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HEAVY METALS TRANSITION COEFFICIENTS AND ANIONIC FATTY ACID CONTENT IN BEE POLLEN (PLANT POLLEN) IN DIFFERENT NATURAL AREAS OF THE CARPATHIAN REGION

The aim of the work was to determine the coefficients of transition of heavy metals from the arable soil layer to bee pollen (plant pollen) and the content of heavy metals and weakly active anionic fatty acids in the mentioned plant material in different natural areas of the Carpathian region. Experimental apiaries of clinically healthy honey bees of the Carpathian breed were selected on the basis of private beekeeping farms in the mountain (urban village Slavsko, Stryj district), foothills (village Nyzhnia Stynava, Stryj district) and forest-steppe (village Myklashiv, Lviv district) zones of Lviv region. The content of Ferrum, Zinc, Copper, Chromium, Cobalt, Nickel, Plumbum and Cadmium in the arable layer of soil and bee pollen (plant pollen) was determined to assess the intensity of man-made load on the environment where the experimental apiaries of honey bees are located. The content of heavy metals in the selected samples of the arable soil layer, bee pollen (plant pollen) was determined on an atomic-absorption spectrophotometer, and anionic fatty acids in bee pollen (plant pollen) – on a gas-liquid chromatograph. It has been established that in the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region the content of Ferrum, Zinc, Copper, Cobalt, Chromium, Nickel, Plumbum and Cadmium in the arable soil layer and bee pollen (plant pollen) increases. At the same time, in the above direction, the coefficients of transition of Zinc, Chromium and Nickel from the arable layer of soil to bee pollen are decreasing; Zinc and Nickel – in dandelion pollen; Zinc – in apple pollen. In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, due to the high level of heavy metals, the value of anionic fatty acids of bee pollen (plant pollen) decreases for bees, beehives and hives. In the above direction due to the increase in the content of heavy metals and anionic forms of fatty acids in bee pollen (plant pollen), decreases the flight-harvest relative to the bee pollen (plant pollen) and honey productivity of worker bees. The high level of heavy metals and anionic fatty acids in bee pollen (plant pollen) obtained from hives located in the foothills and especially the forest-steppe zones of the Carpathian region is a consequence of urbanization and industrialization of the territory. Bee pollen, dandelion and apple pollen in general can be bioindicators of the ecological state of the environment. However, due to the optimal content of heavy metals and fatty acids, the best bioindicator of the ecological state of the environment is dandelion pollen.

Keywords: natural zones of the Carpathian region, bee pollen (plant pollen), heavy metals, anionic fatty acids, heavy metals transition coefficients, bioindicators, productivity of worker bees.

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Коефіцієнти переходу важких металів і вміст аніонних жирних кислот у бджолиному обніжжі (пилку рослин) у різних природних зонах Карпатського регіону

Метою роботи було визначити коефіцієнти переходу важких металів із орного шару ґрунту в бджолине обніжжя (пилок рослин) та вміст важких металів і малоактивних аніонних жирних кислот у згадуваному матеріалі з рослин у різних природних зонах Карпатського регіону. Піддослідні пасіки клінічно здорових медоносних бджіл породи карпатська було підібрано на базі приватних пасічних господарств гірської (смт Славсько Стрийського району), передгірної (с. Нижня Стинава Стрийського району) та лісостепової (с. Миклашів Львівського району) зон Львівської області. Для оцінки інтенсивності техногенного навантаження на довкілля, де знаходяться піддослідні пасіки медоносних бджіл, визначали вміст Феруму, Цинку, Купруму, Хрому, Кобальту, Ніколу, Плюмбуму та Кадмію в орному шарі ґрунту та бджолиному обніжжі (пилку рослин). Вміст важких металів у відібраних зразках орного шару ґрунту, бджолиного обніжжя (пилку рослин) визначали на атомно-абсорбційному спектрофотометрі, а аніонних жирних кислот у бджолиному обніжжі (пилку рослин) – на газо-рідинному хроматографі. Встановлено, що в напрямі від гірської до передгірної та далі до лісостепової зони Карпатського регіону зростає вміст Феруму, Цинку,

