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## MAJOR FACTORS DETERMINING ACCUMULATION OF TOXIC ELEMENTS BY BEES AND HONEY PRODUCTS

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### ABSTRACT

This study presents the results of the main factors that determine the accumulation of toxic elements in the body of bees and some honey products. The following tasks were solved: evaluation of the dynamics of the movement of the heavy metals along with the trophic chain soil-plant-body of bee-honey products; clarification of the main mechanism and the main factors determining the accumulation of heavy metals in the body of bees and honey products. Samples of soil, plants, bees, as well as honey products from areas of the Ryazan region in Russia, were investigated by the method of atomic absorption spectrometry to determine heavy metals. It was found that of all honey products the most environmentally friendly product is honey, and propolis, pollen, and bee bread were most polluted. It was established that the maximum purity of honey was determined by its biochemical composition (mainly carbohydrates, which are secreted by secretory cells of nectaries within a few hours) and by careful draining of pollen grains from nectar by an intermediate valve in a honey sac. It was revealed that the main amount of heavy metals accumulated in the body of bees due to intensive consumption of pollen and bee bread for two weeks, contamination of which was hundreds of times higher than that of nectar and honey, as well as during processing of nectar into honey when draining pollen and entering it into the midgut. The level of pollution of honey products was strongly influenced by the remoteness of the family from the source of pollution. The bee, pollen, and propolis can serve as objective indicators of environmental cleanliness of the environment and the content of heavy metals.

**Disciplinary:** Multidisciplinary (Biology, Ecology/Ecosystem, Environmental Sciences, Agricultural Science, Food Sciences (Nutrition and Toxicity)).

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## 1. INTRODUCTION

At present, in the age of technical progress, there have been violations in the ecology of the Earth. Human activity often adversely affects the environment. Man ceased to feel himself and the environment as a whole within the biosphere.

We live in the era of paradoxes when with the latest achievements of science and technology, we do not improve our living environment, and often pollute it because of insufficient education and culture, and the lack of objective control. Pollution disturbs ecology.

There is a relationship: bees, their environment and the man (the beekeeper). When normal living conditions, humans and vegetation, insects and animals develop normally under the influence of a system or combination of environmental factors. Under their influence, nature forms an organic whole. Ecological distress affects the health of humans and animals. Harmful substances from the environment, entering the body, accumulate in the tissues, aggravate cell metabolism and pollute the internal environment.

One views honey products as ecologically clean when during their production cycle adopted for various stages they correspond to established organoleptic, hygienic, technological and toxicological standards and do not adversely affect the health of men and animals and the environment (Lebedev, 2016).

Contamination of honey products with various substances is due to a multitude of interrelated processes occurring with varying intensity in the interfaced environments and components of the ecosystem.

The unique properties of honey products that have been tested by more than one generation and received a special significance in our age of environmental tension and high-stress loads contribute to the growing interest in them. Honey products are not just natural. They are able to change the functional state of the physiological systems of the human body in fairly low concentrations. By chemical composition, these products are, as a rule, a composition of biologically valuable compounds that are completely absorbed by the body.

Honey products have a wide range of biological properties and a positive physiological effect, synergistic to the human body. To reduce the negative impact of the environment on human health, it is necessary to improve the quality of nutrition and the use of ecologically pure honey products. This is one of the priority ways to solve this problem. And one of the main conditions for obtaining pure honey products is the search for environmentally friendly places of their production.

The degree of pollution by toxicants, as a rule, is determined by analyzing their content in the soil, water, and air, which is associated with significant labor and time costs, but the biotic component of ecosystems remains beyond researchers' attention. When monitoring ecosystems, biological methods that imply the use of honey bees, being common and very sensitive to environmental pollution, are very promising. In this connection, detailed studies of the properties of the indicators are necessary, which allow determining the extent to which bees and their products reflect the state of the ecosystem, as well as tracing the migration of heavy metals along trophic chains, where insects (bees, in particular) are an important link.

Monitoring (from Latin 'monitor' is the one who reminds, warns) implies monitoring of some phenomena, complex observation, and assessment of changes and the state of the biosphere or its individual elements (Efimenko, 2012).

Many researchers are working on the problem of obtaining ecologically pure honey products.

Unfortunately, in our country, there is no reliable and accessible information on the contamination of specific areas of agricultural land with these or those harmful technogenic substances (Burmistrova, 2008).

