ТВАРИННИЦТВО

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REPRODUCTIVE ABILITY OF QUEEN BEES UNDER THE INFLUENCE OF HEAVY METALS AND FATTY ACIDS OF BEE POLLEN

In the literature, there are only fragmentary data on the content of heavy metals and fatty acids in bee pollen and tissues of honey bees kept in different natural zones of the Carpathian region.

The aim of the work was to determine the relationship between the content of Zinc, Copper, Lead, Cadmium and unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families in bee pollen and the intensity of egg-laying by queen bees kept in hives located in separate natural zones of the Carpathian region.

Experimental apiaries of clinically healthy honey bees of the Carpathian breed were selected on the basis of private apiary farms in the mountain, foothill and forest-steppe zones of the Lviv region. In order to assess the intensity of man-made load on the environment, where experimental honey bee apiaries are located, the content of heavy metals in the topsoil, bee comb and abdominal tissues of honey bees was determined. In the second half of the spring period, in each of the above-described natural areas of the Carpathian region, in 3 apiaries and in each of 3 hives, samples of bee pollen and honey bees were taken for laboratory research. In addition, samples of the arable layer of the soil were taken in the radius of the useful flight of honey bees. In each of the above-described natural zones of the Carpathian region, in 3 apiaries and in each of 3 hives in the second half of the spring period, for 36 days, every twelfth day, the intensity of egg-laying of queen bees was studied.

The content of heavy metals, including toxic ones, was determined in selected samples of the topsoil, bee pollen and honey bee abdominal tissues. The content of unsaturated fatty acids in samples of bee pollen and honey bee tissues was determined as well.

It has been established that Cuprum and especially Zinc are characterized by relatively high coefficients of transition from the arable soil layer to bee colony in various natural zones of the Carpathian region. At the same time, the transfer coefficients of Cadmium and especially Plumbum into bee pollen are very low. It was recorded that in the direction from the mountain to the foothills and forest-steppe zone of the Carpathian region, the coefficient of transition of Zinc from the arable layer of the soil to the bee colony decreases. At the same time, the assimilation of Cadmium by plants increases.

In the direction from the mountain to the foothills and forest-steppe zone of the Carpathian region, due to the high concentration of Zinc, Copper, Plumbum and especially Cadmium, the value of unsaturated fatty acids of bee pollen for intensive egg-laying of queen bees decreases. The egg-laying intensity of queen bees kept in hives located in the foothills and especially in the forest-steppe zones of the Carpathian region is lower than that of queen bees in the mountainous zone.

Bee pollen and abdominal tissues of honey bees can serve as a bioindicator of the ecological state of the environment by the content of heavy metals and unsaturated fatty acids. An integrated indicator such as the intensity of egg-laying by queen bees can also serve as a good bioindicator of the ecological state of the environment.

Keywords: natural zones of the Carpathian region, arable soil layer, bee pollen, abdominal tissue of honey bees, heavy metals, fatty acids, egg-laying intensity of bee queens, bioindicator.

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Відтворна здатність бджолиних маток за впливу важких металів і жирних кислот бджолиного обніжжя

У літературі є тільки фрагментарні дані щодо вмісту важких металів і жирних кислот у бджолиному обніжжі та тканинах медоносних бджіл, яких утримують у різних природних зонах Карпатського регіону.

Метою роботи було зафіксувати зв'язок між вмістом Цинку, Купруму, Плюмбуму і Кадмію й ненасичених жирних кислот родин омега-3, омега-6, омега-7 і омега-9 у бджолиному обніжжі та інтенсивністю яйцекладки бджолиних маток, яких утримують у вуликах, розміщених в окремих природних зонах Карпатського регіону.

Піддослідні пасіки клінічно здорових медоносних бджіл породи карпатська було підібрано на базі приватних пасічних господарств гірської, передгірної та лісостепової зон Львівської області. Для оцінки інтенсивності техногенного навантаження на довкілля, де знаходяться піддослідні пасіки медоносних бджіл, визначали вміст важких металів в орному шарі ґрунту, бджолиному обніжжі та тканинах черевця медоносних бджіл. У другій половині весняного періоду в кожній із описаних вище природних зон Карпатського регіону на 3 пасіках і на кожній із 3 вуликів для лабораторних досліджень відбирали зразки бджолиного обніжжя та медоносних бджіл. До того ж у радіусі корисного льоту медоносних бджіл відбирали зразки орного шару ґрунту. Крім того, в кожній із описаних вище природних зон Карпатського регіону на 3 пасіках і на кожній на 3 вуликах у другій половині весняного періоду впродовж 36 діб щодванадцятої доби досліджували інтенсивність яйцекладки бджолиних маток. У відібраних зразках орного шару ґрунту, бджолиного обніжжя та тканин черевця медоносних бджіл визначали вміст важких металів, зокрема токсичних, а в зразках бджолиного обніжжя та тканин медоносних бджіл – ненасичениих жирних кислот.