Купруму, Кобальту, Хрому,Ніколу, Плюмбуму та Кадмію в орному шарі ґрунту та бджолиному обніжжі (пилку рослин). Одночасно в наведеному вище напрямі знижуються коефіцієнти переходу Цинку, Хрому та Ніколу із орного шару ґрунту в бджолине обніжжя; Цинку та Ніколу – у пилок із кульбаби лікарської; Цинку – в пилок із яблуні. У напрямі від гірської до передгірної і далі до лісостепової зони Карпатського регіону через високий рівень важких металів знижується цінність аніонних жирних кислот бджолиного обніжжя (пилку рослин) для організму бджіл, бджолиних стільників і вуликів. У наведеному вище напрямі через зростання вмісту важких металів і аніонних форм жирних кислот у бджолиному обніжжі (пилку) знижується льотно-збиральна щодо обніжжя (пилку) і медова продуктивність робочих бджіл. Високий рівень важких металів і аніонних жирних кислот у бджолиному обніжжі (пилку рослин), отриманому з вуликів, розміщених у передгірній та особливо лісостеповій зонах Карпатського регіону, є наслідком урбанізації та індустріалізації території. Бджолине обніжжя та пилок із кульбаби лікарської і яблуні в загальному можуть бути біоіндикаторами екологічного стану довкілля. Однак через оптимальний вміст важких металів і жирних кислот найкращим біоіндикатором екологічного стану довкілля є пилок із кульбаби лікарської.
Ключові слова: природні зони Карпатського регіону, бджолине обніжжя (пилок рослин), важкі метали, аніонні жирні кислоти, коефіцієнти переходу важких металів, біоіндикатори, продуктивність робочих бджіл.

Introduction. The sources of heavy metals emissions and the ways of their entry into the environment are diverse, but in general they are of man-made origin as a result of urbanization and industrialization. Urbanization and industrialization, in particular the activities of industry, agriculture, energetics and transport, as well as intensive extraction of minerals – all this led to the entry of heavy metals into the air, water, soil and plants, including highly toxic ones (Plumbum, Cadmium and Arsenic) [16, 17].

The migration of heavy metals in objects of the external environment caused their accumulation in soils and plants [14, 16, 17]. As a result, some types of plants gave way to others and the terms of their flowering, and thereby the conditions of honey collection by bees, changed [1, 7].

Zinc, Copper, Cobalt, Chromium and Nickel in acceptable amounts are absolutely necessary for the normal vital activity of plant tissues [19]. But the increased level of toxic Plumbum and Cadmium in the topsoil is certainly able to neutralize the positive effect of probiotic heavy metals on the mentioned tissues. 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 [17, 18, 25], and Cadmium – with the introduction of meliorants and mineral fertilizers, in particular phosphogypsum and superphosphate [5, 12].

The above-mentioned heavy metals are involved in metabolic processes in the tissues and pollen of plants, in the body tissues of bees. In particular, at a high level, Ferrum is able to stimulate peroxide processes in the tissues and pollen of plants and in the tissues of the body of bees, destroying, at the same time, its most valuable components – amino acids, polyunsaturated fatty acids and fat-soluble vitamins [22]. On the other hand, such heavy metals as Zinc and Copper, which in the tissues and pollen of plants and in the tissues of the body of bees, at a physiologically determined level, can act in the opposite way, because both of them are components of such an enzyme as superoxide dismutase, which strongly inhibits the development of peroxide processes [34]. And this despite the fact that Cuprum in the lymph of bees performs the same function as Ferrum in the blood of humans and animals – redox [31]. At a physiologically determined level, such heavy metals as Nickel and Chromium also have an antiperoxide effect [5]. The intensity of protein synthesis in plant and bee tissues depends on Cobalt through the enzyme cyanocobalamin [29]. It is believed that, regardless of the concentration, such heavy metals as Plumbum and Cadmium are toxicants for the body of bees [7].

Through the alimentary canal and wax glands heavy metals enter bee combs. In the latter, heavy metals behave almost the same as in the body of bees [19]. In addition, all heavy metals in large quantities are capable of reducing the strength of the walls of bee combs [33]. The presence of a large amount of toxic Plumbum and Cadmium in the composition of bee combs is undesirable for the body of larvae and adult bees [26]. A large amount of toxic Plumbum and Cadmium in the hive is also a bad sign [32].

It should be noted that at a high level, all studied heavy metals of plant pollen in the body of bees become toxicants [19]. At the same time, heavy metals in bee lymph bind more intensively to sulfhydryl groups of heat-resistant proteins and are transported into chitin [31]. In the latter, heavy metals are deposited.

The problem of heavy metals is as follows. At a physiologically determined level, they are involved in the synthesis, oxidation, deposition and exchange of fatty acids in the tissues and pollen of plants and in the body tissues of bees. In particular, Ferrum is able to stimulate the peroxide processes of polyunsaturated fatty acids in the tissues and pollen of plants and in the body of bees [7]. Zinc and Copper in the tissues and pollen of plants and in the tissues of bees are able to effectively inhibit the course of peroxide processes of the above-mentioned fatty acids [3]. To some extent, the anti-peroxide effect is also characteristic of Nickel and Chromium [5]. Cobalt, through such a vitamin as cobalamin, initiates the synthesis of proteins and related fatty acids in plant and bee tissues [20].

Due to the fact that it is a part of 9-desaturase, in the tissues and pollen of plants and in the tissues of bees, cuprum contributes to the formation of monounsaturated fatty acids of the omega-7 (palmitoleic) and omega-9 (oleic) families, respectively, from saturated palmitic and stearic fatty acids [19].

