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THE IMPACT OF HEAVY METAL MIGRATION ON THE QUALITY OF HONEY IN THE TROPHIC CHAIN

421.03 –TECHNOLOGY OF ANIMAL BREEDING AND LIVESTOCK PRODUCT PRODUCTION

Abstract of the Doctoral Dissertation in Agricultural Sciences

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CONCEPTUAL FRAMEWORK OF THE RESEARCH

Relevance of the Topic. Honey is a natural product produced by bees, and its composition depends on the nectar sources they utilize, as well as various environmental factors. However, the presence of beehives in areas with heavy metal contamination can impact the quality of beekeeping products [13].

Currently, the properties of heavy metals are actively being studied. Different researchers come to conflicting conclusions regarding this issue. Through the food chain, bees accumulate and distribute various mineral elements, including toxic ones, while bee colonies themselves act as indicator organisms [24].

The results of many studies have shown that heavy metals accumulate in honey as a result of atmospheric pollution [1]. Heavy metals from the atmosphere can settle on the bodies of bees or enter them along with nectar, pollen, honeydew, or water during foraging [2]. Therefore, the presence of undesirable heavy metal contaminants in honey indicates that the environment where the beehives are located is polluted.

Studies on the concentration of heavy metals reveal that their accumulation in the bee's body depends on the specific location. After being transferred to the hive, these metals can be found in various beekeeping products such as honey, wax, and propolis [11, 12].

Nectar-producing plants primarily absorb heavy metal ions through their root system. Heavy metals can also enter plants through the air, and they may accumulate and be retained in their leaves. Atmospheric pollution with heavy metals is one of the most pressing environmental issues. This problem has become especially significant recently, as it is closely linked to the issue of producing ecologically clean food products [28].

The study of physicochemical properties of honey from different soil and climatic zones, as well as the migration of micro- and macro-elements and heavy metals in the trophic chain (*soil – nectar plants – honey – pollen – propolis – bee bodies*), holds significant theoretical and practical value for the intensive development of beekeeping, taking into account ecological conditions and the requirements for honey quality and export to the European Union. At the same time, considerable attention is given to the search for new biostimulants, including those of natural origin to increase honey production volumes and improve the winter hardiness of bee colonies, which shapes the relevance of the problem and makes it of great scientific and practical importance.

The aim of the study is: to scientifically justify and assess the quality of honey from different soil and climatic zones, investigate the migration of heavy metals in the food chain, and increase honey production through the use of biostimulants in bee feeding.

The objectives of the research are: to determine the physicochemical properties of bee honey from different soil and climatic zones; to identify the content of micro- and macroelements and heavy metals in honey from various soil and climatic zones; to analyze the amino-acid composition and antibacterial activity of bee honey; to study the migration and content of micro- and macroelements and heavy metals in the trophic chain (soil – nectar plants – honey – pollen – propolis – bee bodies); to evaluate the effectiveness of using natural biostimulants in bee feeding and develop practical recommendations.

The hypothesis of the study. The following hypotheses put forward for the intensive development of beekeeping, taking into account the ecological conditions of the environment:

- to identify ecological zones, honey quality, and the migration of heavy metals in the trophic chain (*soil – nectar plants – honey – pollen – propolis – bee bodies*);

- to increase the production of high-quality honey and improve the winter hardiness of bee colonies through the use of biostimulants in bee feeding.

The synthesis of the research methodology and justification of the chosen research methods. The methodology of the research is based on the principles and methods described and applied in the field of beekeeping [25, 18, 4], which were used to assess the morpho-productive indicators of bee colonies, physicochemical, biochemical, biological, antibacterial, and antifungal properties [15, 14], as well as the quality of bee honey and the migration of micro- and macroelements and heavy metals in the food chain. The obtained results were processed using the method of variance statistics according to MERKUREVA, E. [26] and analyzed with the help of Microsoft Office computer programs. Thus, original results were obtained, and the research objectives and tasks were fully accomplished.

DISSERTATION CONTENTS

The **introduction** outlines the relevance of the topic and describes the current state of the field, presents the aim and objectives of the research, the research hypothesis, the synthesis of the methodology, and the justification of the chosen research methods, as well as a brief overview of the dissertation sections.

1. PHYSICOCHEMICAL PROPERTIES OF HONEY, CONTENT OF MICRO- AND MACROELEMENTS, AMINO ACIDS, MIGRATION OF HEAVY METALS IN THE TROPHIC CHAIN, AND BEE FEEDING

The first chapter summarizes scientific materials presented in the specialized literature on the dissertation topic of beekeeping. It provides information and an analysis of scientific studies by domestic and foreign authors, as well as insights into the physicochemical properties of honey, the migration of heavy metals in the trophic chain, and the use of biostimulants in bee feeding.

2. MATERIAL, METHODS, AND RESEARCH CONDITIONS

2.1. Material and Conditions for Conducting Research

The research focused on the following samples of bee honey collected from various soil and climatic zones to achieve the study objectives: acacia honey from the Southern zone (Comrat), the Central zone (Nisporeni, Călărași, Chișinău); linden honey from the Central zone (Nisporeni, Căpriana, Călărași); and sunflower honey from the Southern zone (Comrat), the Central zone (Nisporeni), and the Northern zone (Bălți, Fălești).

The research also included the collection of samples from soil where sunflowers were grown, from a forest area where white acacia and linden trees grew; flowers of white acacia, linden, and sunflower; pollen; propolis; and worker bees, from which the honey stomach and intestines (digestive tract) were removed. The selected samples were analyzed for physicochemical properties of honey, as well as for the content of micro- and macro-elements, heavy metals, and amino acids. Additionally, the study investigated the content and migration of micro- and macroelements and heavy metals in the trophic chain (*soil – nectar plants – honey – pollen – propolis – bee bodies*).

A series of field experiments were conducted to increase honey production and evaluate the effectiveness of biostimulants in bee feeding:

Experiment I. Four groups of bee colonies were formed in the experiment conducted in 2020 and 2021 at the apiary in the village of Kojushna, Straseni District, with three colonies in each group, including three experimental groups and one control group. In order to replenish the winter food stores (on 13.09.2020), the bee colonies in Group I were given 3.0 liters of a 60% sugar syrup mixture with 1.5 ml/l of biostimulant (*ApiStev*), Group II received 3.0 ml/l, Group III received 4.0 ml/l, and Group IV (control) received pure sugar syrup. In the spring period, the bee colonies were fed with 1 liter of a 50% sugar syrup mixture with biostimulant, when no supporting honey harvest was available: Group I received 1.5 ml/l, Group II received 3.0 ml/l, Group III received 4.0 ml/l, and Group IV (control) received pure sugar syrup, every 7 days starting from April until the main honey harvest. The biostimulant *ApiStev* is a 3% aqueous solution of stevioside glycoside. Stevioside is a natural compound derived from the Stevia plant (*Stevia Rebaudiana*).

Experiment II. In 2022 the research was conducted at the apiary in the village of Brătuleni, Nisporeni District, where 4 groups of bee colonies were formed including three colonies in each group. In the spring period, the bee colonies were fed with one liter of a 1:1 sugar syrup mixture with the biostimulant *CobalStev*, when no supporting honey harvest was available. Group I received 1.0 ml/l, Group II received 2.0 ml/l, Group III received 3.0 ml/l, and Group IV (control) received pure sugar syrup. The biostimulant *CobalStev* contains cobalt(III) hexamine chloride and stevioside.

Experiment III. During 2020 and 2021 the research was conducted at the apiary in the village of Zorile, Orhei District. Four groups of bee colonies were formed for the study and treated to replenish their winter food reserves (on 12.09.2020), with 2 liters of a 60% mixture of inverted corn syrup. Group I received 1.0 ml/l of the biostimulant (*ApiRibo*), Group II received 2.0 ml/l, Group III received 3.0 ml/l, and Group IV (control) received pure inverted corn syrup. In the spring period, when no supporting honey harvest was available, the bee colonies were fed with 1 liter of a 50% mixture of inverted corn syrup with the bioregulator (*ApiRibo*): Group I received 1.0 ml/l, Group II received 2.0 ml/l, Group III received 3.0 ml/l, and Group IV (control) received pure inverted corn syrup, every 7-9 days, starting from April until the main white acacia honey harvest.

The inverted corn syrup solution was prepared by diluting inverted corn syrup with water in a ratio of 1.5:1 (in the fall) and 1:1 (in the spring).

Experiment IV. In 2021 the research was conducted at the apiary in the village of Petichen, Călărași District, four groups of bee colonies were formed with three colonies in each group. The colonies were grouped according to the analogy method based on the number of frames, colony strength, sealed brood, and honey quantity in the hive. During the spring period, when there was no supporting honey flow, the bee colonies were fed 1 liter of a 50% sugar syrup mixture with the biostimulant *ApiDAK*: Group I received 1.0 ml/l, Group II received 2.0 ml/l, Group III received 3.0 ml/l, and Group IV (control) received pure sugar syrup.

Experiment V. The research was conducted at the apiary in the village of Ulmu, Ialoveni District. Four groups of bee colonies were formed, with three colonies in each group. The colonies of the first group were fed with a sugar syrup mixture containing the biostimulant choline chloride at a concentration of 1.25 ml/l, the second group received 2.25 ml/l, the third group received 3.25 ml/l, and the fourth group (control) received pure sugar syrup. During the spring period, the bee colonies were fed 1 liter of a 1:1 sugar syrup mixture with choline chloride biostimulant every 7 days, when there was no supporting honey flow.

Experiment VI. Four groups of bee colonies were formed at the apiary in the village of Petichen, Călărași District, with three colonies in each group, according to the principle of the method of analogs, taking into account the number of combs, colony strength, amount of capped brood, and honey reserves in the hive. During the spring period, when there was no supporting honey flow, the bee colonies were fed with 1 liter of a 50% sugar syrup mixture with a biostimulant (3% solution of glucuronic acid). The first group received 1.30 ml/l, the second group received 2.50 ml/l, the third group received 3.70 ml/l, and the fourth group (control) received pure sugar syrup. The studied biostimulant is a 3% solution, consisting of glucuronic acid (6 grams of glucuronic acid dissolved in 194 grams of water).