Встановлено, що Купруму та особливо Цинку властиві відносно високі коефіцієнти переходу з орного шару ґрунту в бджолине обніжжя в різних природних зонах Карпатського регіону. Водночас коефіцієнти переходу Кадмію та особливо Плюмбуму в бджолине обніжжя є дуже низькими. Зафіксовано, що в напрямі від гірської до передгірної та лісостепової зони Карпатського регіону знижується коефіцієнт переходу Цинку з орного шару ґрунту в бджолине обніжжя. Водночас зростає засвоєння рослинами Кадмію.

У напрямі від гірської до передгірної та лісостепової зони Карпатського регіону через високу концентрацію Цинку, Купруму, Плюмбуму й особливо Кадмію знижується цінність ненасичених жирних кислот бджолиного обніжжя для інтенсивної яйцекладки бджолиних маток. Інтенсивність яйцекладки бджолиних маток, яких утримують у вуликах, розміщених у передгірній та особливо в лісостеповій зонах Карпатського регіону, порівняно з бджолиними матками гірської зони є меншою.

Бджолине обніжжя та тканини черевця медоносних бджіл за вмістом важких металів і ненасичених жирних кислот можуть слугувати біоіндикатором екологічного стану довкілля. Добрим біоіндикатором екологічного стану довкілля може слугувати також такий інтегрований показник, як інтенсивність яйцекладки бджолиних маток.

Ключові слова: природні зони Карпатського регіону, орний шар ґрунту, бджолине обніжжя, тканини черевця медоносних бджіл, важкі метали, жирні кислоти, інтенсивність яйцекладки бджолиних маток, біоіндикатор.

Introduction. The problem of heavy metals and fatty acids in the chain of bee pollen – bee tissues is as follows [19]. Heavy metals and fatty acids are involved in the functional activity of bee body tissues [8]. Heavy metals and fatty acids, depending on the amount and composition, can change the supply of bees' body tissues with energetic, structural, biologically active and antimicrobial material [28]. This is due to the fact that bee tissues are capable of synthesizing only saturated and monounsaturated fatty acids using enzyme systems dependent on heavy metals [14, 34]. Bee tissues are unable to synthesize polyunsaturated fatty acids. Therefore, such essential polyunsaturated fatty acids as linoleic and linolenic should enter their body with food [12, 19, 22, 31, 26].

The main source of indispensable (essential) α-linoleic and α-linolenic acids in diets of bees is bee pollen (plant pollen) [12, 19, 22, 31, 39, 40]. The above mentioned polyunsaturated fatty acids are dominant in the fatty acid composition of bee pollen [14, 40]. Even longer-chain and more unsaturated fatty acids of the omega-6 and omega-3 families, respectively, are synthesized in the tissues of bees from α-linoleic and α-linolenic acids with the help of enzyme systems dependent on heavy metals [22, 31, 34].

A common sign of a deficiency of omega-3 and omega-6 fatty acids in the bee body is a decrease in growth rates, the efficiency of assimilation of feed nutrients, suppression of immunity, and a decrease in productive signs [39, 40].

In the literature, there are only fragmentary data on the content of heavy metals and fatty acids in bee honey taken from hives located in the mountain, foothill and forest-steppe zones of the Carpathian region, where there are different natural and climatic conditions and ecological situation [28].

The aim of the work was to determine the relationship between the content of Zinc, Copper, Plumbum and Cadmium and unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families in bee pollen and the intensity of egg-laying by queen bees kept in hives located 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 on the basis of private apiaries in mountain (Slavsko village, Stryi district), foothills (Nyzhnia Stynava village, Stryi district) and forest-steppe (Myklashiv village, Lviv district) zones of Lviv region.

In order to assess the intensity of man-made load on the environment where experimental honey bee apiaries are located, the content of heavy metals (Zinc, Copper, Plumbum and Cadmium) in the topsoil, bee pollen and abdominal tissues of honey bees was determined.