Environmental pollution with heavy metals has pronounced mutagenic and carcinogenic effects, causing poisoning (often fatal) and disruption of various physiological functions of the body. Therefore, studies of the migration of heavy metals in the biosphere, their movement along trophic chains as well as studies of the mechanisms of entry, accumulation, and utilization in biological objects are very important. Of particular importance are such studies in regions with a developed metallurgical and chemical industry, where the background level of heavy metals in the environment is elevated (Kharitonova, 2015).

Heavy metals belong to the group of substances potentially hazardous to human health. By hazard level chemical elements are divided into three classes:

- 1) highly hazardous substances - arsenic, cadmium, mercury, lead, zinc;
- 2) moderately hazardous substances - copper, molybdenum, chromium, tin;
- 3) low hazardous substances - tungsten, barium, strontium, manganese (John, 2009).

The most dangerous to the biosphere heavy metals include cadmium, nickel, mercury, lead, chromium, copper. Chronic exposure to low doses of toxic substances, like low levels of radioactivity, can cause impaired metabolic processes, immunological status, neurohumoral systems, hereditary properties, etc. (Yagin, 2013).

Lead, mercury, and cadmium, which accumulate in the soil, are absorbed by the roots of plants and along trophic chains enter animal and human organisms. To reduce the concentration of toxicants various preparations that have sorption, ion-exchange and biologically active properties are used. In an organized system of agrotechnical measures, liming and the application of organic fertilizers are used to reduce the mobility of heavy metals and their entry into the plant. Evaluation of the effectiveness of these and other recreational activities, as well as an assessment of the degree of increasing environmental pollution, requires a reliable monitoring system (Eskov, 2012).

Kolbina (2000) analyzed the dynamics of the movement of iron, copper, manganese, and zinc in the system “soil — honey plants — bees — honey, wax, propolis” on the territory of Udmurtia. It has been established that the concentration of heavy metals in plants may increase (zinc) or decrease (iron, manganese, copper) in relation to their content in the soil. The concentration of these elements in the body of bees was higher than in plant tissues. However, the level of heavy metals in honey and wax was significantly lower than in the tissues of bees and did not exceed the MAC, which allowed speaking about the filtering ability of bees. The studies helped to identify both the territories most contaminated with these metals and relatively clean and to conclude that bees and their products can be used in a comprehensive assessment of the environment as an additional information channel of the monitoring system (Kolbina, 2000).

The main sources of heavy metals in the soil are natural parent rock materials. But it should be taken into account that recently, technogenic human activity, which leads to drastic changes in the environment, through pollution with industrial waste (fluorine, sulfur, arsenic, lead, etc.) affects the content of heavy metals in the soil to a far greater degree (Puhalskiy, 2017).

With a high content of pollutants in the soil, they begin to accumulate in plants in excess and migrate along the food chain to the body of bees and, further, through honey products come to the

consumer, causing chronic poisoning and other serious diseases. It is unacceptable to have an apiary near highways and industrial facilities since there is a high probability of toxic elements entering the honey products. It is established that the remoteness of the apiary from the source of pollution has a significant impact on the level of pollution of honey products (Rusakova, 2006; Dubovik, 2011).

Most of the toxic substances that pollute the soil, accumulate in its upper 5-cm layer and then enter the plant. When passing through trophic chains, some substances dissipate, while others accumulate. Concentration and accumulation of toxic substances in this chain are characteristic mainly of radionuclides, heavy metals, and some pesticides that are resistant to decay. Therefore, relatively low concentrations of substances in the soil can become dangerous for humans when moving along food chains from plants to bees and then through honey products to their consumers (Murashova, 2016).

It has been established that the content of heavy metals in entomophilous plants may increase or decrease with respect to their content in the soil (Kovalchuk, 2014).

