (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	26.79 ± 0.607	31.16 ± 0.979*	36.59 ± 1.324**
Zinc, Zn	30.13 ± 0.630	36.30 ± 0.564**	44.00 ± 0.280***
Cuprum, Cu	8.39 ± 0.319	14.35 ± 0.417***	17.66 ± 0.400***
Cobalt, Co	7.22 ± 0.055	7.56 ± 0.078*	7.97 ± 0.084**
Chrome, Cr	6.87 ± 0.098	8.15 ± 0.117**	9.47 ± 0.254***
Nicole, Ni	1.00 ± 0.055	1.47 ± 0.041**	1.78 ± 0.058**
Lead, Pb	0.81 ± 0.030	1.25 ± 0.044**	1.73 ± 0.050***
Cadmium, Cd	0.03 ± 0.003	0.08 ± 0.003***	0.12 ± 0.006***

It should also be noted that the tissues of the abdomen of honeybees are much more active accumulators of heavy metals than the tissues of the

chest and head. Practically all heavy metals are accumulated in 1.61-2.04 times greater quantities in

the tissues of the abdomen of honey bees than in the tissues of the chest and head.

Heavy metals at a physiologically determined level are involved in metabolic processes and the content of fatty acids in the body tissues of bees. In particular, cobalt-initiated protein synthesis in bee tissues, due to the activation of transport and information nucleic acids (Osman et al., 2021), is usually accompanied by the accumulation of polyunsaturated fatty acids necessary for the construction of cytoplasmic and cell membranes.

Since it is part of 9-desaturase, cuprum in the tissues of the body of bees at a physiologically determined level contributes to the formation of palmitic and stearic monounsaturated fatty acids of the omega-7 (palmitoleic) and omega-9 (oleic) families, respectively (Di et al., 2020; Takic et al., 2021).

Linoleic and linolenic acids, sequentially synthesized in plant tissues from oleic acid, are considered indispensable for bees and, therefore, must enter their bodies with food (Hajiahmadi et al., 2020; Hsu et al., 2021; Takic et al., 2021). In the tissues of bees from linoleic and linolenic acids because zinc is part of 2-, 3-, 4-, 5- and 6-desaturases, even longer-chain and more unsaturated fatty acids, respectively, of the omega-6 families (eicosatriene, eicosatetraenoic-arachidonic, docosadiene, and docosatetraenoic) and omega-3 (eicosapentaenoic, docosatrienoic, docosapentaenoic, and docosahexaenoic) (Takic et al., 2021; Hsu et al., 2021).

Longer chains and more unsaturated fatty acids of the omega-3 and omega-6 families are precious for the bee body. As mentioned above, polyunsaturated fatty acids in the body tissues of bees are a source for constructing cytoplasmic and cell membranes and synthesizing biologically active derivatives - prostaglandins, thromboxanes, and leukotrienes (Trinkl et al., 2020; Mărgăoan et al., 2021; Matuszewska et al., 2021). Thus, polyunsaturated fatty acids of the omega-3 and omega-6 families affect the health and vitality of bees.

Precious polyunsaturated fatty acids for bees are fatty acids of the omega-3 family (eicosapentaenoic, docosatrienoic, docosapentaenoic, and docosahexaenoic) (Trinkl et al., 2020; Matuszewska et al., 2021; Takic et al., 2021). These acids in the body tissues of bees are the initiators of synthesizing very strong and fast-acting anti-inflammatory peptide substances - cytokines (Ranneh et al., 2021).

Based on the above, our next task was to study the concentration of esterified fatty acids in the tissues of the abdomen, breast, and head of honey bees obtained from hives located in the mountain, foothill, and forest-steppe zones of the Carpathian region.