Linoleic and linolenic fatty acids, which are sequentially synthesized in the tissues and pollen of plants from oleic acid, are considered indispensable for bees and therefore must enter their body with food [20]. Already in the tissues of bees from linoleic and linolenic acids, due to the fact that Zinc is included in 2-, 3-, 4-, 5- and 6-desaturases, even longer-chain and more unsaturated fatty acids of the omega-6 and omega-3 families, respectively, are synthesized [19, 20].

Longer-chain and more unsaturated fatty acids of the omega-3 and omega-6 families are very valuable for the body of bees, since in their tissues they are mainly used for the construction of cell and cytoplasmic membranes and the synthesis of biologically active derivatives – prostaglandins, thromboxanes and leukotrienes [20] .

In plant organisms, the main biologically active substances involved in reproductive capacity are oxylipins [20], and in bees – prostaglandins [28]. It should be noted that oxylipins and prostaglandins are synthesized, respectively, in plant and bee tissues from polyunsaturated fatty acids – linoleic and linolenic [20, 28].

The low total content of polyunsaturated and monounsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families, respectively, linolenic, linoleic, palmitoleic, and oleic acids in bee pollen and pollen from dandelion and apple tree may through the body of bees, in particular wax glands, contribute to the increase in the fragility of the walls of bee combs [4, 10]. At the same time, a very low content of the above-mentioned fatty acids in plant 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 [4]. It can also help reduce the functional activity of cellular and cytoplasmic membranes of the body of honey bees and thus inhibit their vital activity [4, 23]. The above is reflected in the productivity of bee families and quality indicators of their products [15]. Therefore, the issue of production of ecologically safe beekeeping products is relevant [24]. Moreover, the production of honey bees occupies a prominent place in human life. Very high requirements are placed on the quality indicators of honey bee products, because currently Ukraine has become the main exporter of honey to Europe.

In the literature, there are only fragmentary data on the content of heavy metals and fatty acids in bee honey in various natural zones of the Carpathian region [24].

In view of the above, the aim of the work was to determine the coefficients of transition of heavy metals, including toxic ones, from the arable layer of the soil into bee pollen and pollen from the dandelion and apple tree and the content of heavy metals and low-active anionic forms of fatty acids in the mentioned material from plants in various 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 mountain (Slavsko village, Stryi district), foothills (Nyzhnia Stynava village, Stryi district) and forest-steppe (Myklashiv village) zone apiaries of Lviv region, where natural and climatic conditions and ecological situation are different.

In order to assess the intensity of the man-made load on the environment where experimental honey bee apiaries are located, the content of heavy metals (Ferrum, Zinc, Copper, Chromium, Cobalt, Nickel, Plumbum and Cadmium) in the topsoil, bee pollen and pollen of dandelion (*Taraxacum officinale* Wigg.) and apple tree (*Malus domestica* (Borkh) Borkh) was determined.

To clarify the species affiliation of the selected pollen, identification studies were carried out with the help of computer programs "LUCIA" (Laboratory Color Image Analysis) and "Pollen Data Bank". These programs make it possible to determine the main parameters of a pollen grain, captured by a video camera from a microscope, by overlaying images and comparing them with reference samples.

In the spring-summer 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 were collected for laboratory research using generally accepted methods [15]. At the same time, samples of the arable layer of the soil were taken in the radius of the useful flight of honey bees.

The content of heavy metals, including toxic ones, was determined in selected samples of the arable layer of the soil, bee pollen and pollen from dandelion and apple trees, and anionic forms of fatty acids in samples of bee pollen and pollen from dandelion and apple trees. At the same time, the value of the level of heavy metals, including toxic and anionic forms of fatty acids in bee pollen and pollen from dandelion and apple trees for the body of honey bees, bee combs and beehives in different natural zones of the Carpathian region was analyzed.

The content of heavy metals (Ferrum, Zinc, Copper, Cobalt, Chromium, Nickel, Plumbum, and Cadmium) in selected samples of the arable layer of the soil, bee pollen, and pollen from the dandelion and apple tree was determined on an atomic absorption spectrophotometer (Selmi-115) according to V. V. Vlizlo et al. [9]. The concentration of anionic fatty acids in the investigated bee pollen and plant pollen was determined on a gas-liquid chromatograph (Chrom-5) according to Y. F. Rivis et al. [6].

The obtained digital material was processed by the method of variational statistics using the Student's criterion [10]. Arithmetic mean values (M) and arithmetic mean errors (±m) were calculated. Differences were considered probable at $p<0.05$. The computer program Origin 6.0, Microsoft Excel was used for calculations.

Results and discussion. It was established that in the arable layer of the soil, bee pollen and pollen from the dandelion and apple tree of the foothills and forest-steppe zones of the Carpathian region, compared to the conditionally clean mountain zone, there is a higher content of Ferrum, Zinc, Cuprum, Cobalt, Chromium, Nickel, Plumbum and Cadmium (Tables 1, 2, 3 and 4).