2.2. Methods for Analyzing the Chemical Properties of Honey, Soil, Flowers, Pollen, Propolis, Bee Bodies, and Morphoproductive Indicators of Bee Colonies

The physicochemical parameters were analyzed at the Food Laboratory of the Moldovan Republican Veterinary Diagnostic Center. The water content, invert sugar, sucrose, diastase activity, hydroxymethylfurfurol (HMF) content, and total acidity in honey samples were determined according to the SUST19792-2001 standard.

The levels of micro- and macroelements, as well as the presence of toxic elements, in acacia, linden, and sunflower honey, in the flowers of these melliferous plants, in pollen loads, propolis, and bee bodies, were determined using atomic absorption spectrometry after dry ashing, in accordance with SM SR EN 14082:2006, at the Institute of Chemistry of the Moldovan State University. The accumulation or migration coefficient (K) was calculated as the ratio of the element concentration at a subsequent trophic level to its concentration at the preceding level.

The amino acid content analysis in honey, flowers, pollen loads, propolis, and bee bodies was carried out in the accredited Laboratory of Psychosomatic Relationships at the Institute of Physiology and Sanocreatology of the Moldovan State University. One used the method of ion-exchange liquid chromatography on an amino acid analyzer "AAA T 339M" with a lithium citrate buffer solution. Samples were hydrolyzed with 6 M HCl according to the methodology described in [19].

Antibacterial tests were conducted on reference strains of Gram-positive and Gram-negative bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 700603. The antifungal activity of honey was assessed using the disk diffusion method on agar against the test microorganism *Candida albicans* ATCC 102331.

Microbial Population and Preparation of Honey Concentrates. The microbial population of honey samples was determined using the spread plate method. Honey samples were diluted in sterile distilled water at ratios of 1:2, 1:4, 1:8, and 1:16 to prepare honey concentrations of 33%, 20%, 14%, and 5.9%, respectively. Then the plates were incubated for 24 hours at 37°C.

Determination of Minimum Inhibitory Concentration (MIC). Honey suspensions at concentrations of 33%, 20%, 14%, and 5.9% were introduced into media to assess their effectiveness against microorganisms. Each plate, prepared to a final volume of 5 mL, including honey and the medium, was inoculated and incubated at 37°C for 48 hours. The MIC was determined by identifying the plates with the lowest honey concentration that showed no microbial growth. All MIC values were expressed in % (v/v).

The number of frames, colony strength, sealed brood quantity, and honey productivity were assessed In the experimental groups. Sealed brood was measured using a grid frame (5x5 cm^2 = 100 cells), while honey productivity was evaluated using electronic scales. The study of the morphological and productive traits of the bee colonies was conducted following methodological guidelines and recommendations from leading experts in beekeeping [25, 18, 4]. The results were processed using variation statistical methods according to МЕРКУРЬЕВА, Е.К. [26], and computer software.

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3. IMPACT OF HEAVY METAL MIGRATION IN THE TROPHIC CHAIN ON HONEY QUALITY

3.1. Chemical Composition of Honey from Different Soil and Climatic Zones

Chemical Composition of Acacia Honey. The issue of environmental contamination of honey has become increasingly relevant in recent years.Research findings indicate that acacia honey from the central zone contains an average water content of 16.1%, ranging from 15.6% to 16.5%. The average inverted sugar content is 77.9% (77.3–79.0%), while sucrose levels are at 1.6% (1.0–2.3%). The diastase number is 9.1 Gothe units (5.8–14.1 Gothe units), hydroxymethylfurfurol (HMF) content is 6.2 mg/kg (2.21–12.48 mg/kg), and acidity is 1.1 milliequivalents per 100 g (0.75–1.58 milliequivalents per 100 g). The diastase number indicates bee honey heating or overheating. In the Republic of Moldova, the natural honey diastase number is considered to range from 6 to 13 Gothe units [20].

Southern zone acacia honey had, on average, 1.6% higher water content, 0.2% higher sucrose content, 1.1 mg/kg more hydroxymethylfurfurol, and 1.1 milliequivalents per 100 g more acidity than honey from the Central zone. However, it had 1.5% less invert sugar content and a diastase number were 1.1 units lower. Our average results from 2020-2022 are consistent with previous findings [22].

The chemical composition of sunflower honey revealed that in the Central zone, the highest average water content was 18.0%, which was 0.8% lower in the Northern zone and 1.4% lower in the Southern zone. The mass percentage of invert sugar was higher in the Northern zone at 78.0%, with sucrose content at 2.1%. The average diastase number ranged from 15.7 to 16.59 Gothe units, with minimum-maximum values between 11.19 and 24.29 units, and acidity ranged from 2.1 to 2.73 milliequivalents per 100 g. The highest amount of hydroxymethylfurfurol was found in the Southern zone at 4.25 mg/kg, which was 0.31 mg/kg more than in the Central zone and 0.95 mg/kg more than in the Northern zone.

The chemical composition of linden honey was determined, with the water content from the Central zone varying between 15.2% and 19.9%, which meets the permissible requirements. The invert sugar content ranged from 77.5% to 81%, while the sucrose content ranged from 1.5% to 2.5%. The diastase number was between 5.8 and 15.3 Gothe units, with the hydroxymethylfurfurol content ranging from 1.35 to 4.9 mg/kg, and the acidity ranged from 1.65 to 1.83 milliequivalents per 100g.

Analyzing the data from Table 3.1, it can be noted that the moisture content of the honey varied from 16.93% (acacia honey) to 18.05% (linden honey).

The invert sugar content ranged from 77.18% to 78.50%, the sucrose content from 1.71% to 2.07%, and the diastase number ranged from 8.56 Gothe units (acacia honey) to 16.25 Gothe units (linden honey). Additionally, the acidity ranged from 1.13 to 2.26 milliequivalents per 100g, while hydroxymethylfurfurol content ranged from 3.00 mg/kg (linden honey) to 6.77 mg/kg. These values comply with the established standards for honey and are consistent with the data of other researchers [10].

Table 3.1. Average values of chemical indicators for different types of honey (2020-2022)

Analyzed indicators	Permissible levels according to the standard	Acacia honey	Linden honey	Sunflower honey
Mass fraction of water, %, max.	20,0	16,93±0,551	18,05±1,01	17,05±0,403
Inverted sugar content, %, min.	60,0	77,18±0,634	78,5±0,842	77,65±0,665
Sucrose content, %, max.	7,0	1,71±0,240	2,07±0,217	2,06±0,382
Diastase number, Goethe units, min.	6,5	8,56±1,198	11,75±2,282	16,25±1,81
Hydroxymethylfurfural, mg/kg, max.	20,0	6,77±2,143	3,00±0,732	3,88±0,424
Acidity, milliequivalents per 100 g, not more than that	4,0	1,13±0,132	1,75±0,038	2,26±0,189

3.2. Content of micro-, macroelements, and heavy metals in honey from different soil-climatic zones

Content of microelements in different types of honey. The research results conducted from 2020 to 2023 showed that the amount of manganese in acacia honey from the Central zone's rural areas was on average 0.443 mg/kg, while in urban areas it was 10.657 mg/kg higher. The zinc content was 0.520 mg/kg and 2.14 mg/kg higher, copper – 1.363 mg/kg and 0.137 mg/kg higher, iron – 1.730 mg/kg and 1.230 mg/kg higher. The amount of chromium (<1.5 mg/kg) and nickel (<2.5 mg/kg) was at the same level regardless of the collection area.

There was no significant difference in the manganese content in sunflower honey across the zones, as it was the same (0.56 mg/kg) in both the Southern and Northern zones, while in the Central zone it was 0.09 mg/kg lower. The highest amount of zinc was found in sunflower honey from the Central zone (1.15 mg/kg), copper – 1.21 mg/kg (Southern zone), and iron – 4.09 mg/kg (Northern zone). The content of chromium (<1.5 mg/kg) and nickel (<2.5 mg/kg) in sunflower honey was the same in all three zones, and the locality did not affect the levels of these microelements. The amount of manganese in linden honey from rural areas was 0.22 mg/kg higher than from urban areas, zinc – 0.652 mg/kg higher, copper – 0.01 mg/kg higher, iron – 0.714 mg/kg higher, while chromium and nickel levels were the same.

It was found that the highest amount of the studied microelements, on average over the four years (2020-2023), was in acacia honey – 16.457 mg/kg, while in other types it ranged from 9.117 mg/kg (sunflower honey) to 9.980 mg/kg (linden honey) (Table 3.2).

Table 3.2. Average values of chemical indicators for different types of honey (2020-2023), mg/kg

Microelements	Acacia honey	Sunflower honey	Linden honey
Manganese (Mn)	3,661 ± 2,040	0,536 ± 0,032	0,513 ± 0,045
Zinc (Zn)	1,896 ± 0,979	0,870 ± 0,132	1,073 ± 0,295
Copper (Cu)	1,413 ± 0,120	1,153 ± 0,139	1,348 ± 0,095
Iron (Fe)	5,487 ± 2,590	2,559 ± 0,620	3,045 ± 0,606
Chromium (Cr)	<1,5	<1,5	<1,5
Nickel (Ni)	<2,5	<2,5	<2,5
Total amount	**16,457±4,074**	**9,117±0,728**	**9,980±0,614**

The amount of manganese in honey ranged from 0.513 mg/kg (linden honey) to 3.661 mg/kg (acacia honey), zinc from 0.870 mg/kg (sunflower honey) to 1.896 mg/kg (acacia honey), copper from 1.153 mg/kg to 1.413 mg/kg, and iron from 2.559 mg/kg to 5.487 mg/kg.