In the second half of the spring period, in each of the above-described natural zones of the Carpathian region, in 3 apiaries and in each of 3 beehives, samples of bee pollen and honey bees were taken for laboratory research using generally accepted methods [5]. There were 2-year-old queen bees in each experimental hive. In addition, within the useful flight radius (2-4 km) of honey bees, samples of the arable layer of the soil were taken.

In each of the above-described natural zones of the Carpathian region, in 3 apiaries and in each of 3 hives in the second half of the spring period, the intensity of egg-laying of queen bees was studied every twelfth day for 36 days using a generally accepted method [5]. In particular, the area of closed brood was measured on all nest frames using a 25 cm^2 (5x5 cm) grid measuring frame. Taking into account that this frame covers 100 bee cells, their total number was calculated, which corresponds to the total egg production of the queen bee during 12 days.

The content of Zinc, Copper, Plumbum, and Cadmium was determined in the selected samples of the topsoil, bee comb and honey bee abdomen tissues, and in the samples of bee comb and honey bee abdomen tissues – unsaturated fatty acids of the omega-3, omega-6, and omega-7 and omega-9 families. At the same time, the significance of the level of Zinc, Copper, Plumbum and Cadmium and unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families of bee pollen for the reproductive capacity of bees in different natural zones of the Carpathian region was analyzed.

The content of Zinc, Copper, Plumbum, and Cadmium in selected samples of the arable layer of the soil, bee pollen, and abdominal tissues of honey bees was determined on an atomic absorption spectrophotometer (Selmi-115) according to V. V. Vlizlo et al. [2]. The concentration of unsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families in the studied bee pollen and abdominal tissues of honey bees was determined on a gas-liquid chromatographic apparatus (Chrom-5) according to Y. F. Rivis et al. [37].

The obtained digital material was processed by the method of variational statistics using the Student's criterion [4]. Arithmetic mean values (M) and arithmetic mean errors (±m) were calculated. Differences were considered probable at $p<0.05$. Computer program Origin 6.0 and Microsoft Excel was used for calculations.

Results and discussion. It was found that in the arable layer of the soil and bee hives of the foothills and forest-steppe zones of the Carpathian region, compared to the conditionally clean mountain zone, there is a higher content of Zinc, Copper, Plumbum and Cadmium (tables 1 and 2). It is also clear from these tables that the top layer of the soil and bee pollen of the forest-steppe zone of the Carpathian region contains the highest level of the studied heavy metals. At the same time, the content of Plumbum and Cadmium in the arable layer of the soil in the above zone is 1.1 times higher than the maximum permissible concentration [3]. The level of the dangerous element of the first class of toxicity, Cadmium, increases especially significantly in the arable layer of the soil and bee hives, compared to the conditionally clean mountain environment.

1. The content of Zinc, Copper, Plumbum and Cadmium in the topsoil in different natural zones of the Carpathian region, g·10^{-3}/kg of air-dry mass (M±m, n=3)

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Zinc, Zn	47,58±4,488	78,52±3,722**	96,13±4,890***
Copper, Cu	21,60±1,391	34,56±1,828**	45,64±2,264***
Plumbum, Pb	19,37±0,784	25,83±1,442*	33,30±2,870***
Cadmium, Cd	2,03±0,088	2,60±0,115*	3,20±0,271***

Note: here and further the differences are probable compared to the mountain zone: * P<0,05, ** P<0,01, *** P<0,001.

2. The content of Zinc, Copper, Plumbum and Cadmium in bee pollen in different natural zones of the Carpathian region, g·10^{-3}/kg of air-dry mass (M±m, n=3)

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Zinc, Zn	34,39±1,91	39,20±0,900*	42,72±0,872**
Copper, Cu	2,01±0,089	3,02±0,169*	4,20±0,170***
Plumbum, Pb	0,13±0,007	0,16±0,009*	0,21±0,012**
Cadmium, Cd	0,04±0,003	0,07±0,007*	0,10±0,009**

It is believed that the increase in the content of Plumbum in the arable layer of the soil is associated with the intensive movement of motor vehicles [6], and Cadmium – with the introduction of meliorants and mineral fertilizers, in particular phosphogypsum and superphosphate [7]. It is obvious that phosphoric acid residues present in phosphogypsum and superphosphate are able to retain large amounts of harmful Cadmium.