1. The content of heavy metals, including toxic ones, in the topsoil in different natural zones of the Carpathian region, g·10^{-3}/ kg air-dry mass (M±m, n=3)

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Ferrum, Fe	14325,00±294,214	15184,29±454,862*	16573,04±294,429**
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***
Cobalt, Co	11,76±0,375	13,63±0,560**	17,20±1,830***
Chromium, Cr	41,69±2,283	63,65±3,584**	87,53±4,163***
Nickel, Ni	21,24±1,625	41,33±2,512***	59,42±3,214***
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.

The above tables also show that the top layer of the soil, bee pollen and pollen from the dandelion and apple tree of the forest-steppe zone of the Carpathian region contains the highest level of investigated heavy metals. At the same time, the content of Plumbum and Cadmium in the arable layer of the soil in the above zone is slightly higher than the maximum permissible concentration.

The level of the dangerous element of the first class of toxicity Cadmium increases especially significantly in bee pollen and pollen from dandelion and apple tree – by 2.5-4.0 times, compared to a conditionally clean mountain environment. The concentration of Chromium, an element of the second class of toxicity, also noticeably increases – by 1.6–2.7 times.

It should also be noted that dandelion pollen is a much more active accumulator of heavy metals, including toxic ones, compared to apple pollen. Practically all heavy metals accumulate in an average twice as much amount in dandelion pollen than in apple pollen.

2. The content of heavy metals, including toxic ones, 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
Ferrum, Fe	33,52±0,830	37,11±0,781*	43,39±2,253**
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***
Cobalt, Co	1,01±0,029	1,14±0,050*	1,44±0,112***
Chromium, Cr	4,10±0,177	5,02±0,180*	6,68±0,149***
Nickel, Ni	0,58±0,015	0,65±0,015*	0,74±0,023**
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**

3. The content of heavy metals, including toxic ones, in dandelion 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
Ferrum, Fe	30,48±0,876	41,78±0,922***	51,77±1,230***
Zinc, Zn	42,02±1,020	54,35±0,640***	63,81±0,856***
Copper, Cu	3,17±0,111	5,17±0,091***	6,89±0,214***
Cobalt, Co	10,25±0,494	12,37±0,452*	13,83±0,235**
Chromium, Cr	3,21±0,121	5,53±0,184***	6,76±0,300***
Nickel, Ni	0,43±0,020	0,67±0,026**	0,95±0,040***
Plumbum, Pb	0,98±0,035	1,93±0,042***	2,69±0,123***
Cadmium, Cd	0,04±0,006	0,11±0,006**	0,15±0,009***

The obtained data characterize the level of man-made environmental pollution in the experimental territories. The high level of Ferrum, Zinc, Copper, Cobalt, Chromium, Nickel, Plumbum and Cadmium in the air and soil is the reason for their increased concentration in bee pollen and dandelion and apple pollen obtained 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.

4. The content of heavy metals, including toxic ones, in pollen from apple trees 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
Ferrum, Fe	14,16±0,440	19,24±0,338***	24,68±0,288***
Zinc, Zn	16,72±0,323	23,54±0,336***	29,00±0,480***
Copper, Cu	1,19±0,046	2,06±0,073***	2,94±0,049***
Cobalt, Co	1,06±0,056	1,30±0,043*	1,48±0,037**
Chromium, Cr	1,10±0,057	2,34±0,192**	2,97±0,051***
Nickel, Ni	0,11±0,010	0,22±0,011*	0,33±0,015***
Plumbum, Pb	0,42±0,011	0,67±0,029**	1,08±0,030***
Cadmium, Cd	0,02±0,003	0,04±0,003*	0,08±0,005**

It was established that in the Carpathian region, Zinc has a very high coefficient of transition from the arable layer of the soil to bee pollen and pollen from the dandelion and the apple tree (Tables 5, 6 and 7). Copper and Chromium have much lower coefficients, other metals are even lower. At the same time, Ferrum has a very low coefficient of transition from the arable layer of the soil to bee pollen and pollen from the dandelion and the apple tree.

It should be noted that in the Carpathian region Cobalt also has a very high coefficient of transition from the arable layer of the soil to dandelion pollen (Table 6). This is possibly due to the high level of protein synthesis in dandelion tissues. As is known, Cobalt is part of such an enzyme as cyanocobalamin, which stimulates the processes of protein synthesis in plant and animal tissues [29].

The increased coefficient of Zinc transition from the arable layer of the soil to bee pollen and pollen from the dandelion and apple tree is obviously caused by the fact that this mineral element is extremely necessary for the normal functioning and high activity of male gametophytes [11]. It may also be absolutely necessary for the normal functioning and high activity of female gametophytes. The above is apparently related to the fact that Zinc is part of enzymes that contribute to the formation of a number of biologically active substances from polyunsaturated fatty acids, which are involved in the reproductive capacity of plant organisms [11].