It was found that of all the honey types, the highest concentration of microelements was found in acacia honey, with a total of 16.421 mg/kg. This included manganese – 3.661 mg/kg, zinc – 1.860 mg/kg, copper – 1.413 mg/kg, iron – 5.487 mg/kg, chromium – <1.5 mg/kg, and nickel – <2.5 mg/kg, while the lowest concentration was found in sunflower honey, with 9.118 mg/kg [21].

The content of macroelements in different types of honey. The results of the studies showed that among all the examined types of honey, the highest calcium content was found in sunflower honey – 82.42 mg/kg. In acacia honey, the calcium content was 50.81 mg/kg lower than in sunflower honey, with the difference being statistically significant (*P1 ≥ 0.95). The magnesium content ranged from 10.962 mg/kg (acacia honey) to 39.883 mg/kg (sunflower honey). The highest potassium content was found in linden honey – 1168.967 mg/kg, which is 902.275 mg/kg higher than in acacia honey (*P2 ≥ 0.99). The sodium content ranged from 17.20 mg/kg (linden honey) to 26.10 mg/kg (sunflower honey), and the phosphate content ranged from 148.85 mg/kg (linden honey) to 228.68 mg/kg (sunflower honey) (Table 3.3).

Table 3.3. Content of macroelements in different types of honey (2020-2023), mg/kg

Macroelements	Acacia honey	Sunflower honey	Linden honey
Calcium (Ca^{2+})	31,618±13,190	82,42±9,908*	77,99±18,211
Magnesium (Mg^{2+})	10,962±1,817	39,883±14,457	22,183±3,763
Potassium (K^{+})	266,217±41,086	553,050±175,345	1168,967±207,411*
Sodium (Na^{+})	24,767±5,848	26,10±4,751	17,20±1,813
Phosphates (P_2O_5)	150,25±29,928	228,68±7,115*	148,85±37,563
Total amount	**483,81±50,250**	**930,14±187,372**	**1435,19±182,466****

Ca: sunflower honey / acacia honey– *$P_1 \geq 0{,}95$; K: linden honey / acacia honey– *$P_2 \geq 0{,}99$; P_2O_5: sunflower honey / acacia honey – *$P_1 \geq 0{,}95$; Total amount of macroelements: linden honey / acacia honey – **$P_2 \geq 0{,}99$.

The total content of the studied macroelements in linden honey was significantly higher by 951.38 mg/kg compared to acacia honey (**P2 ≥ 0.99). It was found that the total sum of all macroelements in different honey types ranged, on average, from 483.81 mg/kg (acacia) to 1435.19 mg/kg (linden).

It was found that the calcium content in different types of honey ranged from 31.618 to 82.42 mg/kg, magnesium from 10.962 to 39.883 mg/kg, potassium from 266.217 to 1168.967 mg/kg, sodium from 17.20 to 26.10 mg/kg, and phosphates from 148.85 to 228.68 mg/kg [21].

The content of heavy metals in different types of honey. The results of the studies showed that the lead content in acacia honey ranged from 0.344 mg/kg to <0.5 mg/kg, cadmium from 0.0065 to <0.06 mg/kg, zinc from 0.520 to 3.017 mg/kg, copper from 1.363 to <1.5 mg/kg, and ash content from 0.014% to 0.031%. In sunflower honey, the lead content ranged from 0.344 to <0.5 mg/kg, cadmium from 0.041 to 0.06 mg/kg, zinc from 0.76 to 0.997 mg/kg, and copper from 0.980 to <1.5 mg/kg. In linden honey, the lead content ranged from 0.033 to 0.415 mg/kg, cadmium from 0.0065 to 0.049 mg/kg, zinc from 0.53 to 1.182 mg/kg, copper from 1.34 to 1.35 mg/kg, and ash content from 0.322 to 0.87 mg/kg.

The total amount of heavy metals in different types of honey ranged on average from 2.507 mg/kg (sunflower honey) to 3.837 mg/kg (acacia honey) [21] (Table 3.4). The lead content in honey ranged from 0.351 mg/kg to 0.433 mg/kg, cadmium from 0.042 mg/kg to 0.052 mg/kg, zinc from 0.870 mg/kg to 1.896 mg/kg, copper from 1.153 mg/kg to 1.413 mg/kg, and ash content from 0.139% to 0.413%.

Table 3.4. The total amount of heavy metals in different types of honey (2020-2023), mg/kg

Heavy metals	Acacia honey	Sunflower honey	Linden honey
Lead (Pb)	0,433±0,067	0,433±0,067	0,351±0,094
Cadmium (Cd)	0,052±0,008	0,052±0,008	0,042±0,011
Zink (Zn)	1,896±0,979	0,870±0,132	1,073±0,295
Copper (Cu)	1,413±0,120	1,153±0,139	1,348±0,095
Total amount	**3,837±1,017**	**2,507±0,280**	**2,733**±0,332
Ash content, %	0,139±0,080	0,166±0,037	0,413±0,146

It can be noted that the highest amount of heavy metals was found in acacia honey from the Southern zone of the rural area – 5.010 mg/kg, in the Central zone of the urban area – 4.72 mg/kg, and in the rural area of the same zone – 2.27 mg/kg. In sunflower honey, the total amount of heavy metals ranged from 2.362 mg/kg (Central zone) to 2.82 mg/kg (Northern zone).

3.3. Amino acid composition and antibacterial activity of honey

The amino acid composition in different types of honey. It has been determined that in acacia honey, obtained from the southern rural zone, the highest proportion of amino acids is taurine, which accounts for 20.74% of the total amino acid content. In contrast, honey from the urban area of the central zone contains 28.86% taurine. The levels of proline varied from 11.25% to 19.41%, glutamic acid – from 5.56% to 11.43%, and aspartic acid – from 4.44% to 11.54%. Proline is unique because it primarily comes from the bees during the process of converting nectar into honey. The amount of proline is one of the honey maturity indicators [16].

In acacia honey, the average content of amino acids includes leucine (2.60-4.07%), alanine (1.93-3.55%), serine (1.85-3.49%), phenylalanine (2.52-3.16%), valine (1.68-3.04%), arginine (2.33-3.02%), and threonine (1.35-2.91%) of the total amino acid content. Phenylalanine plays a key role in the formation of aromatic components [23].

The total content of essential amino acids in different types of honey ranged from 0.663 mg/g to 1.093 mg/g, non-essential amino acids from 0.357 mg/g to 0.402 mg/g, immunoactive amino acids from 0.444 mg/g to 0.738 mg/g, glucogenic amino acids from 0.330 mg/g to 0.481 mg/g, ketogenic amino acids from 0.214 mg/g to 0.229 mg/g, proteinogenic amino acids from 1.019 mg/g to 1.495 mg/g, and sulfur-containing amino acids from 0.248 mg/g to 0.374 mg/g.

It was determined that the total amino acid content in different types of honey was as follows: acacia honey – 1.352 mg/kg, sunflower honey – 1.741 mg/kg, and linden honey – 1.756 mg/kg, with the highest proportions of proline – 17.90-23.65%, taurine – 11.0-21.89%, glutamic acid – 9.76-16.95%, and aspartic acid – 9.89-11.38% of the total amino acids amount.

Antimicrobial properties of honey. The conducted analysis revealed that sunflower honey samples inhibit *Pseudomonas aeruginosa* even at significant dilutions. Similar to *Staphylococcus aureus*, inhibition of this bacterium was observed at a dilution of 1:16 (2.5%). Linden honey samples showed lower biological activity, with bacterial inhibition occurring at a sample dilution of 1:8 (5%). Acacia honey samples exhibited inhibitory properties at a concentration not exceeding 10% of honey.

Additionally, good results were obtained when testing the honey samples for their ability to inhibit *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, types of bacteria commonly found in the environment, including soil, water, and plants. These bacteria are opportunistic pathogens, meaning they can cause infections in individuals with weakened immune systems, and they are a frequent cause of nosocomial (hospital-acquired) infections [3].

The honey samples used in these experiments exhibited weak to moderate antibacterial activity against *E. coli*. The ability of honey to inhibit fungal infections was also studied using *Candida albicans* as the pathogen. It was shown that the tested honey samples exhibited weak antifungal activity against *C. albicans*. The minimum inhibitory concentration (MIC) was observed at a dilution of 1:2. As with the previous cases, sunflower honey showed higher antifungal activity than acacia honey.

It should be mentioned that the clusters of compounds in the study showed a significant difference in the MIC for the bacteria *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. For the MIC against *S. aureus*, there were no differences between the two clusters. The greatest difference was observed for *K. pneumoniae*, followed by *P. aeruginosa*.

3.4. The content and migration of microelements, macroelements, and heavy metals in the trophic chain (*soil – flowering plants – honey – pollen – propolis – bee bodies*)

Worker bees forage for nectar and pollen from plants over an extensive area, covering up to 25 km². The presence of various environmental pollutants can cause pathological changes in bees, and these contaminants can also enter the products they produce [17].

Micro-, macroelements, and heavy metals in soil. The results of the studies showed that the total amount of all studied microelements was highest in the soil of the Central zone, at 9.35 mg/kg. The amount of manganese (<0.7 mg/kg), zinc (<0.75 mg/kg), and chromium (<1.5 mg/kg) was the same in both zones. In the Central zone soil, the amount of iron was 1.8 mg/kg higher, and nickel was 0.93 mg/kg higher compared to the soil of the Southern zone.

The total amount of macroelements in the soil averaged 228.62 mg/kg, with the highest concentration found in the Southern zone at 263.66 mg/kg. The amount of calcium in the soil of the Southern zone was 1.47 times higher, potassium 1.22 times higher, and sodium 1.87 times higher compared to the soil of the Central zone. However, the amount of magnesium in the soil of the Central zone was 1.05 mg/kg higher, and phosphates were 4.13 mg/kg higher compared to the soil of the Southern zone.