The content of esterified forms of saturated fatty acids with an even (caprylic, capric, lauric, myristic, palmitic, and stearic) and odd (pentadecanic) amount of carboxylic acids was studied in the tissues of the abdomen, breast, and head of honey bees of the foothills and forest-steppe zones of the Carpathian region, compared to the mountain zone. Atoms in the chain, monounsaturated fatty acids of the omega-7 (palmitoleic) and omega-9 (oleic and eicosanoic) families, and polyunsaturated fatty acids of the omega-3 (linolenic, eicosapentaenoic, docosatrienoic, docosapentaenoic and docosahexaenoic) and omega-6 (linoleic, eicosadienoic) families, eicosatrienoic, eicosatetraenoic-arachidonic, docosadiene and docosatetraenoic). Data on their content are presented in Tables 6, 7, and 8.

It should be noted that the most significant amount of esterified forms of fatty acids is found in the tissues of the abdomen, breast, and head of honey bees (Desbois & Smith, 2010; Giri et al., 2018; Burdge, 2018; Saranchuk, 2020; Corby-Harris et al., 2021). Esterified forms of fatty acids in the mentioned tissues of honey bees are included in the composition of phospholipids, esterified cholesterol, mono-, di- and triacylglycerols (Desbois & Smith, 2010; Corby-Harris et al., 2021). It was established (Tables 6, 7 and 8) that the total concentration of esterified forms of fatty acids in the tissues of the abdomen, chest and head of honey bees obtained from hives located in the foothills (respectively 17.67, 18.57 and 18.33 g/kg of raw mass) and forest-steppe (16.98, 17.69 and 17.31) zones of the Carpathian region, compared with the tissues of the abdomen, breast and head of honey bees selected from hives located in the mountain zone (21.72, 22, 69 and 23.17), due to the lower level of saturated fatty acids in their composition (2.24, 2.32 and 1.99 and 2.09, 2.12 and 1.96 versus 2.93, 2.97 and 2.97) and an odd (0.07, 0.07 and 0.09 and 0.06, 0.05 and 0.08 versus 0.09, 0.09 and 0.13) number of carbon atoms in the chain, of monounsaturated fatty acids of the omega-7 (0.05, 0.05 and 0.05 and 0.04, 0.04 and 0.04 versus 0.08, 0.07 and 0.07) and omega-9 (3.53, 3.31 and 3.12 and 3.36, 3.20 and 3.03 vs. 4.12, 4.00 and 3.78) and polyunsaturated fatty acids of omega-3 families (6.18, 6, 62 and 7.29 and 5.97, 6.42 and 6.98 against 7.61, 8.01 and 9.10) and about mega-6 (respectively 5.60, 6.10 and 5.79 and 5.46, 5.86 and 5.22 against 6.89, 7.55 and 7.12 g/kg raw weight).

In terms of energy, lipids are much more valuable than proteins and carbohydrates (Ruedenauer et al., 2021; Stabler et al., 2021). Literature sources indicate that the greater the number of fatty acids contained in tissues, the

greater their energy value for the body of honey bees (Ruedenauer et al., 2021). It was established (Tables 6, 7, and 8) that the most significant amount of esterified forms of saturated, monounsaturated, and polyunsaturated fatty acids are contained in the tissues of the abdomen, breast, and head of honey bees in the mountainous zone of the

Carpathian region (respectively 21.72, 22.69 and 23, 17 g/kg of raw weight), a smaller amount of them is in the tissues of the abdomen, chest, and head of honey bees in the foothills (17.67, 18.57 and 18.33), and even less in the forest-steppe (respectively 16.98, 17, 69 and 17.31 g/kg raw weight).

Table 6. The content of esterified fatty acids in the abdominal tissues of honey bees in different natural zones of the Carpathian region at the beginning of the summer period, g/kg of raw mass ($M \pm m$, $n = 3$)