It is known that plants accumulate heavy metals not only from the soil but also from the air. That is why their concentration in plants may exceed or be lower than the content in the soil. Leaf vegetable absorbs especially much lead from the air, up to about 95 %. With increased soil pollution by lead (about 50 mg/kg), herbaceous honey plants accumulate about a tenth of this amount. And they actively absorb zinc. The amount of this element in them can be several times greater than its content in the soil. Herbaceous plants of natural biocenoses have the greatest ability to concentrate heavy metals. Morpho-physiological features of plants in natural lands have more favorable factors for the root entry of pollutants compared to agrocenoses. On arable cultivated lands, radionuclides and heavy metals are distributed over the arable horizon more evenly, and, consequently, reduce their entry into agricultural crops from 2-5 times. The introduction of potash fertilizer also reduces the amount of toxic elements in the soil. Accordingly, the content of heavy metals in fodder crops on arable land is much lower than in natural biocenoses (Eskov, 2001; Sokolskiy, 2012; Kharitonova, 2015).

It has been established that pollutants of the food consumed by bees accumulate mainly in their rectums (Eskov, 2012).

Bees are constantly affected by a variety of factors, especially when pollinating entomophilous plants and collecting nectar. Other important factors include humidity and ambient temperature, solar radiation, wind, the state of nectar and pollen sources (Fatkulkin, 2017).

A particular threat to the purity of products of bee colonies is caused by environmental pollution by road transport and industrial enterprises for various purposes. Bees infect themselves and produce polluted, hazardous products, collecting nectar and pollen from honey plants infected with toxic substances (Yadav, 2010).

The quality of bee products depends on a variety of conditions. It is extremely important to comply with the technological requirements during their production, storage, and transportation. The most important condition is the proper placement of the apiary taking into account the environment: field pollution with pesticides, taking into account the sanitary condition of apiaries, the health of bee colonies and the use of various preventive and therapeutic means (Osintseva, 2008).

Honey products obtained along highways are prone to lead contamination. Many authors have noted that lead is not transported by plants and can get into honey products, mainly from the atmosphere through direct contact with nectar and pollen. The most important sources of cadmium for honey products are the metallurgical industry and atmospheric pollution caused by burning

household and industrial wastes. Cadmium is also transported by plants from the soil and only a small part of cadmium can get into honey and other honey products from the atmosphere, this happens mainly if the apiary is located in the immediate vicinity of refuse incinerators (Jose, 2009; Nanti Bolan, 2014).

When certification and standardization of honey products problems that are associated with a variety of collection conditions and, as a consequence, their chemical composition often appear.

Among other constituent elements determining the safety and therapeutic value of such products, there may be heavy metals with a relative atomic mass of more than 40, or density of  $5 \text{ g/cm}^3$  according to the technical classification. This group includes toxic metals (cadmium, lead) and trace elements that have physiological and biochemical significance (zinc, copper). Research works in recent decades in the field of environmental monitoring have shown that honey products, like bees, are indicators of pollutant accumulation in biocenoses (Murashova, 2004; Lebedev & Murashova, 2016; Eskov, 2016).

The main argument in favor of using bees in bioecological monitoring is that bees actively collect nectar and pollen in a radius of about 3 km. However, there are certain limitations. Namely, bees form their food reserves only when, under favorable weather conditions, entomophilous plants secrete nectar and produce pollen. Most often this happens only on an apiary part. Honey plants are blooming for a short time, from several days to several weeks, and therefore the bee family does not have the opportunity to replenish feedstocks for most of the annual cycle. Thus, environmental monitoring with the help of bees will be fragmentary both in time and in space (Osintseva, 2015).

In this regard, the use of trophic motivation of bees focused on the replenishment of feedstocks is significantly limited by this fact (Dimou, 2007).

A lot of information indicates the concentration of heavy metals in the system “water - soil - plants - bees - honey products”, but data on their content in various honey products are contradictory (Rusakova, 2006).

All over the world, the requirements for environmental cleanliness of honey products are getting stringent. In our country, sanitary and hygienic requirements for food products (SanPiN 2.3.2. 1078-01) normalize the amount of lead, arsenic, and cadmium for natural honey, pollen and dietary supplements based on honey products, as well as the concentration of mercury for pollen and dietary supplements based on it (Kharitonova, 2015).

However, it should be noted that in this regulatory document there are no requirements for wax that are in direct contact with honey and other honey products. Specific features of honey products are not taken into account, and requirements for the content of copper and zinc are not limited (Efimenko, 2012).

Since they serve as components of the mineral composition of all honey products, it is very important to establish the dependence of the quantitative content of these elements on environmental conditions. In our country, environmental studies have started relatively recently, but it is already possible to talk about the feasibility of using bees in bioecological monitoring. The tissues of the organs of bees, honey, bee-bread, pollen and propolis, in which radioactive nuclides, pesticides, heavy metals, and other toxic substances are concentrated, provide information about environmental pollution (Dzhambulov, 2010).