It was found that the amount of heavy metals in the soil averaged 2.09 mg/kg, with variations ranging from 2.11 mg/kg (Central zone) to 2.07 mg/kg (Southern zone). The amount of lead was at the same level – <0.5 mg/kg, and cadmium – <0.06 mg/kg in the soils of both the Southern and Central zones. The amount of copper in the soil of the Southern zone was 1.5 times higher than in the soil of the Central zone

Micro-, macroelements, and heavy metals in nectar-producing plants

Microelements content. It was found that in acacia flowers, the average amount of microelements was 165.52 mg/kg, with fluctuations ranging from 138.2 mg/kg to 195.78 mg/kg. The highest content in the flowers of acacia from the Southern zone was zinc – 32.43 mg/kg, copper – 7.15 mg/kg, and iron – 128.87 mg/kg. In the Central zone, the highest content was nickel – 7.55 mg/kg, while in the urban area (Chișinău), the amount of manganese was 22.6 mg/kg. The amount of chromium was the same in both zones at <1.5 mg/kg.

The highest amount of all microelements in both acacia and sunflower flowers was found in the Southern zone – 166.40 mg/kg, consisting of manganese – 16.85 mg/kg, iron – 110.25

mg/kg, and nickel – 2.75 mg/kg. Zinc predominated in sunflower flowers from the Central zone, with 34.10 mg/kg, while in the Northern zone, copper was most prevalent with 11.60 mg/kg [5].

In linden flowers, the amount of manganese was 32.90 mg/kg, zinc – 18.80 mg/kg, copper – 6.53 mg/kg, iron – 53.23 mg/kg, chromium – <1.5 mg/kg, and nickel – 1.98 mg/kg. In the urban area, the total amount of microelements was 15.36 mg/kg lower than in the Central zone. However, the manganese content was higher, reaching 43.0 mg/kg, and zinc content was also greater at 21.6 mg/kg.

Macroelements content. The average content of macroelements in acacia flowers was 38738.67 mg/kg, with variation ranging from 37278.47 mg/kg (Central Zone) to 40198.87 mg/kg (Southern Zone). However, the total amount was 41894.5 mg/kg in the urban area (Chisinau). In sunflower flowers, the average content of macroelements was 38489.1 mg/kg, with variation from 34183.5 mg/kg to 48869.3 mg/kg. The highest concentration of macroelements was found in sunflower flowers from the Northern Zone, at 48869.3 mg/kg, while the lowest was in the urban area (Chisinau) at 33089.20 mg/kg.

It was found that the content of macroelements in linden flowers varied from 29936.6 mg/kg to 45126.23 mg/kg. In the urban area, the amount of calcium in linden flowers was 1.32 times higher than in the rural Central Zone, magnesium was 1.24 times higher, potassium was 1.06 times higher, and phosphates were 1.23 times higher. However, sodium content was 2.73 times higher in the rural area.

Heavy metals content. It was found that the content of heavy metals in acacia flowers averaged 35.58 mg/kg, ranging from 29.06 mg/kg (Chisinau) to 40.14 mg/kg (Southern Zone). The amount of lead was the same, at <0.5 mg/kg, and cadmium at <0.06 mg/kg in the acacia flowers from the Southern and Central zones. The zinc content in acacia flowers from the Southern Zone was 32.43 mg/kg, which was 8.0 mg/kg higher than in the Central Zone, while the copper content was 7.15 mg/kg, which is 1.13 mg/kg higher. The ash content ranged from 5.9% (Southern Zone) to 6.7% (Chisinau).

The content of heavy metals in sunflower flowers averaged 41.46 mg/kg, ranging from 35.61 mg/kg (Southern Zone) to 48.96 mg/kg (Northern Zone). The levels of lead in sunflower flowers were consistent across all zones at <0.5 mg/kg, and cadmium at <0.06 mg/kg. Zinc content varied from 28.1 mg/kg (Southern Zone) to 36.8 mg/kg (Northern Zone), copper from <1.5 mg/kg (Chisinau) to 11.6 mg/kg (Northern Zone), and ash content ranged from 5.07% (Chisinau) to 7.35% (Central Zone). The content of heavy metals in linden flowers averaged 25.89 mg/kg, which is 2.23 mg/kg higher than in the urban area (Chisinau).

Microelements, macroelements, and heavy metals in pollen load

Microelements content. It has been established that the amount of microelements in pollen loads depends on the plant species and averaged 117.45 mg/kg, ranging from 85.45 mg/kg (sunflower) to 168.95 mg/kg (acacia). The manganese content in the pollen loads varied depending on the plant species from which the pollen was collected, ranging from 10.5 mg/kg (sunflower, urban zone, Chisinau) to 50.0 mg/kg (acacia), zinc from 33.27 mg/kg (sunflower) to 41.40 mg/kg (acacia), copper from <1.5 mg/kg (sunflower, urban zone, Chisinau) to 14.9 mg/kg (linden), and iron from 30.10 mg/kg (sunflower) to 65.25 mg/kg (acacia). The chromium content was the same across all plant species at <1.5 mg/kg, and nickel was <2.5 mg/kg.

Macroelements content. It has been found that the content of macroelements in the pollen loads of the Central zone averaged 18,802.60 mg/kg, with variation from 14765.43 mg/kg (sunflower) to 24392.98 mg/kg (acacia). The content of macroelements in pollen loads depends on the type of pollen-bearing plants and varies as follows: calcium – from 1133.07 mg/kg (sunflower) to 1657.30 mg/kg (acacia); magnesium – from 397.0 mg/kg (sunflower, urban area) to 890.95 mg/kg (acacia); potassium – from 3312.97 mg/kg (sunflower) to 6733.18 mg/kg (acacia); sodium – from 22.6 mg/kg (sunflower, urban area) to 33.0 mg/kg (linden), and phosphates – from 5,765.33 mg/kg (sunflower) to 15,085.40 mg/kg (acacia).

Heavy metals content. It has been found that the content of heavy metals in pollen loads averages 45.671 mg/kg, with variation from 39.26 mg/kg (sunflower, urban area) to 52.365 mg/kg (linden). The highest concentration of heavy metals was found in the pollen loads of linden – 52.365 mg/kg and acacia – 51.66 mg/kg. The levels of lead in the pollen loads ranged from 0.065 to <0.5 mg/kg, cadmium from 0.06 to 0.40 mg/kg, zinc from 31.30 to 41.40 mg/kg, and copper from <1.5 to 14.9 mg/kg.

Micro-, macroelements, and heavy metals in propolis

Microelements content. The research results showed that the content of microelements in propolis averages 1114.39 mg/kg, with variations ranging from 619.72 mg/kg (urban area, Chișinău) to 2081.54 mg/kg (Central and Southern zones). The amount of manganese ranged from 11.47 to 28.8 mg/kg, zinc from 85.3 to 142.85 mg/kg, copper from 3.07 to 4.71 mg/kg, iron from 459.55 to 1958.7 mg/kg, chromium from <1.5 to 3.52 mg/kg, and nickel from 2.27 to 2.5 mg/kg.

Macroelements content. It was found that the content of macroelements in propolis averaged 5946.87 mg/kg. The highest amount of macroelements was found in propolis collected in the Southern zone – 8993.8 mg/kg, which is 4809.3 mg/kg more than in the Central zone. The content of calcium in propolis ranged from 1290.70 mg/kg to 4770.0 mg/kg, magnesium from 230.9 to 419.7 mg/kg, potassium from 954.55 to 1553.4 mg/kg, and sodium from 80.8 to 98.6 mg/kg

Heavy metals content. The total amount of heavy metals in propolis averaged 123.524 mg/kg. It was found that in the urban area (Chișinău), the amount of heavy metals was 1.49 times higher than in the Southern zone and 1.22 times higher than in the Central zone. The amount of lead in propolis from the Southern zone was 9.85 mg/kg, which is 3.07 times higher than in the Central zone, while zinc content was 142.85 mg/kg (urban area, Chișinău), which is 1.67 times higher than in the Southern zone. A slight variation was observed in the content of cadmium (<0.06-0.063 mg/kg) and copper (3.07-4.74 mg/kg).

Micro-, macroelements, and heavy metals in the body of bee

Microelements content. The study results showed that the total amount of studied microelements in the bodies of bees averaged 232.11 mg/kg. It was found that the amounts of manganese were 28.0 mg/kg, zinc – 63.62 mg/kg, copper – 11.47 mg/kg, iron – 126.27 mg/kg, chromium – <1.5 mg/kg, and nickel – <2.15 mg/kg.

Macroelements content. It was found that the total amount of macroelements in the bodies of bees averaged 35029.47 mg/kg, ranging from 31131.0 mg/kg to 39204.3 mg/kg. The amounts of calcium were 875.27 mg/kg, magnesium – 705.40 mg/kg, potassium – 8736.70 mg/kg, sodium – 461.17 mg/kg, and phosphates – 24250.93 mg/kg.

Heavy metals content. The total amount of heavy metals in the bodies of bees was 75.57 mg/kg, including lead – 0.394 mg/kg, cadmium – 0.074 mg/kg, zinc – 63.62 mg/kg, and copper – 11.47 mg/kg.

The migration of micro-, macroelements, and heavy metals in the trophic chain

Migration of microelement in the trophic chain. Understanding the distribution and migration of microelements in the trophic chain is important for assessing environmental health and its impact on species like honeybees. Iron shows a relatively higher concentration in the soil (2.20 mg/kg) compared to other microelements, but its concentration significantly increases in the flowers of honey plants and pollen baskets. Iron is crucial for various metabolic processes in plants, with the highest migration observed from the soil to the flowers of honey plants and from the flowers to the bee body, with concentrations of 74.32 mg/kg and 126.27 mg/kg, respectively, indicating substantial absorption by plants. However, its migration into honey is very low (3.70 mg/kg), suggesting limited transfer into the honey. The highest accumulation occurs in propolis (975.14 mg/kg). The significant concentration of iron in propolis suggests its strong absorption and retention through the trophic chain. The high migration of iron in this case may be related to its important role in various metabolic processes and its efficient use by both plants and bees.