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 some heavy metals by plants decreases. This fact is confirmed by the decrease in the coefficients of the transition of Zinc, Chromium and Nickel from the arable layer of the soil to bee pollen; Zinc and Nickel - in dandelion pollen; Zinc - in apple pollen (Tables 5, 6 and 7). At the same time, it has been recorded that the assimilation of some heavy metals by plants is increasing in the above direction. This fact is confirmed by the increase in the coefficients of the transition of Cadmium from the arable layer of the soil to bee pollen; Plumbum and Cadmium – in dandelion pollen; Chromium and especially Copper, Plumbum and Cadmium in apple pollen (Tables 5, 6 and 7).

5. Coefficients of transition of heavy metals, including toxic ones, from the arable layer of the soil to the bee pollen in different natural zones of the Carpathian region

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Ferrum, Fe	0,002	0,002	0,003
Zinc, Zn	0,723	0,499	0,444
Copper, Cu	0,093	0,087	0,092
Cobalt, Co	0,086	0,084	0,084
Chromium, Cr	0,098	0,079	0,076
Nickel, Ni	0,027	0,016	0,012
Plumbum, Pb	0,007	0,006	0,006
Cadmium, Cd	0,020	0,027	0,031

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 probiotic Zinc, Copper, Cobalt, Chromium and Nickel. The above-mentioned heavy metals in acceptable quantities are absolutely necessary for the normal vital activity of plant tissues [19]. 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.

6. Transfer coefficients of heavy metals, including toxic ones, from the topsoil to dandelion pollen in different natural zones of the Carpathian region

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Ferrum, Fe	0,002	0,003	0,003
Zinc, Zn	0,883	0,692	0,664
Copper, Cu	0,147	0,150	0,151
Cobalt, Co	0,872	0,908	0,804
Chromium, Cr	0,076	0,087	0,077
Nickel, Ni	0,020	0,016	0,016
Plumbum, Pb	0,051	0,075	0,081
Cadmium, Cd	0,020	0,042	0,047

7. Transfer coefficients of heavy metals, including toxic ones, from the topsoil to apple pollen in different natural zones of the Carpathian region

Metal and its symbol	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Ferrum, Fe	0,001	0,001	0,001
Zinc, Zn	0,351	0,300	0,302
Copper, Cu	0,055	0,060	0,064
Cobalt, Co	0,090	0,095	0,086
Chromium, Cr	0,026	0,037	0,034
Nickel, Ni	0,005	0,005	0,006
Plumbum, Pb	0,022	0,026	0,032
Cadmium, Cd	0,010	0,015	0,025

We studied the content of anionic forms of saturated fatty acids with an even (caprylic, capric, lauric, myristic, palmitic, and stearic) and odd (pentadecanoic) number of carbon atoms in the chain, monounsaturated fatty acids of the omega-7 families in bee honey and pollen from dandelion and apple tree (palmitoleic) and omega-9 (oleic) and polyunsaturated fatty acids of the omega-3 (linolenic) and omega-6 (linoleic) families.

It was established (Tables 8, 9 and 10) that the higher total concentration of anionic forms of fatty acids in bee pollen and pollen from dandelion and apple tree, obtained from beehives located in the foothills (2071.4, 2332.8 and 1130.4 g $\cdot 10^{-3}$/kg of air-dry mass) and forest-steppe (2285.7, 2380.4 and 1154.6) zones of the Carpathian region, compared to bee pollen and pollen from dandelion and apple tree, selected from beehives located in the mountain zone (1798.0, 2153.2 and 1080.8 g·10^{-3}/kg of air-dry mass), caused by a higher level of saturated fatty acids with steam (543.4, 458.6 and 208.6 and 601.0, 472.5 and 218.2 vs. 486.6, 418.7 and 186.9 and odd (96.8, 0.4 and 0.7 and 119.2, 0.6 and 0.8 against 54.2, 0.2 and 0.4) by the number of carbon atoms in the chain, monounsaturated fatty acids of the omega-7 families (73.8, 8.5 and 5.7 and 79.9, 9.0 and 6.7 against 66.1, 7.4 and 4.3) and omega-9 (177.2, 167.4 and 89.4 and 198.4, 167.4 and 89.4 against 137.6, 153.4 and 83.1) and polyunsaturated fatty acids of the omega-3 family (797.6, 1174.1 and 585.5 and 867.2, 1155.1 and 591.2 vs. 742.2, 1066.7 and 574.3) and omega-6 (respectively 382.6, 523.8 and 240.5 and 420.0, 528.9 and 245.8 against 329.3, 506.8 and 231.8 g·10^{-3}/kg of air-dry mass).