Acid and its code	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Caprylic acid, 8:0	0.08 ± 0.003	0.04 ± 0.003**	0.03 ± 0.003**
Capric acid, 10:0	0.05 ± 0.003	0.03 ± 0.003*	0.02 ± 0.003**
Lauric acid, 12:0	0.06 ± 0.003	0.04 ± 0.003*	0.03 ± 0.003**
Myristic acid, 14:0	0.07 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Pentadecanoic acid, 15:0	0.09 ± 0.003	0.07 ± 0.003*	0.06 ± 0.003**
Palmitic acid, 16:0	1.20 ± 0.028	0.93 ± 0.024**	0.92 ± 0.012***
Palmitoleic acid, 16:1	0.08 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Stearic acid, 18:0	1.30 ± 0.032	1.04 ± 0.045**	0.95 ± 0.024***
Oleic acid, 18:1	3.91 ± 0.085	3.36 ± 0.031**	3.21 ± 0.059**
Linoleic acid, 18:2	2.79 ± 0.074	2.28 ± 0.080**	2.23 ± 0.038**
Linolenic acid, 18:3	4.05 ± 0.113	3.31 ± 0.052**	3.20 ± 0.067**
Arachinic acid, 20:0	0.17 ± 0.007	0.11 ± 0.005**	0.10 ± 0.006**
Eicosaenoic acid, 20:1	0.21 ± 0.006	0.17 ± 0.003*	0.15 ± 0.003**
Eicosadienoic acid, 20:2	0.21 ± 0.009	0.17 ± 0.003*	0.15 ± 0.003**
Eicosatrienoic acid, 20:3	0.31 ± 0.009	0.23 ± 0.009**	0.21 ± 0.006**
Arachidonic acid, 20:4	3.18 ± 0.076	2.67 ± 0.035**	2.60 ± 0.032**
Eicosapentaenoic acid, 20:5	2.17 ± 0.071	1.74 ± 0.023**	1.71 ± 0.017**
Docosadienoic acid, 22:2	0.28 ± 0.012	0.21 ± 0.006**	0.19 ± 0.007**
Docosatrienoic acid, 22:3	0.32 ± 0.010	0.23 ± 0.006**	0.21 ± 0.006**
Docosatetraenoic acid, 22:4	0.32 ± 0.009	0.25 ± 0.006**	0.23 ± 0.007**
Docosapentaenoic acid, 22:5	0.53 ± 0.015	0.43 ± 0.006**	0.40 ± 0.007**
Docosahexaenoic acid, 22:6	0.54 ± 0.009	0.47 ± 0.015*	0.45 ± 0.007**

Table 7. The content of esterified fatty acids in the breast tissues of honey bees in different natural zones of the Carpathian region at the beginning of the summer period, g/kg of raw weight ($M \pm m$, $n = 3$)

Acid and its code	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Caprylic acid, 8:0	0.03 ± 0.003	0.02 ± 0.003*	0.01 ± 0.000**
Capric acid, 10:0	0.04 ± 0.003	0.02 ± 0.003*	0.01 ± 0.003**
Lauric acid, 12:0	0.05 ± 0.003	0.03 ± 0.003*	0.02 ± 0.006*
Myristic acid, 14:0	0.09 ± 0.003	0.06 ± 0.003**	0.04 ± 0.003***
Pentadecanoic acid, 15:0	0.09 ± 0.006	0.07 ± 0.003*	0.05 ± 0.003**
Palmitic acid, 16:0	1.29 ± 0.026	1.08 ± 0.022**	0.97 ± 0.098**
Palmitoleic acid, 16:1	0.07 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Stearic acid, 18:0	1.30 ± 0.032	1.02 ± 0.050**	0.97 ± 0.047**
Oleic acid, 18:1	3.74 ± 0.066	3.13 ± 0.073**	3.04 ± 0.084**
Linoleic acid, 18:2	2.95 ± 0.064	2.37 ± 0.049**	2.28 ± 0.052**
Linolenic acid, 18:3	3.58 ± 0.118	3.02 ± 0.058**	2.92 ± 0.04**
Arachinic acid, 20:0	0.17 ± 0.006	0.11 ± 0.006**	0.10 ± 0.006**
Eicosaenoic acid, 20:1	0.26 ± 0.014	0.18 ± 0.009**	0.16 ± 0.012**
Eicosadienoic acid, 20:2	0.28 ± 0.009	0.20 ± 0.015*	0.19 ± 0.015*
Eicosatrienoic acid, 20:3	0.21 ± 0.009	0.16 ± 0.006**	0.15 ± 0.006**
Arachidonic acid, 20:4	3.51 ± 0.082	3.00 ± 0.050**	2.80 ± 0.128**
Eicosapentaenoic acid, 20:5	2.43 ± 0.050	1.98 ± 0.041**	1.97 ± 0.044**
Docosadienoic acid, 22:2	0.23 ± 0.009	0.18 ± 0.007*	0.17 ± 0.007**