The obtained data characterize the level of man-made environmental pollution in the experimental territories. The high level of Zinc, Copper, Plumbum and Cadmium in the air and soil is the reason for the increase of their content in bee honey collected in the foothills and forest-steppe zones of the Carpathian region. All this is a consequence of greater urbanization and industrialization of the above territories.

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 has a fairly high content of probiotics Zinc and Copper. These heavy metals in acceptable amounts are absolutely necessary for the normal functioning of plant and animal tissues [32]. But the increased level of toxic Plumbum and Cadmium in the topsoil is apparently able to neutralize the positive effect of probiotic heavy metals on the mentioned tissues.

Data from the literature indicate that although Zinc, Copper, Plumbum and Cadmium are divalent mineral elements, they have very different coefficients of transition from the topsoil to the root system of plants, from the root system to the stem and leaves, from the stem and leaves to the inflorescence, from inflorescence to pollen [6]. It was established that in the Carpathian region Zinc has a very high coefficient of transition from the arable layer of the soil to the bee colony (Table 3). Copper has a much lower coefficient, Cadmium and especially Plumbum have even lower coefficients.

3. Coefficients of transition of Zinc, Copper, Plumbum and Cadmium from the arable layer of the soil to bee pollen in different natural zones of the Carpathian region

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Zinc, Zn	0,723	0,499	0,444
Copper, Cu	0,093	0,087	0,092
Plumbum, Pb	0,007	0,006	0,006
Cadmium, Cd	0,020	0,027	0,031

The increased coefficient of the transition of Zinc from the arable layer of the soil to bee pollen may be caused by the fact that this mineral element is extremely necessary for the normal functioning and high activity of male gametophytes [32]. It may also be extremely necessary for the normal functioning and high activity of female gametophytes [9].

The above is apparently related to the fact that Zinc is part of enzymes that, on the one hand, contribute to the protection of unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families from excessive peroxidation, and on the other – to the formation from α-linolenic, α-linoleic and α-oleic acids of even longer-chain and more unsaturated fatty acids of the omega-3, omega-6 and omega-9 families, respectively, and from even longer-chain and more unsaturated fatty acids of the omega-3 and omega-6 families formation a number of biologically active substances, primarily oxylipins and prostaglandins, which are involved in the reproductive capacity of plants and bees, respectively [25, 30, 32, 34].

Oxylipins in plant tissues are synthesized from such polyunsaturated fatty acids as linoleic and linoleic, which have 18 carbon atoms in their chain [31, 34, 35].

The lipoxygenase reaction and the formation of oxyacids are the primary link in the synthesis of oxylipins in plant tissues [35]. Synthesized oxylipins greatly intensify metabolic processes in tissues, growth and death of plants [31, 35].

Prostaglandins are synthesized in every tissue of the bee body only from polyunsaturated fatty acids, which have 20 or more carbon atoms in their chain [27, 29, 30]. During the synthesis of prostaglandins, longer-chain and unsaturated derivatives of linoleic and linolenic acids in the places where double bonds are found, with the help of enzymes such as cyclases, form a ring that has so-called tails, which on the one hand are hydrophilic due to the carboxyl group, and with the second is hydrophobic on the part of the methyl group [34]. Hydrophilic and hydrophobic tails of prostaglandin molecules have different lengths and, as a result, functional activity [27, 30]. Due to the hydrophilic tails of the prostaglandin molecules in the body tissues of bees, the exchange processes of proteins and amino acids are well and strongly regulated, and the hydrophobic tails are of lipids and fatty acids [27, 29, 30]. The most active prostaglandins in the body of bees belong to groups E and F [23, 29, 30]. In addition, very active prostaglandins are marked with symbols α or β. Thus, the very active prostaglandin $F_{2\alpha}$ is directly related to the reproductive capacity of drones and queen bees [23]. The mentioned type of prostaglandin is synthesized in the tissues of bees from eicosatetraenoic-arachidonic acid, which has 20 carbon atoms in its chain. In turn, eicosatetraenoic-arachidonic acid is synthesized in bee tissues from linoleic acid, which has only 18 carbon atoms in its chain [26]. As is known, linoleic acid is not synthesized in the tissues of bees and must enter their body with food [14, 17]. Prostaglandin $F_{2\alpha}$ in the genital tracts of drones and queen bees strongly regulates sperm growth and oocyte growth and fertilization, respectively [10, 23]. The specificity of the action of the mentioned prostaglandin is that it is quickly synthesized in the tissues of the genital organs of drones and bee queens and quickly loses its activity [10, 18].