8. The content of anionic forms of fatty acids in bee pollen obtained from beehives located in different natural zones of the Carpathian region, g·10^{-3}/kg of air-dry mass (M±m, n=3)

Fatty acids and their code	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Capric, 10:0	25,4±1,51	30,9±1,31*	36,2±1,24**
Lauric, 12:0	87,3±2,20	100,1±2,35*	109,7±2,28**
Myristic, 14:0	68,4±3,49	80,2±2,24*	90,6±2,84**
Pentadecanoic, 15:0	54,2±6,26	96,8±3,31**	119,2±3,42**
Palmitic, 16:0	168,2±8,92	195,9±4,08*	221,2±3,89**
Palmitoleic, 16:1	66,1±1,99	73,8±1,42*	79,9±1,36**
Stearic, 18:0	119,3±7,02	135,3±7,81**	143,3±7,14**
Oleic, 18:1	137,6±11,03	177,2±3,41**	198,4±6,36**
Lino leic, 18:2	329,3±17,93	382,6±7,64*	420,0±11,07**
Linolenic, 18:3	742,2±21,19	797,6±8,03*	867,2±15,28**

The highest total content of anionic forms of fatty acids was found in bee pollen, dandelion pollen and apple pollen obtained from beehives located in the forest-steppe zone.

It should be noted that dandelion pollen contains a much larger amount of hard-to-reach anionic forms of saturated, monounsaturated and polyunsaturated fatty acids compared to apple pollen. Practically, anionic forms of long-chain fatty acids are accumulated in an average of 2.1 times more in dandelion pollen than in apple pollen.

9. The content of anionic forms of fatty acids in dandelion pollen obtained from beehives located in different natural zones of the Carpathian region, g·10^{-3}/kg of air-dry mass (M±m, n=3)

Fatty acids and their code	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Capric, 10:0	21,2±0,65	27,4±0,78**	29,0±0,61**
Lauric, 12:0	84,8±2,19	96,8±1,28**	100,9±1,23**
Myristic, 14:0	6,3±0,14	8,0±0,20**	8,8±0,34**
Pentadecanoic, 15:0	0,2±0,03	0,4±0,03*	0,6±0,03**
Palmitic, 16:0	213,8±1,86	224,6±1,27**	229,2±1,07**
Palmitoleic, 16:1	7,4±0,14	8,5±0,14*	9,0±0,12**
Stearic, 18:0	92,6±1,06	101,8±1,41**	104,6±1,17**
Oleic, 18:1	153,4±2,30	167,4±1,44**	174,3±1,27**
Lino leic, 18:2	506,8±9,35	523,8±4,62*	528,9±1,68**
Linolenic, 18:3	1066,7±14,89	1174,1±4,94*	1195,1±4,09*

10. The content of anionic forms of fatty acids in apple pollen obtained from beehives located in different natural zones of the Carpathian region, g·10^{-3}/kg of air-dry mass (M±m, n=3)

Fatty acids and their code	Natural zones of the Carpathian region		
	mountain	foothill	forest-steppe
Caprylic, 8:0	6,3±0,13	7,3±0,27*	7,5±0,21*
Capric, 10:0	14,2±0,23	16,1±0,17**	16,6±0,29**
Lauric, 12:0	48,1±0,28	54,5±0,37**	57,0±0,52***
Myristic, 14:0	4,3±0,17	5,2±0,09**	6,3±0,34**
Pentadecanoic, 15:0	0,4±0,03	0,7±0,03**	0,8±0,03**
Palmitic, 16:0	96,8±1,15	105,3±0,93**	108,0±0,78**
Palmitoleic, 16:1	4,3±0,17	5,7±0,23**	6,7±0,15***
Stearic, 18:0	17,2±0,43	20,2±0,56*	22,8±1,05**
Oleic, 18:1	83,1±1,05	89,4±0,90*	91,9±0,64**
Lino leic, 18:2	231,8±1,30	240,5±0,92**	245,8±0,78***
Linolenic, 18:3	574,3±1,79	585,5±1,04**	591,2±1,47**

In terms of energy, lipids are much more valuable than proteins and carbohydrates [20]. Literature sources indicate that the greater the amount of fatty acids contained in plant pollen, the greater its energy value for the body of honey bees [4, 20]. We established (Tables 8, 9 and 10) that the largest amount of anionic forms of fatty acids, which perform energy functions, is contained in bee pollen and in pollen from dandelion and apple in the forest-steppe zone of the Carpathian region (respectively (2285.7, 2380 ,4 and 1154.6 g·10^{-3}/kg of air-dry mass), a smaller amount of them is in bee pollen and in pollen from dandelion and apple in the foothills (2071.4, 2332.8 and 1130.4) , even smaller in the mountain (respectively 1798.0, 2153.2 and 1080.8 g·10^{-3}/kg of air-dry mass).

The above indicates that in the direction from the forest-steppe zone of the Carpathian region to the foothills and further to the mountains, the energy value of the fatty acids of bee pollen and pollen from the dandelion and apple tree for the body of bees decreases.