Docosatrenoic acid, 22:3	0.27 ± 0.012	0.19 ± 0.007**	0.17 ± 0.009**
Docosatetraenoic acid, 22:4	0.37 ± 0.014	0.29 ± 0.009**	0.27 ± 0.009**
Docosapentaenoic acid, 22:5	0.81 ± 0.034	0.67 ± 0.009*	0.63 ± 0.019*
Docosahexaenoic acid, 22:6	0.92 ± 0.029	0.76 ± 0.019**	0.73 ± 0.017**

Table 8. The content of esterified fatty acids in the tissues of the head of honey bees in different natural zones of the Carpathian region at the beginning of the summer period, g/kg of raw weight ($M \pm m$, $n = 3$)

Acid and its code	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Caprylic acid, 8:0	0.03 ± 0.003	0.02 ± 0.003*	0.01 ± 0.003*
Capric acid, 10:0	0.04 ± 0.003	0.02 ± 0.003*	0.01 ± 0.003**
Lauric acid, 12:0	0.05 ± 0.003	0.03 ± 0.003*	0.02 ± 0.003**
Myristic acid, 14:0	0.09 ± 0.003	0.06 ± 0.003**	0.05 ± 0.003**
Pentadecanoic acid, 15:0	0.13 ± 0.003	0.09 ± 0.003**	0.08 ± 0.007**
Palmitic acid, 16:0	1.52 ± 0.084	0.98 ± 0.030*	0.94 ± 0.029**
Palmitoleic acid, 16:1	0.07 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Stearic acid, 18:0	1.05 ± 0.038	0.86 ± 0.014*	0.83 ± 0.015**
Oleic acid, 18:1	3.54 ± 0.089	2.95 ± 0.045**	2.87 ± 0.026**
Linoleic acid, 18:2	2.77 ± 0.068	2.15 ± 0.036**	2.03 ± 0.037***
Linolenic acid, 18:3	3.54 ± 0.075	3.00 ± 0.054**	2.92 ± 0.046**
Arachinic acid, 20:0	0.18 ± 0.012	0.11 ± 0.007**	0.10 ± 0.007**
Eicosaenoic acid, 20:1	0.24 ± 0.012	0.17 ± 0.006**	0.16 ± 0.003**
Eicosadienoic acid, 20:2	0.19 ± 0.006	0.14 ± 0.006**	0.13 ± 0.006**
Eicosatrienoic acid, 20:3	0.13 ± 0.003	0.10 ± 0.003**	0.09 ± 0.003**
Arachidonic acid, 20:4	3.35 ± 0.072	2.85 ± 0.087*	2.44 ± 0.042***
Eicosapentaenoic acid, 20:5	2.47 ± 0.081	1.94 ± 0.058**	1.87 ± 0.037**
Docosadienoic acid, 22:2	0.22 ± 0.009	0.17 ± 0.006**	0.16 ± 0.006**
Docosatrenoic acid, 22:3	0.23 ± 0.006	0.18 ± 0.006**	0.16 ± 0.006**
Docosatetraenoic acid, 22:4	0.46 ± 0.014	0.38 ± 0.006**	0.37 ± 0.006**
Docosapentaenoic acid, 22:5	1.24 ± 0.038	0.91 ± 0.029**	0.86 ± 0.032**
Docosahexaenoic acid, 22:6	1.62 ± 0.058	1.26 ± 0.029**	1.17 ± 0.038**