Considering that copper and zinc compounds are often used in agriculture as fungicides and foliar fertilizers, determining the concentrations of these metals in the studied objects and their subsequent migration is of particular interest. It has been shown that zinc is present in the soil in minimal amounts (<0.75 mg/kg), but its concentration increases sharply in flowers (20.89 mg/kg), especially in the bee's body (63.62 mg/kg) and propolis (114.63 mg/kg) along the trophic chain. It can be concluded that environmental contamination with zinc may pose a particular threat to bees considering the significant accumulation of zinc in the bee's body even at low concentrations in the earlier links of the trophic chain.

Manganese shows a relatively low concentration in the soil (<0.7 mg/kg), but demonstrates significant accumulation, especially in the bodies of bees (28.0 mg/kg) and pollen baskets (23.18 mg/kg), indicating considerable absorption from nectar plants (21.85 mg/kg). The transfer from flowers to pollen and then to the bee body highlights the movement of manganese through the food chain.

All microelements demonstrate significant accumulation from the soil into the flowers of nectar plants. Iron shows the highest migration from the soil to the flowers of nectar plants, with a transfer or migration coefficient of 33.78, indicating significant absorption by the plants. However, its migration from the flowers to honey is very low. Overall, the cumulative coefficients indicate the effectiveness of migration and bioaccumulation of micronutrients in bees and their related products, with iron showing the highest accumulation levels, followed by zinc, manganese, and copper.

Thus, it can be noted that the highest concentrations of manganese (28.0 mg/kg) and copper (11.47 mg/kg) were found in the bodies of bees, zinc (114.627 mg/kg), iron (975.14 mg/kg), and chromium (2.523 mg/kg) in propolis, and nickel (3.90 mg/kg) in the flowers of nectar plants.

Migration of macroelements in the trophic chain. The calcium baseline concentration in the soil is recorded at 160.62 mg/kg. The relatively low calcium concentration in the soil compared to subsequent trophic levels indicates that calcium is efficiently absorbed by plants. The flowers of honey plants show a sharp increase in calcium concentration, reaching 6604.10 mg/kg. This significant rise confirms that honey plants have a high affinity for calcium, which is vital for various physiological processes, leading to its accumulation in such large amounts. From the flowers, calcium migrates to pollen pellets, where the concentration decreases to 1459.72 mg/kg. In bee bodies, the concentration is 875.27 mg/kg. This value reflects the intake of calcium through pollen and nectar and its critical role in the physiological processes of bees. Honey exhibits the lowest calcium concentration among the samples, at 64.01 mg/kg. This low concentration suggests that only a minimal amount of calcium migrates from bee bodies to honey.

However, the magnesium concentration in the soil is also relatively low (14.87 mg/kg). It significantly increases in the flowers of honey plants (2084.86 mg/kg) similar to calcium. This substantial increase indicates that magnesium is readily absorbed by plants from the soil. The concentration decreases in bee bodies to 705.40 mg/kg and to 300.60 mg/kg in propolis. This suggests that magnesium is efficiently transferred from the soil to bees and propolis. However, honey shows a much lower concentration (24.34 mg/kg), indicating minimal transfer of magnesium from bees to honey.

Potassium is an essential macroelement for both plants and animals, playing a critical role in various physiological processes. The potassium concentration in the soil is 37.97 mg/kg. This relatively low-level increases sharply in the flowers of honey plants, reaching 17475.71 mg/kg. This significant uptake highlights the crucial role of potassium in plant physiology. In bee bodies, the potassium concentration reaches 8736.70 mg/kg, indicating its vital role in the metabolic processes of bees.

The potassium concentration in honey is quite high at 662.74 mg/kg, making it the primary contributor to the mineral content, a finding consistent with other studies [33].

The phosphates migration through the trophic chain associated with bees demonstrates significant accumulation, with notable changes at each stage. Phosphate levels increase sharply from the soil to the flowers of honey plants, rising from 4.7 mg/kg in the soil to 10615.05 mg/kg in honey plant flowers. This considerable increase highlights the efficient phosphate uptake by honey plants. Phosphate concentrations show a modest rise to 10929.74 mg/kg from the flowers to the pollen pellets, indicating that pollen grains act as a concentrated reservoir for phosphates.

The most significant increase in phosphate concentration occurs when moving from the pollen pellets to the bee's body, reaching 24250.93 mg/kg. This highlights the bee's ability to intensely accumulate and utilize phosphates. Thus, phosphates show a clear pattern of accumulation, particularly peaking in the bee's body, similar to other macroelements such as potassium and magnesium.

The baseline sodium concentration in the soil is recorded at 10.45 mg/kg. Although sodium is not a primary nutrient like potassium or calcium, it still plays a crucial role in the physiology of plants and animals. The sodium concentration increases to 38.10 mg/kg in the flowers of honey plants. This rise in sodium levels indicates that plants absorb sodium from the soil, albeit less efficiently compared to other macroelements such as calcium or potassium. There is a significant increase in the bee's body, reaching 461.17 mg/kg while the sodium concentration in pollen pellets slightly decreases to 26.83 mg/kg. The minimal migration of sodium from the bee’s body into honey aligns with the general observation that honey (22.69 mg/kg) primarily serves as an energy source rather than a significant carrier of mineral elements. The limited sodium content in honey is consistent with other studies, which have identified low mineral concentrations in honey, reflecting its composition and the selective retention of specific elements by bees [33].

It can be noted that the highest concentrations of calcium (6604.10 mg/kg), magnesium (2084.86 mg/kg), and potassium (17475.71 mg/kg) are found in the flowers of honey plants (acacia, linden, sunflower), while sodium (461.17 mg/kg) and phosphates (24250.93 mg/kg) are most concentrated in the bee’s body, analyzing the trophic chain (*soil – flowers of honey plants – honey – pollen pellets – propolis – bee body*).

The migration of heavy metals in the trophic chain. Lead, a highly toxic metal, had a relatively low concentration in the soil at 0.426 mg/kg and at a concentration of <0.5 mg/kg in the flowers of honey plants. This suggests that plants in this region, due to selective absorption mechanisms (carbonate soil), prevent the accumulation of lead, which is a positive outcome considering the toxic nature of this metal. However, lead was detected both in pollen grains and in the bodies of bees at a concentration of 0.394 mg/kg, indicating some level of bioaccumulation as it moves from plants to bees. Despite this, the concentration of lead in honey remains low at 0.406 mg/kg, comparable to the level in the soil. The most significant accumulation of lead is observed in propolis, where the concentration reaches 5.125 mg/kg.

Cadmium has a very low concentration in the soil, at 0.051 mg/kg, and at <0.06 mg/kg in the flowers of honey plants, suggesting limited absorption by plants. However, the concentration of cadmium in the pollen grains increases to 0.145 mg/kg, indicating some bioaccumulation as it moves from the soil into the pollen. Interestingly, this concentration slightly decreases in the bee's body to 0.074 mg/kg, which may suggest that bees have mechanisms for regulating or excreting cadmium to avoid toxicity.

It was found that copper also shows noticeable accumulation in the bee's body (11.47 mg/kg) compared to its initial concentration in the soil (0.940 mg/kg) although in smaller amounts compared to zinc. This trend indicates the bioavailability and mobility of copper in the ecosystem, albeit to a lesser extent than zinc.

Chromium becomes toxic at higher concentrations. According to our research, the chromium levels in the soil were <1.5 mg/kg, suggesting a low baseline presence in the environment. Despite this, chromium was found at a concentration of 1.42 mg/kg in the flowers of honey plants, indicating some absorption from the soil. Interestingly, the highest concentration of chromium was found in propolis – 2.52 mg/kg, which supports the idea that propolis acts as an absorber of potentially harmful metals, preventing their migration into honey.

Nickel (Ni), like chromium, is essential in small amounts but can be toxic when accumulated in the body. The concentration of nickel is 2.04 mg/kg in the soil, and it reaches 3.90 mg/kg in the flowers of honey plants, reflecting higher absorption by plants compared to other heavy metals. The concentration of nickel in propolis is 2.42 mg/kg, indicating some accumulation, although it is only marginally higher than in the soil. Nickel is below detection limits (<2.5 mg/kg) in honey.

Zinc shows significant accumulation from the soil into the honey plant flowers with a bioaccumulation factor of 39.64, and copper with a factor of 7.55. However, their migration from the flowers to the honey is very low. Zinc exhibits high transfer from the honey plants to the bee's body (BFA = 2.39) and into propolis (BFA = 4.31) (Fig. 3.1).

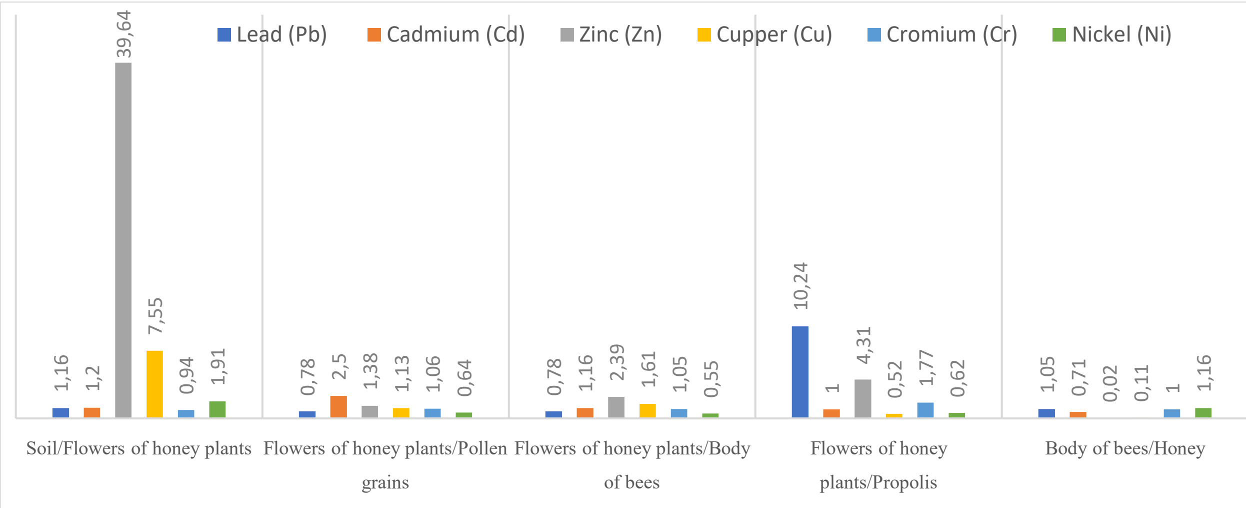

Figure 3.1. Diagram of migration coefficients of elements in the trophic chain

The relatively low concentration of copper in propolis compared to honey plant flowers (BFA = 0.52) indicates that copper tends to accumulate and is more difficult to expel, or alternatively, that this amount is required for essential biological functions.