It was recorded that in the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, the assimilation of Zinc by plants decreases. This fact is confirmed by the decrease in the coefficient of Zinc transition from the arable layer of the soil to the bee colony (Table 3). Cadmium can be seen to have such an inhibitory effect on the Zinc transport pathway. This is possibly due to the fact that the divalent mineral elements Zinc and Cadmium in plant tissues are competitors for a place in exposure reactions.

It was also established that the assimilation of Cadmium by plants increases in the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region. This result is indicated by the obtained data on the increase in the coefficient of transition of Cadmium from the arable layer of the soil to the bee colony (Table 3). Perhaps in this case, due to the high level of Cadmium present in plants, plant tissues can no longer protect themselves from excessive intake of the mentioned mineral element.

The high level of Zinc, Copper, Lead, and Cadmium in bee honey, in turn, is the reason for the increase in their content in the tissues of honey bees (Table 4). At the same time, in the tissues of the abdomen of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment, there is a higher level of dangerous elements of the first toxicity class - Plumbum (by 1.38–1.70 times) and Cadmium (by 1.78–2, 33 times). Data from the literature indicate that the level of heavy metals in some tissues of honey bees and queen bees corresponds to their concentration in other tissues [34].

4. The content of Zinc, Copper, Plumbum and Cadmium in the abdominal tissues of honey bees in different natural zones of the Carpathian region, g·10^{-3}/kg of raw weight (M±m, n=3)

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Zinc, Zn	77,08±1,190	91,32±1,536**	104,24±2,060***
Copper, Cu	0,34±0,012	0,47±0,014**	0,59±0,014***
Plumbum, 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**

The problem of heavy metals is as follows. At a physiologicall level, they are involved in the synthesis, oxidation, deposition and exchange of fatty acids in plant tissues and in the body tissues of bees. In particular, the content of unsaturated fatty acids linolenic, linoleic, palmitoolenic, oleic and eicosaenoic was determined in bee honey, and in the tissues of the abdomen of honey bees – unsaturated fatty acids of the omega-3 family (eicosapentaenoic, docosatrienoic, docosapentaenoic and docosahexaenoic), omega-6 family (eicosatrienoic, eicostetraenoic-arachidonic, docosadienoic and docosatetraenoic), omega-7 family (palmitoleic) and omega-9 family (oleic and eicosaenic). Unsaturated fatty acids in bee pollen and abdominal tissues of honey bees include phospholipids, non-esterified fatty acids, mono-, di- and triacylglycerols [14, 26, 31]. The listed unsaturated fatty acids in bee honey are also included in the composition of esterified phytosterol, and in the tissues of the abdomen of honey bees – esterified cholesterol [11, 26, 31].

It was recorded that the total content of unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families in bee honey obtained from beehives located in foothills (21.09 g/kg of air-dry mass) and especially forest-steppe (18.19) zones of the Carpathian region, compared to bee honey taken from hives located in the mountain zone (24.32 g/kg of air-dry mass), is smaller (Table 5).

5. The content of unsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families in bee honey in different natural zones of the Carpathian region, g/kg of air-dry mass (M±m, n=3)

Acid and its code	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Polyunsaturated fatty acids of the omega-3 family			
Linolenic, 18:3	16,20±0,358	14,23±0,535*	12,32±0,388**
Polyunsaturated fatty acids of the omega-6 family			
Linoleic, 18:2	3,54±0,136	2,98±0,078*	2,56±0,113**
Monounsaturated fatty acids of the omega-7 family			
Palmitoleic, 16:1	0,25±0,012	0,19±0,012*	0,16±0,009**
Monounsaturated fatty acids of the omega-9 family			
Oleic, 18:1	3,48±0,162	2,95±0,081*	2,53±0,084**
Eikosaenic, 20:1	0,85±0,038	0,74±0,020*	0,62±0,026**

Bees feel the smell and taste of various fatty acids and amino acids of plant pollen [39]. The smell of amino acids and especially fatty acids, unlike pollen sterols, strongly attracts bees to honey plants [26, 39, 40]. At the same time, among the fatty acids of plant pollen, which attract the special attention of honey bees, there are monounsaturated and polyunsaturated fatty acids containing 16 or more carbon atoms in their composition [40]. At the same time, the fatty acids of plant pollen are much more valuable for the body of bees in terms of energy than amino acids [14, 22, 39, 40]. The attractive function of fatty acids in plant pollen is due to their relatively high volatility and specific smell [34], and the energetic function is due to the high ratio of hydrogen and carbon atoms to oxygen atoms in their composition [34, 40].