Studies show that environmental pollution by toxic elements significantly affects the purity of

propolis and pollen, and in honey and wax, these substances do not accumulate. The highest content of copper and zinc in the body of the bee indicates the accumulation of these elements by them. The unique structure of the body of the bee, its physiological characteristics allow it to concentrate in its body a significant portion of heavy metals and other toxic elements. This is due to the special permeability of the walls of the honey sac. Together with the water that is absorbed by the hemolymph from the content of the honey sac through its walls, toxic substances also pass through. They accumulate partially in the fat body and partially in other structures of the bee's body. Some of them are removed by excretory organs (Ai-Rashdi, 2013).

Significantly increased levels of some toxic elements in bees, soil, plants, as compared to honey, wax and royal jelly indicate that bees produce environmentally friendly products due to their vital activity. According to statistics, the content of heavy metals in forest honey is higher than in floral honey (Suresh Kumar, 2015).

Together with nectar, honeydew, pollen, and water, they are brought into the hive, getting into honey, propolis, wax, and pollen (Fatkullin, 2017).

It is important that food products, including honey products, are environmentally friendly. Consequently, it is necessary to control the content of heavy metals in products of general consumption systematically and carefully. In addition, the certification of food products, raw materials, and honey products also provides for their safety parameters, for which they establish the maximum allowable concentration (MAC) of the content of toxic elements, radionuclides, and pesticides (Eskov, 2016).

Honey, as a natural product, has no equivalents in the number of ash elements. About forty macro- and microelements are found in it, however, their set and relative content are subject to considerable fluctuations depending on the origin of this product. Thus, the values for magnesium, lead, copper and some other elements differ 100-500 times, and for tin and zinc - 900-2,000 times. Honey contains relatively much potassium, calcium, sulfur, and magnesium. Copper, zinc, aluminum, cobalt, nickel, etc. have also been found in it. The amount and composition of minerals in honey depends on their geographical and botanical origin.

Foreign researchers argue that the content of heavy metals depends on their presence in nectar. The latter fact is, in turn, affected, among other reasons, by the type of flower (male or female), its age, location on the plant and the structure of nectaries. The amount of metals contained in the honey from various plants varies widely. However, in all cases, there are more metals in polyfloral honey than in monofloral ones.

Bulgarian researchers give some data on the content of heavy metals in the honey of various botanical origin (Table 1).

**Table 1.** The content of some chemical elements in various types of honey (mg/kg).

Type of honey	Number of samples	Tin M±m	Cadmium M±m	Copper M±m	Zinc M±m	Iron M±m
Acacia	41	0.07±0.03	0.007±0.002	0.14±0.04	0.84±0.28	11.5±5.1
Polyfloral	75	0.08±0.03	0.008±0.003	0.20±0.07	0.86±0.31	18.1±5.1
Honeydew	13	0.08±0.03	0.004±0.001	0.34±0.05	1.13±0.51	20.6±7.3

It can be seen from the table that tin and cadmium are close in values in different types of honey. Cadmium content in honeydew honey is lower than in other types of honey - acacia and polyfloral. According to the authors, these elements are background pollutants of honey and get into it,

regardless of the season and the type of honey plants. There is significantly more copper, zinc and iron in honeydew than in acacia and polyfloral honey (Burmistrova, 2008).

The results of the spectral analysis of samples of domestic honey for the presence of various elements in them indicate a wide range of chemical elements in the presented honey samples. Of the 16 elements studied, no samples have lead, tin, nickel, cobalt. Silver is present in only one of 10 samples, and potassium in two. The remaining elements are contained in honey, which indicates its multi-component mineral composition, as well as the nutritional properties of honey. Of heavy metals, copper is found in almost all samples and lead, as mentioned above, is absent. Arsenic and cadmium cannot be determined (Rusakova, 2006).

In addition to the natural content of metals in honey, their number is also influenced by grown honey plants. Deforestation, the expansion of areas under wind-pollinated crops, the chemicalization of agriculture, the increase in toxic emissions by industrial enterprises and radioactive contamination create prerequisites for substances in honey harmful to people's health.