The migration process of lead shows migration coefficients of 1.16 along the trophic chain from soil to honey plant flowers, with the maximum 10.24 in propolis. Cadmium demonstrates accumulation up to the pollen pellets with migration coefficients of 2.5, but decreases as it moves through the chain, reaching 0.71 in honey.

Chromium and nickel have lower initial absorption by plants, with migration coefficients from soil to honey plant flowers of 0.94 and 1.91, respectively, in the bee's body – 1.05 and 0.55, in propolis – 1.77 and 0.62, and in honey – 1.0 and 1.16. The migration of elements from the bee's body to honey either does not occur or occurs at very low concentrations.

The total amount of heavy metals in the trophic chain was 2.089 mg/kg in the soil, 34.223 mg/kg in the honey plant flowers, 3.026 mg/kg in honey, 45.661 mg/kg in pollen pellets, 123.524 mg/kg in propolis, and 75.57 mg/kg in the bodies of bees.

These results highlight the importance of understanding the selective migration and retention of heavy metals in the trophic chain. The low concentrations of these metals in honey indicate effective barriers against their transfer, which is crucial for maintaining the safety of honey as a consumable product.

The study was conducted in ecologically friendly regions of the Republic of Moldova, which likely contributes to the low initial concentrations of heavy metals in the environment. However, even in such regions, it is important to monitor the migration of these metals, as they pose a significant health risk if accumulated in higher concentrations.

Thus, worker bees, consuming honey and pollen pellets (bee bread) from which they absorb all the necessary elements for metabolic processes, can serve as indicators of the environmental conditions within their productive flight radius of 2-3 km, which covers an area of 1250-2580 hectares.

4. INCREASING HONEY PRODUCTION VOLUMES USING BIOSTIMULANTS IN BEE FEEDING

4.1. Use of Biostimulants *ApiStev, CobalStev, ApiRibo, and ApiDAK* in Bee Feeding

Use of the *Apistev* biostimulant in bee feeding

Experiment I. On September 13, 2020 at the apiary in the village of Kozhushna, the average strength of the bee colonies was 8.7-9.0 frames before use of the biostimulant *ApiStev* in bee feeding. During the autumn inspection on October 28, the strength decreased by 2 frames in Group I, by 1.5 frames in Group II, by 4 frames in Group III, and by 3 frames in Group IV. It was noted that the winter hardiness was 80.95% in Group I, 86.6% in Group II, 95.24% in Group III, and 85.71% in the control Group IV, comparing the strength of the bee colonies during the autumn inspection (October 28, 2020) and the spring inspection (March 28, 2021). Therefore, the bee colonies in Groups II and III showed better winter hardiness compared to the control group.

The spring feeding of the bee colonies had a positive effect on the queen's egg-laying and brood development. The highest number of eggs was observed in the bee colonies of Group II – 180.0 frames just before the white acacia flowering, which is 77.69% higher than the control group. The queen fertility in the experimental groups ranged from 1352 to 1500 eggs during this period, which is 60.19% to 77.72% higher than in the control group, which laid 844 eggs in 24 hours.

Therefore, the experimental colonies were better prepared for the main honey harvest from the white acacia.

A significant amount of honey was collected from the white acacia by the bee colonies in Group II (35.2 kg), which is 55.75% more than the control group (Figure 4.1). Increasing the dose of the natural bioregulator *ApiStev* to 4.0 ml/l did not affect the honey production [7].

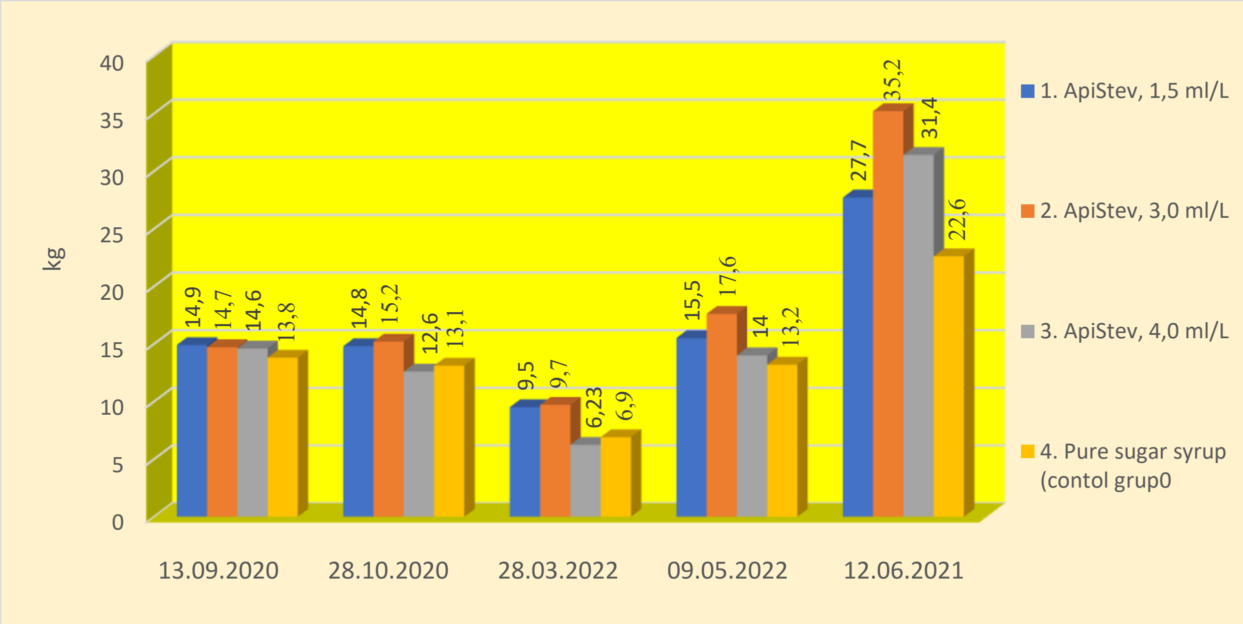

Figure 4.1. The honey reserves dynamics in the bee colonies.

Therefore, the optimal dose of the natural biostimulant *ApiStev* is 3.0 ml/l for bee feeding. Autumn feeding of bees with the biostimulant *ApiStev* boosts immunity and winter hardiness by 0.89-9.53%, while in the spring, it increases colony strength by 18.3-21.8%, brood production by 77.7%, and honey productivity by 22.6-55.7% compared to the control group [7].

The CobalStev biostimulant use in bee feeding

Experiment II. Результаты исследований показали, что при весенней ревизии пчелиных семей 9 апреля 2022 года до стимулирующей подкормки в гнезде было в среднем 6,7-7,0 сотов, сила семей – 5,7-6,0 улочек, печатного расплода – 57,7-60,0 кв. и резервы меда – 2,3-2,7 кг. При контрольном осмотре пчелиных семей, проведенном до цветения акации белой 19 мая 2022 г., в гнезде насчитывалось 11,3-13,0 сот, сила составляла 10,3-11,7 улочек. The research results have shown that during the spring inspection of the bee colonies on April 9, 2022, before the stimulating feeding, there was an average of 6.7-7.0 frames in the hive, the colony strength was 5.7-6.0 frames, the capped brood was 57.7-60.0 frames, and the honey reserves were 2.3-2.7 kg. The hive contained 11.3-13.0 frames, and the colony strength was 10.3-11.7 frames during the control inspection of the bee colonies, carried out before the flowering of white acacia on May 19, 2022. The bee colonies of groups I and II produced an average of 84.0 and 82.7 frames of capped brood, which is 8.11% and 6.43% more than in the control group IV. The honey reserves ranged from 2.7 to 3.7 kg on average in the bee colonies, which confirms the absence of a supporting honey harvest.

It was found that the bee colonies in groups II and III had an average of 20 frames, with a colony strength of 19.0 frames, which was 7.34% higher than in the control group at the end of the honey harvest from white acacia on June 6, 2022. The group II bee colonies produced 123.0 squares of capped brood, with the queens laying 1025 eggs in 24 hours, while the control group produced 877 eggs. The spring feeding stimulated an increase in queen fertility and brood production by 16.81%. The bee colonies of experimental group II collected an average of 28.1 kg of honey per colony, which is 9.77% more compared to the control group IV under challenging climatic conditions (high temperatures and drought).

Thus, it has been established that the optimal dosage of the biostimulator *CobalStev* is 2.0 ml per liter of sugar syrup in bee feeding. Using a mixture of 1:1 sugar syrup and *CobalStev* at a dose of 2.0 ml/l, enhances colony strength by 7.34%, increases sealed brood production by 16.81%, and boosts honey yield by 9.77% implementing the proposed feeding method in spring, during periods of limited hive food reserves.