In addition, monounsaturated and polyunsaturated fatty acids of plant pollen provide antibacterial and antifungal protection of the body of honey bees, bee combs and beehive [14, 19, 25]. Non-esterified forms of fatty acids are particularly active in this regard [15, 38].

Unsaturated fatty acids (linolenic, linoleic, palmitoleic, oleic, and eicosaenic) of bee pollen have an antimicrobial effect due to their high ability to increase the surface activity of the tissues of microorganisms and thus, under normal osmotic pressure of the surrounding environment, strongly inhibit their vital activity [16, 19].

It was established that the content of linolenic, linoleic, palmitoleic, oleic and eicosaenic acids, which are an attractive and energetic material for bees and provide antimicrobial protection of their body, bee combs and hive, in bee honey obtained from beehives located in the foothills (21.09 g/kg of air-dry mass) and forest-steppe (18.19) zones of the Carpathian region, compared to bee honey taken from hives located in the mountain zone (24.32 g/kg of air-dry mass), is smaller (table. 5).

The main mass of polyunsaturated fatty acids of the omega-3 and omega-6 families in the composition of phospholipids is included in the structure of cellular and cytoplasmic membranes of body tissues and ensures their functional activity and ultimately the vital activity of bees [34]. At the same time, linolenic acid and its longer-chain and unsaturated fatty acids in the body of bees are the initiators of anti-inflammatory processes, and linoleic acid and its longer-chain and unsaturated derivatives are pro-inflammatory [34, 46]. Linolenic and linoleic acids and their longer-chain and unsaturated derivatives act on the body of bees through the corresponding anti-inflammatory and pro-inflammatory cytokines of a peptide nature [21, 36] and such anti-inflammatory and pro-inflammatory eicosanoids as various types of prostaglandins [19].

The effects of pro-inflammatory cytokines, which are abundant in the body of bees, can be characteristic of each tissue separately, are inhibited by certain types of anticytokines [19]. At the same time, there are very few anti-inflammatory cytokines in the body of bees, since they exert their effect on all tissues and do not have inhibitory endogenous anticytokines.

It should be emphasized that pro-inflammatory eicosanoids (certain groups of prostaglandins) in the body of bees are synthesized from polyunsaturated fatty acids of the ω-6 family (eicosatrienoic, eicosatetraenoic-arachidonic, docosatetraenoic) [19]. Data from the literature indicate that the most active pro-inflammatory eicosanoid in the body of bees, especially queen bees, is prostaglandin $F_{2\alpha}$, which is synthesized in their tissues from eicosatetraenoic-arachidonic acid and, in turn, from linoleic acid [19, 24]. Pro-inflammatory prostaglandin $F_{2\alpha}$ promotes the growth and maturation of sperm and the growth and fertilization of oocytes in the reproductive tracts of drones and queen bees, respectively [24]. Thus through functionally active cell and cytoplasmic membranes and biologically active substances, in particular prostaglandins, unsaturated fatty acids, primarily polyunsaturated fatty acids of the omega-3 and omega-6 families, have the most complete and pronounced effect on the reproductive capacity of bees.

Table 5 shows that the largest amount of structural, biologically active and anti-inflammatory forms of linoleic and linolenic acids is contained in bee pollen of the mountain zone of the Carpathian region (19.74 g/kg of air-dry mass), a smaller amount is found in bee pollen in the foothill zone (17.21), even smaller in the forest-steppe zone (14.88 g/kg of air-dry mass).

A very low level of unsaturated fatty acids, in particular polyunsaturated fatty acids of the omega-3 and omega-6 families in plant pollen, which is the basis of bee pollen, 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 in it [14, 31]. In turn, a low level of unsaturated fatty acids in bee pollen can contribute to a decrease in the functional activity of cellular and cytoplasmic membranes of the honey bee body and thus inhibit their vital activity [26, 31].

It was established that the total content of unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families in the abdominal tissues of honey bees obtained from hives located in the foothills (17.29 g/kg of raw mass) and especially forest-steppe (16.36) zones of the Carpathian region, compared to the abdominal tissues of honey bees selected from hives located in the mountain zone (21.33 g/kg of raw mass), is smaller (Table 6). Data from the literature indicate that the relative level of unsaturated fatty acids in some tissues of honey bees and queen bees corresponds to their relative concentration in other tissues [34].