Esterified forms of polyunsaturated fatty acids of the omega-3 and omega-6 families in the composition of phospholipids are included in the structure of cellular and cytoplasmic membranes of the body and ensure their functional activity and, ultimately, the vital activity of bees (Trinkl et al., 2020). At the same time, the esterified form of linoleic acid and its longer-chain and unsaturated fatty acids in the body of bees is the initiator of pro-inflammatory processes (Mărgăoan et al., 2021), and linoleic acid and its longer-chain and unsaturated fatty acids are anti-inflammatory (Ranneh et al., 2021). Esterified forms of linoleic and linolenic acids and their longer-chain and unsaturated fatty acids act on the bees' bodies through the corresponding pro-inflammatory and anti-inflammatory peptide cytokines (Ranneh et al., 2021).

It was recorded (Tables 6, 7, and 8) that the most significant amount of esterified forms of polyunsaturated fatty acids of the omega-3 and omega-6 families are contained in the tissues of the abdomen, breast, and head of honey bees of

the mountain zone of the Carpathian region (respectively 7.61, 8.01 and 9.10 and 6.89, 7.55 and 7.55 g/kg of raw weight), a smaller number of them are in the tissues of the abdomen, chest, and head of honey bees in the foothill zone (6.18, 6.62 and 7, 29 and 5.60, 6.10 and 5.79), even less in the forest-steppe (respectively 5.97, 6.42 and 6.98 and 5.46, 5.86 and 5.22 g/kg raw weight). The mentioned esterified forms of polyunsaturated fatty acids of the omega-6 and omega-3 families in the exact quantities in bee tissues are initiators of pro-inflammatory and anti-inflammatory processes, respectively.

Esterified forms of polyunsaturated fatty acids linolenic and linoleic in the tissues of the bee body are primarily the precursors of a whole series of longer-chain and more unsaturated fatty acids, respectively, of the families of omega-3 (eicosapentaenoic, docosatrenoic, docosapentaenoic and docosahexaenoic) and omega-6 (eicosatrienoic, eicosatetraenoic-arachidonic, docosadiene and docosaterpene). Zinc takes an active part in the reactions of

conversion of linoleic and linolenic acids into their longer-chain and more unsaturated derivatives in bee tissues, as it is part of 2-, 3-, 4-, 5-, and 6-desaturases (Di et al., 2020; Hsu et al., 2021). At the same time, the ratio of the content of esterified forms of linolenic and linoleic acids to the content of their longer-chain and unsaturated fatty acids of the omega-3 and omega-6 families, respectively, indicates the intensity of transformation of the less functionally and biologically active former into more active latter (Hsu et al., 2021).

It was established (Tables 6, 7, and 8) that the esterified form of linolenic and linoleic acids in the abdominal tissues of honey bees kept in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain ones, are less wholly transformed into their longer-chain and unsaturated fatty acids of the families omega-3 (1.15 and 1.16 vs. 1.14, respectively) and omega-6 (0.69 and 0.69 vs. 0.68, respectively). The same direction of transformations of the esterified form of linolenic acid is observed in the breast tissues of honey bees (0.82 and 0.83 versus 0.81). At the same time, in the breast tissues of honey bees of the forest-steppe zone of the Carpathian region, compared to breast tissues of honey bees of the mountain zone, the intensity of conversion of the esterified form of linoleic acid into its longer-chain and unsaturated fatty acids of the omega-6 family is sharply reduced (0.64 vs. 0.60). A similar direction of transformations of the esterified form of linoleic acid is observed in the tissues of the head of honey bees of the foothill zone of the Carpathian region (0.64 versus 0.59). At the same time, in the tissues of the head of honey bees, which are kept in the foothills and especially in the forest-steppe zones of the Carpathian region, compared to the mountains, the intensity of the conversion of the esterified form of linolenic acid into its longer-chain and unsaturated fatty acids of the omega-3 family (respectively 0.70 and 0.72 vs. 0.64).