Tables 8, 9 and 10 show that the largest amount of structural and biologically active anionic forms of linoleic and linolenic acids [18, 19, 27] is contained in bee pollen and in pollen from dandelion and apple in the forest-steppe zone of the Carpathian region (respectively, 1287.2, 1724.0 and 837.0 g·10^{-3}/kg of air-dry mass), a smaller amount of them is in bee pollen and in pollen from dandelion and apple in the foothills (1182.2, 1697.9 and 826.0), it is even smaller in the mountain (respectively 1071.5, 1573.5 and 806.1 g·10^{-3}/kg of air-dry mass). This indicates that in the direction from the forest-steppe zone of the Carpathian region to the foothills and further to the mountains, the biological value of polyunsaturated fatty acids of bee pollen and pollen from dandelion and apple for the organism of bees decreases.

The total content of functionally active anionic polyunsaturated and monounsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families, respectively linolenic, linoleic, palmitooleic, and oleic acids [4, 11] in bee pollen, dandelion and apple pollen, obtained from beehives located in the foothills and forest-steppe zones of the Carpathian region, compared to bee honey and pollen from dandelion and apple tree, selected from beehives located in the mountain zone, is greater (respectively 1357.4, 1865.3 and 915.4 and 1485.1, 1898.3 and 928.9 against 1209.7, 1726.9 and 889.2 g·10^{-3}/kg of air-dry mass). Their content increases the most in bee pollen and in pollen from dandelion and apple, obtained from beehives located in the forest-steppe zone of the Carpathian region (Tables 8, 9 and 10).

Among the organic substances of plant pollen, which attract special attention of honey bees, there are fatty acids. In particular, saturated fatty acids of plant pollen, which have 14 or fewer carbon atoms in their chain, and saturated, monounsaturated and polyunsaturated fatty acids, which contain 18 or more carbon atoms in their composition, perform an attractive function [21]. The attractive function of saturated, monounsaturated and polyunsaturated fatty acids of plant pollen is due to their relatively high volatility and specific smell. We established (Tables 8, 9 and 10) that the total content of anionic forms of fatty acids that have 14 or fewer carbon atoms in their chain, and fatty acids that contain 18 or more carbon atoms in their composition and that perform an attractive function, in bee pollen and in dandelion and apple pollen obtained from beehives located in the foothills (fatty acids with 14 and fewer carbon atoms in the chain in bee pollen, dandelion and apple pollen, respectively there is 211.2, 132.2 and 83.1, and fatty acids containing 18 or more carbon atoms in bee pollen, dandelion and apple pollen are 1492.7, 1967.1 and 935.6 g·10^{-3}/kg of air-dry mass respectively) and forest-steppe (236.5, 138.7 and 87.4 and 1628.9, 2002.9 and 951.7) zones of the Carpathian region, compared to bee pollen, dandelion and apple tree pollen, selected from beehives located in the mountain zone (fatty acids that have 14 or less in their chain and volumes of carbon in bee pollen, dandelion and apple pollen are 181.1, 112.3, and 72.9, respectively, and fatty acids containing 18 or more carbon atoms in bee pollen and pollen from dandelion and apple tree, respectively, are 1328.4, 1819.5 and 906.4 g·10^{-3}/kg of air-dry mass), is larger. Thus, in the forest-steppe natural zone of the Carpathian region, among other zones, the attractive value of saturated, monounsaturated and polyunsaturated fatty acids of bee pollen and pollen from dandelion and apple trees for bees is most intensively reduced.

Saturated, monounsaturated and polyunsaturated fatty acids caprylic, capric, lauric, myristic, oleic, linoleic and linolenic provide antibacterial and antifungal protection of the body of honey bees, bee combs and beehive. In particular, the high antibacterial activity of the above acids against bee rot was found [23].

Caprylic and, to a lesser extent, capric and, to a yet lesser extent, lauric and, to a very small extent, myristic acids have an antimicrobial effect due to their high ability to reduce the concentration of hydrogen ions. Linolenic and, to a lesser extent, linoleic and, to a yet lesser extent, oleic acids – to increase the surface activity of the tissues of microorganisms and thereby strongly inhibit their vital activity under normal osmotic pressure of the surrounding environment [23].

We established (Tables 8, 9 and 10) that the content of anionic forms of caprylic, capric, lauric, myristic, oleic, linoleic and linolenic acids, which provide antibacterial and antifungal protection of the body of bees, bee combs and beehives, in bee pollen and in pollen from dandelion and apple tree obtained from beehives located in foothills (respectively 1568.6, 1997.5 and 938.5 g·10^{-3}/kg of air-dry mass) and forest-steppe (1721.6, 2037.0 and 1016.3) zones of the Carpathian region, compared to bee pollen and pollen from dandelion and apple tree, selected from beehives located in the mountain zone (respectively 1390.2, 1839.2 and 962.1 g·10^{-3}/kg of air-dry mass), is larger. The content of anionic fatty acids, which have an antimicrobial effect, increases the most in bee pollen and in pollen from dandelion and apple tree, obtained from beehives located in the forest-steppe zone of the Carpathian region. The above indicates that in the direction from the forest-steppe zone of the Carpathian region to the foothills and further to the mountains, the value of saturated, monounsaturated and polyunsaturated fatty acids of bee pollen and pollen from dandelion and apple tree, which provide antibacterial and antifungal protection of the body of bees and bee combs, decreases.