6. The content of unsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families in the abdominal tissues of honey bees in different natural zones of the Carpathian region, g/kg raw weight (M±m, n=3)

Acid and its code	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
1	2	3	4
Polyunsaturated fatty acids of the omega-3 family			
Linolenic, 18:3	4,62±0,155	3,71±0,058*	3,51±0,060*
Eicosapentaenic, 20:5	2,48±0,140	1,94±0,026*	1,88±0,020*
Docosatrienic, 22:3	0,34±0,006	0,26±0,005*	0,23±0,010*
Docosapentaenic, 22:5	0,53±0,015	0,43±0,006**	0,40±0,007**
Docosahexaenic, 22:6	0,54±0,009	0,47±0,015*	0,45±0,007**
Polyunsaturated fatty acids of the omega-6 family			
Linoleic, 18:2	3,19±0,140	2,55±0,091*	2,45±0,042*
Eicosadienic, 20:2	0,24±0,015	0,19±0,003*	0,16±0,003**
Eicosatrienic, 20:3	0,33±0,015	0,26±0,008*	0,23±0,007*
Eicosatetraenic-arachidonic, 20:4	3,63±0,150	2,99±0,040*	2,87±0,026*
Dokosadienic, 22:2	0,32±0,010	0,23±0,010*	0,20±0,008*

1	2	3	4
Dokozatetraenic, 22:4	0,32±0,009	0,25±0,006**	0,23±0,007**
Monounsaturated fatty acids of the omega-7 family			
Palmitoleic, 16:1	0,09±0,03	0,06±0,003*	0,05±0,006*
Monounsaturated fatty acids of the omega-9 family			
Oleic, 18:1	4,46±0,155	3,76±0,033*	3,54±0,064*
Eikosaenic, 20:1	0,24±0,005	0,19±0,010*	0,16±0,005**

It can be seen from Table 6 that the largest amount of monounsaturated and polyunsaturated fatty acids, which can serve as energy and antimicrobial material, is contained in the abdominal tissues of honey bees of the mountain zone of the Carpathian region (21.33 g/kg of raw weight), a smaller amount is in the abdominal tissues bees in the foothill zone (17.29), even less in the forest-steppe zone (16.36 g/kg of raw weight).

It was recorded (Table 6) that the highest level of polyunsaturated fatty acids of the omega-3 and omega-6 families, which are part of the structure of cell and cytoplasmic membranes and are precursors of prostaglandins and initiators of anti-inflammatory and pro-inflammatory processes, respectively, is contained in the abdominal tissues of honey bees of the mountain zone of the Carpathian region (respectively 8.51 and 8.03 g/kg of raw mass), their level is lower in the tissues of the abdomen of honey bees in the foothills (6.81 and 6.47), and even lower in the forest-steppe zone (6.47 and 6.14 g/kg of raw weight).

It should be noted that in the abdominal tissues of honey bees of the mountain zone of the Carpathian region, compared to the foothills and forest-steppe zone, there is a 1.2–1.3 times higher content of the direct precursor of the highly active prostaglandin $F_{2\alpha}$ – eicosatetraenic-arachidonic acid (Table 6). Prostaglandin $F_{2\alpha}$ synthesized from this acid can maintain the reproductive capacity of drones and especially queen bees at a very high level [21].

Thus, in the forest-steppe natural zone of the Carpathian region, among other zones, due to a decrease in the content, the value of monounsaturated and polyunsaturated fatty acids of bee pollen for the organism of bees, bee combs and beehive decreases most intensively. At the same time, the value of the mentioned fatty acids in the tissues of the abdomen of bees decreases.

The decrease in the content of monounsaturated and polyunsaturated fatty acids in bee pollen and in the tissues of honey bees of the foothills and especially the forest-steppe zones of the Carpathian region, compared to the bee pollen and tissues of honey bees in the mountain zone, is clearly related to their greater transition to the anionic form and the formation of salts. This is due primarily to the binding of fatty acids by heavy metal cations [13, 26].

High levels of heavy metals, including toxic ones, but low levels of unsaturated fatty acids, in particular polyunsaturated fatty acids of the omega-3 and omega-6 families, in bee pollen and abdominal tissues of honey bees obtained from hives located in foothill and especially forest-steppe zones of the Carpathian region, is a consequence of the urbanization and industrialization of the territory.