The above-mentioned indicates the negative influence of several heavy metals present, primarily toxic Lead and Cadmium, on the synthesis in the body tissues of honeybees of very active biologically and functionally longer chain and unsaturated fatty acids of the omega-3 and omega-6 families.

The low total content of esterified polyunsaturated and monounsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families can contribute to the increase in the fragility of bee comb walls through the bee body, in particular the wax glands (Didaras et al., 2020; Castaños et al., 2022). At the same time, the shallow content of the above fatty acids in the tissues of the abdomen, chest, and head of honeybees can cause a decrease in the permeability of its structural components to water and water-soluble substances and thereby inhibit the intensity of metabolic processes (Bakour et al., 2022; Castaños et al., 2022). It can also contribute to a decrease in the functional activity of cellular and cytoplasmic membranes of the body of honey bees and thereby inhibit their vital activity (Corby-Harris et al., 2021; Ricigliano et al., 2021; Wang et al., 2021).

It was established that the total content of esterified polyunsaturated and monounsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families in the tissues of the abdomen, breast, and head of honey bees obtained from hives located in the foothills (respectively 15.36, 16.08 and 16.25 g/kg of raw weight) and forest-steppe (14.83, 15.52 and 15.27) zones of the Carpathian region, compared to the tissues of the abdomen, breast, and head of honey bees selected from hives located in the mountain zone (18.70, 19.63 and 20.07 g/kg raw weight, respectively), is smaller (Tables 6, 7 and 8).

Saturated, monounsaturated, and polyunsaturated fatty acids caprylic, capric, lauric, myristic, palmitoleic, oleic, linoleic, linolenic, eicosaenic, eicosadiene, eicosatriene, eicosatetraenoic-arachidonic, eicosapentaenoic, docosadiene, docosatrienoic, docosatetraenoic, docosapentaenoic, and docosahexaenoic provide antibacterial protection of the body honey bees (Didaras et al., 2020; Rothman et al., 2020; Mărgăoan et al., 2021; Bakour et al., 2022). In particular, the high antibacterial activity of the above acids against bee rot was found (Rothman et al., 2020).

Caprylic and, to a lesser extent, capric and, to a lesser extent, lauric and, to a lesser extent, myristic acids have an antimicrobial effect due to their high ability to reduce the concentration of hydrogen ions (Didaras et al., 2020;

Rothman et al., 2020; Bakour et al., 2022), and palmitoleic, oleic, linoleic, linolenic, eicosaic, eicosadiene, eicosatriene, eicosatetraeno-arachidone, eicosapentaeno, docosadiene, docosatrieno, docosatetraenoic, docosapentaenoic, and docosahexaenoic - with the growth of this series, increase the surface activity of the tissues of microorganisms and thereby strongly suppress their vital activity under the normal osmotic pressure of the surrounding environment (Wang et al., 2021; Mărgăoan et al., 2021).

It was established that the total content of esterified caprylic, capric, lauric, myristic, palmitoleic, oleic, linoleic, linolenic, eicosenoic, eicosadienoic, eicosatrienoic, eicosatetraenoic-arachidonic, eicosapentaenoic, docosadienoic, docosatrienoic, docosatetraenoic, docosapentaenoic, and docosahexaenoic acids, which provide antibacterial protection of the bee organism, in the tissues of the abdomen, chest, and head of honey bees obtained from hives located in the foothills (respectively 15.36, 16.31 and 16.38 g/kg of raw mass) and forest-steppe (15.31, 15.60 and 15.36) zones of the Carpathian region, compared to the tissues of the abdomen, chest, and head of honey bees selected from hives located in the mountain zone (respectively 19.16, 19.84 and 20.28 g/kg of raw mass), there is less (Tables 5, 6 and 7). This significantly affects, as evidenced by the scientific literature (Didaras et al., 2020; Mărgăoan et al., 2021; Wang et al., 2021), the antibacterial and antifungal activity of the body tissues of honey bees.