Use of the ApiRibo Biostimulator in bee feeding

Experiment III. The effects of the *ApiRibo* biostimulator were studied in relation to winter hardiness, growth, early spring development, and honey productivity. *ApiRibo* is a commercially available product consisting of a 3% aqueous solution of the glycoside rebaudioside A extract. This biostimulator was developed at the Institute of Chemistry of the State University of Moldova.

On September 12, 2020, it was observed before feeding that the nests of bee colonies in the experimental groups contained an average of 7.33–7.67 frames, colony strength was 6.33–6.67 combs, sealed brood occupied 19.33–26.0 squares, and honey reserves totaled 12.67–13.03 kg. The best overwintering results were recorded in the colonies of experimental group II, which were fed inverted corn syrup supplemented with the *ApiRibo* biostimulator at a dose of 2 ml/L. These colonies exhibited a winter survival rate of 68.33%, which was 11.66% higher than that of group IV (control). Spring feeding of bee colonies with inverted corn syrup and the *ApiRibo* biostimulator at doses of 1–2 ml/L increased queen egg-laying and brood rearing by 19.6–23.6% compared to group IV (control). On June 8, 2021, the bee colonies in group II showed the best development by the end of the white acacia honey harvest. On average, these colonies had 18 frames, reared 6.3 artificial frames per colony, achieved a colony strength of 16.7 combs, sealed brood covering 161.0 squares, and honey reserves of 27.57 kg.

Thus, it was determined that the optimal dose of the *ApiRibo* biostimulant for bee feeding, both for replenishing winter food reserves and during the spring period, is 2.0 ml per liter of inverted corn syrup.

Fall feeding of bees with a mixture of inverted corn syrup and *ApiRibo* biostimulant at a dose of 2.0 ml/l in a 1.5:1 ratio, totaling 2.0 liters, results in an increase in immunity and winter hardiness by 11.6%. In the spring, feeding with the same mixture increases colony strength by 28.5%, capped brood by 37.6%, queen fertility by 37.6%, and honey production by 52.5% at a rate of 1.0 liter per bee colony every 7-9 days, compared to the control group [9].

Use of the *ApiDAK* Biostimulator in bee feeding

Experiment IV. The impact of the *ApiDAK* biostimulant on growth, early development, and the efficiency of honey collection by bee colonies was studied to determine the optimal conditions for its use. The bioregulator *ApiDAK* is a 3% aqueous solution of a substance derived from suspended dihydroabietic acid, dissolved in an aqueous KOH solution at room temperature, and is applied in doses of 1.0-3.0 ml/l of sugar syrup.

It was found that the average number of frames in the hives was 5.0-5.67, the colony strength was 4.0-4.67 frames, and the honey reserves were 1.17-1.33 kg, as a result of the control inspection of bee colonies on April 18, 2021. The hives contained 9.0-14.7 frames, and the colony strength ranged from 8.0 to 13.7 frames before the bloom of white acacia on May 11, 2021. Bee colonies in the I, II, and III experimental groups reared an average of 142.0-167.0 square centimeters of sealed brood, which was 5.97-24.63% more than the control IV group. Feeding bees with sugar syrup mixed with the *ApiDAK* biostimulant at doses of 1.0-3.0 ml/l of syrup increased queen fertility by 5.91-24.62% compared to the IV control group. At the end of the white acacia honey harvest on June 15, 2021, it was found that the bee colonies in groups I and II had an average of 25.5-28.0 frames, with colony strength ranging from 24.5 to 27.0 frames, which was 11.36-22.73% higher than the control group. The bee colonies in group II collected the most honey from white acacia – 50.55 kg, which was 62.91% more than the control IV group.

Thus, it was determined that the optimal dosage of the natural biostimulant *ApiDAK* for feeding bees during the spring season is 2.0 ml/l of sugar syrup. Feeding bees in the springtime with a mixture of sugar syrup and *ApiDAK* at a dose of 2.0 ml/l, providing 1.0 liter of the mixture per bee colony every 7 days, increases colony strength by 11.36-22.73%, sealed brood production by 5.97-24.63%, queen fertility by 5.91-24.62%, and honey productivity by 62.91%, compared to the control group [8].

4.2. Use of *Choline Chloride* Biostimulant in Bee Feeding

Experiment V. Spring feeding of bees with a mixture of sugar syrup and the biostimulant choline chloride, starting in March and continuing until the main honey flow, boosts colony strength by 4.12-17.65%, increases brood rearing by 17.48-43.98%, and enhances honey productivity by 22.88%.

4.3. Use of Biostimulants (3% Glucuronic Acid Solutions) in Bee Feeding

Experiment VI. The use of the developed method for spring feeding of bees with a mixture of 50% sugar syrup and a biostimulant (3% glucuronic acid solution), provided at a rate of one liter every 10 days, increases colony strength by 19.05%, enhances queen fertility by 57.7%, and boosts honey production by 21.4-28.74%.

4.4. Effectiveness of Biostimulants in Bee Feeding The use of biostimulants in bee feeding can yield a net profit ranging from 176.0 MDL (II-*CobalStev*) to 1159.6 MDL (IV-*ApiDAK*), representing an increase of 10.62-46.71%, compared to the control group.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

1. The research results on the migration of heavy metals within the trophic chain and compliance with honey quality standards, as well as requirements for the EU export, made it possible to identify regions in the Republic of Moldova rich in honey plants and enhance honey production. It was found that the moisture content of honey from various soil and climatic zones ranged from an average of 16.93% (acacia honey) to 18.05% (linden honey). The invert sugar content was 77.18–78.50%, sucrose content ranged from 1.71–2.07%, and the diastase number varied between 8.56 Gothe units (acacia honey) and 16.25 Gothe units (sunflower honey). Additionally, acidity was measured at 1.13–2.26 milliequivalents per 100 g, and hydroxymethylfurfurol (HMF) content ranged from 3.00 mg/kg (linden honey) to 6.77 mg/kg (acacia honey). As a result, all indicators meeting established honey standards.

2. It was established that the highest average concentration of the studied trace elements over four years (2020–2023) was found in acacia honey at 16.457 mg/kg, including manganese at 3.661 mg/kg, zinc at 1.860 mg/kg, copper at 1.413 mg/kg, iron at 5.487 mg/kg, chromium at <1.5 mg/kg, and nickel at <2.5 mg/kg. The lowest concentration was observed in sunflower honey at 9.118 mg/kg.

The macroelements content of various types of honey ranges on average from 483.81 mg/kg (acacia honey) to 1435.19 mg/kg (linden honey), including calcium at 31.618–82.42 mg/kg, magnesium at 10.962–39.883 mg/kg, potassium at 266.217–1168.967 mg/kg, sodium at 17.20–26.10 mg/kg, and phosphates at 148.85–228.68 mg/kg. The microelement and macroelement composition of honey depends on nectar sources and soil-climatic zones.

It has been determined that the content of heavy metals in acacia honey, obtained from the Southern region and urban areas (Chisinau), is at the upper limits of permissible levels. Higher quality honey (acacia, linden, and sunflower) was produced in the Central rural area, which meets the requirements of all standards.

3. It was found that the total amino acid content in different types of honey ranged from 1.352 mg/g (acacia) to 1.756 mg/g (linden). Acacia, linden, and sunflower honey exhibit high antibacterial activity against *S. aureus* and *P. aeruginosa* even when diluted to 1:16 (2.5%). The samples tested showed weak antifungal activity against *Candida albicans*, with the MIC determined at a dilution of 1:2 (20%). The linden and sunflower honey samples exhibited higher antifungal activity than the acacia honey. The samples with the best biological activity (sunflower honey) contain higher amounts of free acids, which results in lower pH values of the honey solution, and these samples also had the highest content of OMF [6].

4. It has been shown that the total amount of trace elements in the trophic chain (soil – flowers of honey plants – honey – pollen baskets – propolis – bee bodies) varied and averaged as follows: in the soil – 8.19 mg/kg, in the flowers of honey plants it increased 16.50 times, in honey – 1.45 times, in pollen baskets – 14.34 times, in propolis – 136.07 times, and in bee bodies – 28.34 times compared to the soil. The amount of macroelements in the trophic chain averaged 228.62 mg/kg in the soil, increased to 38,473.40 mg/kg in the flowers of honey plants, 949.71 mg/kg in honey, 18,802.60 mg/kg in pollen baskets, 5,946.87 mg/kg in propolis, and 28,747.92 mg/kg in bee bodies. The amount of heavy metals in the soil was 2.089 mg/kg, increased to 34.223 mg/kg in the flowers of honey plants, 3.026 mg/kg in honey, 45.661 mg/kg in pollen baskets, 123.52 mg/kg in propolis, and 75.57 mg/kg in bee bodies.

5. Feeding bees with a mixture of sugar syrup and biostimulants in the amount of 2.0-3.0 liters per bee colony in the fall contributes to an increase in winter hardiness by 0.89-9.53%. Spring feeding with sugar syrup and biostimulants, at a rate of 1.0 liter every 7-10 days, leads to an increase in colony strength by 4.12-22.73%, queen fertility by 3.29-77.7%, and honey productivity by 9.77-62.91% compared to the control group [7].

Feeding bees in the fall with a mixture of inverted corn syrup, sugar syrup, and natural biostimulants at a dose of 2.0 ml/l in a 1.5:1 ratio, in the amount of 2.0 liters, increases winter hardiness by 11.6%. Spring feeding with the same mixture in the amount of 1.0 liter per colony increases colony strength by 15.05-36.56%, brood area and queen fertility by 11.72-46.11%, and honey production by 5.98-69.3% [9].

The obtained main result contributes to solving an important scientific problem related to determining the quality of honey from different soil-climatic zones and nectar plants, the content of micro- and macroelements, and the migration of heavy metals in the food chain. It also helps establish the optimal quantity of biostimulants used in bee feeding, which led to the development of new methods that enhance honey production and the winter hardiness of bee colonies.