From the above follows that in the direction from the mountainous zone of the Carpathian region to the foothills and further to the forest-steppe zone, due to the high level of heavy metals, including toxic ones, the attractive, energetic, structural, biologically active, antimicrobial and anti-inflammatory value of the unsaturated fatty acids of bee pollen decreases for the organism of bees, bee combs and beehive. In the direction mentioned above, the energetic, structural, biologically active, antimicrobial and anti-inflammatory value of unsaturated fatty acids in the tissues of the abdomen of honey bees also decreases for the specified reason. Thus, a high level of Zinc, Copper, Plumbum, and especially Cadmium, but a low level of unsaturated fatty acids, in particular polyunsaturated, in bee honey and in the tissues of the abdomen of bees affects the reproductive capacity of queen bees kept in hives located in the mentioned areas of the Carpathian region.

Table 7 shows that the egg-laying intensity of queen bees kept in hives located in the foothills and especially in the forest-steppe zone of the Carpathian region, compared to queen bees in the mountain zone, in the second half of the spring period is lower by 1.12 and 1.17 times respectively.

A decrease in the egg-laying intensity of queen bees kept in hives located in the foothills and especially in the forest-steppe zone of the Carpathian region, compared to queen bees in the mountain zone, as in the second half of the spring period, due to a decrease in the number of worker bees, can in the future have an extremely negative effect on productive and reproductive features of the bee family.

The decrease in the egg-laying intensity of bee queens is clearly an integrated indicator of the influence of the ecological state of the environment, since the latter also equally affects the reproductive capacity of drones, in particular, their quantitative and qualitative indicators of sperm production. Other scientists also point to the same negative impact of territories polluted with heavy metals on the reproductive characteristics of bees [17].

7. Egg-laying intensity of queen bees in the second half of the spring period in different natural zones of the Carpathian region, pieces of eggs (M±m, n=3)

Natural zones of the Carpathian region		
Mountain	foothill	forest-steppe
Accounting period, April 19		
826,0±24,62	739,3±19,60*	702,7±11,02**
Accounting period, May 1		
1117,7±20,94	930,2±13,56*	879,3±15,24**
Accounting period, May 13		
1391,0±11,52	1307,7±10,10*	1274,0±6,81**
Together for the accounting period, April 19-May 13		
3334,7	2977,2	2856,0

All over the world, the search for means of bioindication of the ecological state of the environment is underway [1, 17]. This is due to the fact that heavy metals, like other environmental pollutants, have a different level of transition in the soil–plant–bee tissue–bee product chain.

It was previously indicated [8] 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 you to determine different levels of accumulation of heavy metals and fatty acids and thus gives more information. The intensity of egg-laying by queen bees can also serve as a bioindicator of the ecological state of the environment in terms of the content of heavy metals and unsaturated fatty acids.

Conclusions

In the direction from the mountain to the foothills and forest-steppe zone of the Carpathian region, the level of Zinc, Copper, Plumbum, and especially Cadmium increases in the topsoil, bee pollen, and abdominal tissues of honey bees.

In the mentioned direction, the coefficient of transition of Zinc from the arable layer of the soil to the bee colony decreases. At the same time, the assimilation of Cadmium by plants increases.

In the direction from the mountain to the foothills and forest-steppe zone of the Carpathian region, the level of unsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families in bee pollen and abdominal tissues of honey bees decreases.

High levels of heavy metals, but low levels of unsaturated fatty acids in bee pollen and abdominal tissues of honey bees obtained from hives located in the foothills and especially the forest-steppe zones of the

Carpathian region are a consequence of the urbanization and industrialization of the territories.

Zinc, Copper, Plumbum and Cadmium affect the value of unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families of bee pollen and affects the reproductive capacity of honey bees in the Carpathian region. In particular, in the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, due to the high concentration of Zinc, Cuprum, Plumbum, and especially Cadmium, both in the second half of the spring period and in the second half of the summer period, the value of unsaturated fatty acids of the omega-3, omega-6, omega-7 and omega-9 families in bee pollen decreases for intensive egg-laying of bee queens.

In the second half of the spring period, the egg-laying intensity of queen bees kept in hives located in the foothills and especially in the forest-steppe zones of the Carpathian region is 1.12 and 1.17 times lower, respectively, compared to queen bees in the mountain zone.

Bee pollen and abdominal tissues of honey bees can serve as a bioindicator of the ecological state of the environment by the content of heavy metals and unsaturated fatty acids. An integrated indicator such as the intensity of egg-laying by queen bees can also serve as a good bioindicator of the ecological state of the environment.

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