Thus, as a result of an increase in the artificial load on the environment and the accumulation of heavy metals, primarily toxic, in the components of the ecosystem, the energy, structural, biological, and antimicrobial value of the central mass of long-chain fatty acids for the body of bees decreases.

The decrease in the content of esterified forms of fatty acids in the tissues of honey bees kept in hives located in the foothills and especially the forest-steppe zones of the Carpathian region, compared to the tissues of honey bees selected from hives located in the mountainous zone, is associated with their transition to anionic form. This is due primarily to the

binding of fatty acids by heavy metal cations (Giri et al., 2018).

It was established that the honey productivity of worker bees in the mountainous zone of the Carpathian region is at the level of 46.6 ± 0.95 kg, in the foothills - 36.6 ± 1.04 , $p < 0.05$, and in the forest-steppe - 31.2 ± 0.56 kg, $p < 0.01$ per bee colony per season. The honey productivity of worker bees decreases due to the high level of heavy metals, including toxic but low - esterified fatty acids, in the tissues. Other scientists also point to the negative influence of territories polluted with heavy metals on the productive characteristics of honey bees (Gizaw et al., 2020; Monchanin et al., 2021).

All over the world, there is a search for means of bioindication of the ecological state of the environment (Komarova, 2018; Costa et al., 2021). This is because heavy metals, like other environmental pollutants, have different transfer rates from the soil to the root system, from the root system to the stem, from the stem to the inflorescence, and from the inflorescence to pollen and nectar.

It was previously indicated (Saranchuk & Ravis, 2008) that *Taraxacum officinale* Wigg pollen can serve as a bioindicator of the ecological state of the environment in the conditions of the Carpathian region due to the optimal content of heavy metals and fatty acids. The positive thing about this bioindicator is that it allows to determine different levels of accumulation of heavy metals and fatty acids and thus gives more information. The body tissues of honey bees can also serve as a bioindicator of the ecological state of the environment in terms of the content of heavy metals and esterified forms of fatty acids.

CONCLUSIONS

The total content of the studied heavy metals in the tissues of honey bees in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain zone, is 1.23-1.50 times higher. The level of dangerous elements of the first class of toxicity - Lead (by 1.33-4.00 times) and Cadmium (by 1.78-4.00 times) - is especially significant in the tissues of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment).

In the Carpathian region, all heavy metals accumulate 1.61-2.04 times more in the abdominal

tissues of honey bees than in the breast and head tissues.

In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, a decrease in the content of esterified forms of fatty acids is observed in the tissues of honey bees (21.72-23.17, 17.67-18.57 and 16.98-17.69 g/kg raw mass).

Esterified forms of linolenic and linoleic acids in the abdominal tissues of honey bees kept in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain ones, are less completely converted into their longer-chain and unsaturated fatty acids of the omega-3 families (respectively 1, 15 and 1.16 vs. 1.14) and omega-6 (0.69 and 0.69 vs. 0.68, respectively). At the same time, the intensity of conversion of the esterified form of linoleic acid into its longer chain and unsaturated derivatives of the omega-6. Also, the intensity of the conversion of the esterified form of linolenic acid into its longer-chain and unsaturated fatty acids of the omega-3 family in the tissues of the head of honey bees in the foothills and especially in the forest-steppe zones (0.70 and 0.72 vs. 0.64, respectively) is sharply reduced.

As a result of the accumulation of heavy metals in tissues, energy (by 21.0-44.6%), structural (21.2-45.7), biological (8.7-24.7), and antimicrobial (by 21.6-32.1%) values of esterified forms of fatty acids for the body of honey bees kept in hives located in the foothills and especially forest-steppe zones of the Carpathian region, compared to the tissues of bees kept in apiaries located in the mountainous zone.

The honey productivity of worker bees per beehive per season is lower in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain, by 27.3% and 49.4%, respectively.

Body tissues of honey bees can serve as a bioindicator of the ecological state of the environment in terms of the content of heavy metals and esterified forms of fatty acids

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