Recommendations:

It is recommended to place the hives during migration to the nectar collection areas in the Central rural zone, in environmentally clean regions, and as far away from highways as possible considering the migration of heavy metals in the trophic chain and the need for high-quality honey.

It is recommended to use feeding methods with a mixture of sugar syrup and the biostimulant *ApiStev* – 3.0 ml/l, in the fall, 3.0 liters per colony and 1.0 liter in the spring [7]; use mixtures of inverted corn syrup with the biostimulant *ApiRibo* – 2.0 ml/l, 2.0 liters per colony in the fall and 1.0 liter in the spring [9]; and apply mixtures of sugar syrup and the biostimulant *ApiDAK* – 2.0 ml/l, 1.0 liter per colony every 7 days in the spring in order to improve the immunity, winter hardiness of bee colonies, and honey production [8].

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20. EREMIA N., **KOSHELEVA O.,** NEICOVCENA I., MAKAEV F. Physico-Chemical Properties of Honey and Sunflower Flowers of Various Soil and Climatic Zones of The Republic Of Moldova. IV. International Agriculture Congress. ABSTRACT BOOK. 16-17 December 2021 www.azimder.org.tr http://utak2021.com Editors Dr. Emrah GÜLER, Dr. Tuba BAK. Comrat, 2021, p. 45. ISBN: 978-605-80128-5-1

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- **Invention patents**

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ADNOTARE

COȘELEVA Olga, ”Influența migrației metalelor grele în lanțul trofic asupra calității mierii de albine”. Teza de doctor în științe agricole, Chișinău, 2024.

Structura tezei: introducere, patru capitole, concluzii generale şi recomandări, bibliografie din 308 de titluri, 9 anexe, 119 pagini de text de bază, 17 figuri şi 43 tabele. Rezultatele obținute sunt publicate în 25 lucrări științifice.

Cuvinte-cheie: familii de albine, sol, florile plantelor melifere, miere, ghemotoace de polen, albine, indici morfoproductivi, hrană stimulatoare, biostimulatori.

Scopul lucrării: constă în argumentarea științifică și evaluarea calității mierii din diferite zone pedoclimatice, migrației metalelor grele în lanțul trofic, sporirii producției mierii cu utilizarea biostimulatorilor în hrana albinelor.

Obiectivele cercetării: determinarea indicilor fizico-chimici a mierii de albine din diferite zone pedoclimatice; identificarea conținutului de micro-, macroelemente și metale grele în miere din diferite zone pedoclimatice; determinarea compoziției de aminoacizi și a activității antibacteriene a mierii de albine; aprecierea migrației și conținutului micro-, macroelementelor și metalelor grele din lanțul trofic (*sol – florile plantelor melifere – miere – ghemotoace de polen – propolis – albin*e); evaluarea eficacității utilizării biostimulatorilor naturali în hrănirea albinelor și elaborarea recomandărilor practice.

Noutatea şi originalitatea științifică constă în argumentarea științifică a evaluării calității mierii din diferite zone pedoclimatice și a surselor melifere, precum și migrației metalelor grele în lanțul trofic, sporirii producției mierii, creşterii rezistenței la iernare a familiilor de albine prin utilizarea biostimulatorilor și elaborarea noilor procedee de hrănire a albinelor (Brevete de invenție MD 1607; MD 1611; MD 1612).

Rezultatul obținut care contribuie la soluționarea unei probleme ştiinţifice importante privind *determinarea* calității mierii din diverse zone pedoclimatice și surse melifere, revelarea conținutului micro-, macroelementelor, migrației metalelor grele în lanțul trofic, *optimizarea* utilizării unor biostimulatori, ceea ce a stat la baza *elaborării* procedeelor noi de hrănire a albinelor, care asigură creşterea producției de miere și rezistenței la iernare a familiilor de albine.

Semnificația teoretică: în premieră au fost efectuate cercetări științifice complexe în evaluarea calitíții mierii din diferite zone pedoclimatice, migrația metalelor grele în lanțul trofic și sporirea producției de miere cu utilizarea biostimulatorilor în hrana albinelor.

Valoarea aplicativă a tezei constă în identificarea zonelor ecologice pentru obținerea mierii de calitate superioară, stabilirea migrației metalelor grele în lanțul trofic și sporirea producției de miere utilizând biostimulatori în hrănirea albinelor.

Implementarea rezultatelor științifice s-a realizat în diverse stupine din raioanele Nisporeni, Strășeni, Călărași, Orhei și în procesul de învățământ la Universitatea Tehnică a Moldovei.

АННОТАЦИЯ

КОШЕЛЕВА Ольга, "Влияние миграции тяжелых металлов в трофической цепи на качество пчелиного меда". Докторская диссертация сельскохозяйственных наук, Кишинев, 2024.

Структура диссертации: введение, четыре главы, общие выводы и рекомендации, библиография из 308 наименований, 9 приложений, 119 страниц основного текста, 17 рисунков и 43 таблицы. Результаты исследования были опубликованы в 25 научных статьях.

Ключевые слова: пчелиные семьи, почва, цветки медоносов, мед, пыльцевые обножки, пчелы, морфо-продуктивные показатели, подкормки, биостимуляторы.

Цель работы: состоит в научном обосновании и оценке качества меда разных почвенно-климатических зон, миграции тяжелых металлов в пищевой цепи и повышение производства меда с применением биостимуляторов в подкормке пчел.

Задачи исследования: определение физико-химических показателей пчелиного меда из разных почвенно-климатических зон; выявление содержание микро-, макроэлементов и тяжелых металлах в меде из разных почвенно-климатических зон; определение аминокислотного состава и антибактериальной активности пчелиного меда; выявление миграции и содержания микро-, макроэлементов и тяжелых металлов в трофической цепи (*почва – цветки медоносных растений – мед – пыльцевые обножки – прополис – тело пчел)*; оценка эффективности использования природных биостимуляторов в подкормки пчел и разработать практические рекомендации.

Научная новизна и оригинальность заключается в научной аргументации и оценке качества меда разных почвенно-климатических зон и источников медоносов, а также миграция тяжелых металлов в пищевой цепи, увеличения производства меда, повышения зимостойкости пчелиных семей путем использования биостимуляторов и разработка новых способов подкормке пчел (патенты на изобретения MD 1607; MD 1611; MD 1612).

Полученный основной результат способствует решению важной научной задачи по *определению* качества меда из различных почвенно-климатических зон и медоносов, выявлению содержания микро-, макроэлементов и миграции тяжелых металлов в пищевой цепи, *оптимизации* использования биостимуляторов, что послужило основой для *разработки* новых способов, обеспечивающих повышению производство меда и зимостойкости пчелиных семей.

Теоретическая значимость: впервые проведены комплексные исследование по оценке качества меда разных почвенно-климатических зон, миграции тяжелых металлов в пищевой цепи и повышение производства меда с применением биостимуляторов в подкормке пчел.

Практическая значимость работы заключается в выявлении экологических зон для получения меда высокого качества, установлении миграции тяжелых металлов в пищевой цепи и увеличения производства меда с использованием биостимуляторов в подкормке пчел.

Внедрение научных результатов проводились на различных пасеках в районах Ниспорены, Страшены, Калараш, Орхей и в учебном процессе – в Техническом Университете Молдовы.

ANNOTATION

KOSHELEVA Olga, "The impact of heavy metal migration on the quality of honey in the trophic chain". PhD Thesis in Agricultural Sciences, Chişinau, 2024.

Thesis structure: introduction, four chapters, conclusions and recommendations, bibliography of 308 titles, 9 appendices, 119 pages of main text, 17 figures and 43 tables. The results of the study were published in 25 scientific articles.

Key words: bee families, soil, nectar-producing flowers, honey, pollen loads, bees, morphoproductive indicators, supplements, biostimulants.

The purpose of research: to scientifically substantiate and evaluate the quality of honey from different soil and climatic zones, investigate the migration of heavy metals in the food chain, and enhance honey production through the implementation of biostimulants in bees' nutrition.

Research Goals: to determine the physicochemical properties of honey from different soil and climatic zones; identify the content of microelements, macroelements, and heavy metals in honey from different soil and climatic zones; define the amino acid composition and antibacterial activity of honey; investigate migration and content of microelements, macroelements, and heavy metals in the trophic chain (*soil – nectar – producing flowers – honey – pollen loads – propolis – bee body*); assess the effectiveness of natural biostimulants usage in bee nutrition and provide practical recommendations.

Novelty and originality: lie in the scientific reasoning and evaluation of honey quality from different soil-climatic zones and nectar sources, as well as the migration of heavy metals in the food chain, increasing honey production, increasing the winter hardiness of bee colonies by using biostimulants and developing new methods of feeding bees (patents for inventions MD 1607; MD 1611; MD 1612).

The main result contributes to the solution of an important scientific problem of *determining* the quality of honey from various soil-climatic zones and nectar sources, identifying the content of micro- and macroelements and the migration of heavy metals in the food chain, *optimizing* the use of biostimulants, which served as the basis for the *development* of new methods to increase honey production and improve the winter hardiness of bee colonies.

Theoretical significance: for the first time, complex scientific research was carried out in the evaluation of the quality of honey from different pedoclimatic zones, the migration of heavy metals in the food chain and the increase of honey production with the use of biostimulators in bee feeding.

Practical significance of the research lies in identifying ecological zones for obtaining high-quality honey, determining the migration of heavy metals in the food chain, and increasing honey production through the use of biostimulants in bee feeding.

The implementation of scientific results was carried out at different apiaries in the regions of Nisporeni, Straşeni, Calaraşi, Orhei and applied to the academic programs at the Technical University of Moldova.

KOSHELEVA OLGA

THE IMPACT OF HEAVY METAL MIGRATION ON THE QUALITY OF HONEY IN THE TROPHIC CHAIN

421.03 – TECHNOLOGY OF ANIMAL BREEDING AND LIVESTOCK PRODUCT PRODUCTION

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