An increase in the concentration of less active anionic forms of fatty acids in bee pollen, dandelion and apple pollen obtained from beehives located in the foothills and forest-steppe zones may indicate a decrease in the content of highly active non-esterified forms of fatty acids. Data from the literature indicate that anionic forms of fatty acids are the least available for the body of bees, bee combs and beehives in metabolic and functional terms [4, 14, 20]. This is due to the fact that anionic forms of fatty acids are mainly connected with metals.

Thus, as a result of an increase in the man-made load on the environment and the accumulation of heavy metals in the components of the ecosystem, in particular in honey plants, the energetic, structural, biological, attractive and antimicrobial value of the anionic forms of fatty acids of bee pollen and pollen from dandelion and apple trees for the body of bees decreases, structural and antimicrobial – for bee hives and antimicrobial – for beehives in the foothills and especially forest-steppe zones of the Carpathian region, compared to the mountain. Data from the literature [4, 20] also indicate a similar effect of the environment polluted by heavy metals.

It was established that the flying and collecting productivity of worker bees in relation to pollen in the mountain zone of the Carpathian region is 3.8±0.15 kg, in the foothills – 3.5±0.17, $p<0.05$, and in the forest-steppe – 2.9±0.15 kg, $p<0.01$ per bee colony per season. At the same time, the honey productivity of worker bees in the mountain zone of the Carpathian region is observed at the level of 40.0±0.95 kg, in the foothills – 36.6±0.92, $p<0.05$, and in the forest-steppe – 31.4± 1.84 kg, $p<0.01$ per bee colony per season. It can be seen that due to the high level of heavy metals, including toxic and less active anionic forms of fatty acids in bee pollen and in pollen from dandelion and apple, the flight-gathering relative to pollen and honey productivity of worker bees decreases. Other scientists also point to the same negative impact of territories polluted with heavy metals on the productive characteristics of honey bees [11].

All over the world, the search for means of bioindication of the ecological state of the environment is underway [4]. This is due to the fact that heavy metals, like other environmental pollutants, have a different level of transition 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 the pollen [27].

We believe that in our conditions, the best bioindicator of the ecological state of the environment, due to the optimal content of heavy metals and essential fatty acids, which are essential for the body of honey bees, is dandelion pollen (*Taraxacum officinale* Wigg.). Dandelion pollen has been used by us for bioindication of the ecological state of the environment for a long time [14]. 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. Now, other scientists have started to use this pollen for bioindication of the ecological state of the environment [8]. But for bioindication of the ecological state of the environment, other scientists use the indicators of the change in the shape of the pollen grain from disk-shaped to lenticular and the characteristics of the germination of dandelion seeds (*Taraxacum officinale* Wigg.). These indicators do not establish the level of influence of the studied environmental factors, but only determine the transition of morphological and functional indicators of plant pollen from one state to another. That is, this method of bioindication of the ecological state of the environment is of a lower level.

Conclusions

In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, the ecological condition of the environment deteriorates. This is confirmed by the level of heavy metals in the topsoil, bee pollen, and pollen from dandelion and apple trees.

Zinc, Cobalt and Copper are absolutely necessary for the normal functioning of plant tissues. This is generally confirmed by a relatively high coefficient of the transition of the mentioned mineral elements from the arable layer of the soil to bee pollen and pollen from the dandelion and apple tree in various natural zones of the Carpathian region. At the same time, the transfer coefficients of Nickel, Plumbum and Cadmium, and especially Ferrum, into bee pollen (plant pollen) are very low.

In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, the coefficients of the transition of Zinc, Chromium and Nickel from the arable layer of the soil to the bee pollen decrease; Zinc and Nickel – in dandelion pollen; Zinc – in apple pollen.

The content of heavy metals and anionic forms of fatty acids in bee pollen affects the life 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 heavy metals, the value of the fatty acids of bee pollen (pollen) for the organism of bees, bee combs and beehives decreases.

Each type of plant has its own ability to deposit heavy metals and anionic forms of fatty acids in tissues. Practically all heavy metals and fatty acids accumulate in dandelion pollen several times more than in apple pollen.

In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, due to the increase in the content of heavy metals and anionic forms of fatty acids in bee pollen, the flight-gathering relative to pollen and honey productivity of worker bees decreases.

The high level of heavy metals and anionic forms of fatty acids in bee pollen, dandelion and apple pollen obtained from beehives located in the foothills and especially forest-steppe zones of the Carpathian region is a consequence of the urbanization and industrialization of the territory.

Bee pollen, dandelion and apple pollen in general can be bioindicators of the ecological state of the environment. However, due to the optimal content of heavy metals and fatty acids, dandelion pollen is the best bioindicator of the ecological state of the environment. Bee pollen due to polyflority, and apple pollen due to its low concentration of heavy metals and fatty acids are less suitable for bioindication.

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