A bee's nest gives a wide and unique range of environmental characteristics during its active state. The bee, collecting nectar, pollen from flowers and resinous substances within a radius of 3-5 km from the apiary, naturally transfers all substances that pollute the environment into the products of its vital activity: honey, pollen, bee bread, royal jelly, propolis, thus testifying local contaminated areas. In addition, the bee population itself, when metabolizing these products, can act as an integrating indicator of the state of the environment.

Currently, the mineral composition of bees, honey, and pollen collected by bees is considered as an indicator of the presence of some metals in this zone.

In recent years, many publications have appeared on the relationship between the bees, the products they produce and the traces of metals in their environment. It is noted that honey collected near large industrial enterprises and highways contains much more lead than that collected far from them (Rusakova, 2006).

Honey and bees from apiaries located near a zinc smelting plant in Poland were contaminated with zinc, cadmium, and lead. At the same time, lead was found in corpses of bees more often than its natural content by 5-7 times, zinc and cadmium by 3.5 times, and the honey contained less lead by 100 times, zinc by 5 and cadmium 10 times than the bees. This indicated the influence of the environment on bees and their products, as well as the fact that bees effectively delayed the entry of heavy metals into honey, even due to their own death.

In another paper, Polish researchers indicated that samples of honey obtained far from the highway showed a very low content of iron, zinc, magnesium, copper, nickel, lead, cadmium and cobalt. Similar data were obtained in the United States. The lead content in flowers and bees in the area of the busy highway was more than 20 times higher than 850 m from it. It is obvious that it is impossible to keep bees in cities and subsidiary farms located near large factories, highways, and airfields (Burmistrova, 2008).

Due to the fact that this condition is often violated, systematic careful control of honey and other honey products to determine heavy metals is necessary.

Hygienic requirements for the safety of food raw materials and food quality, approved in 1996, set standards for the content of only two toxic elements in honey: lead (no more than 1.0 mg/kg) and cadmium (no more than 0.05 mg/kg). These requirements are not documented for wax, although this

product is in direct contact with honey and is widely used in the perfumery and pharmaceutical industry (Dubovik, 2011).

Determining copper and zinc is not provided for by sanitary requirements, while these elements can penetrate into honey products from both the equipment they come into contact with and the environment (Rusakova, 2006).

Due to contradictory data on the effect of various polluting factors on the ecological purity and safety of honey products, this issue requires particularly careful study.

Taking into account the above, the aim of these investigations was to study the basic mechanisms and factors that determine the accumulation of toxic elements in the body of bees and some honey products in the conditions of the Ryazan region.

- to evaluate the dynamics of heavy metals movement along the trophic chain: soil-plant-body of a bee - honey products;

- to find out the main mechanism and the main factors that determine the accumulation of heavy metals by bees and some honey products.

## 2. MATERIALS AND METHODS

The main experimental part of the work was done in the apiaries of the Ryazan region, in laboratories of FSBRI “Federal Research Center for Beekeeping” and its basic enterprises during a two-year period.

The objects of research were samples of soil, plants, and honey products, such as honey, pollen, bee bread, wax, propolis and royal jelly, which were selected from different areas of the Ryazan region.

Samples of honey, pollen, royal jelly, wax, and propolis were collected in apiaries when preparing samples of soil, plants and bees.

The soil, plants, and bees were pre-dried and then mineralized. When preparing the samples of wax, the acid extraction method was used. In the case of propolis sampling, the dry ashing method was used. Samples of honey, pollen and royal jelly were prepared by dissolving using an ultrasonic homogenizer.

Honey samples were investigated by atomic absorption spectrometry to determine lead, cadmium, copper, zinc, mercury and arsenic in the laboratory (spectrophotometer Specter AA 220 FS of the company “Varian”) on the basis of FSBRI “Federal Research Center for Beekeeping”.

Experimental and control groups were formed by the method of selection of pairs of bee families-analogs of the Priokskiyinterbreed type of the Central Russian breed of bees.

Based on the obtained data, the results were analyzed. Then tables and graphs were made, which presented the information on the quantitative content of toxic substances in the analyzed samples of soil, plants, bees, honey, and other honey products.

## 3. RESULTS AND DISCUSSION

An analysis of the experimental data obtained over two years has shown that the concentration of heavy metals in the recorded bee plants is significantly reduced compared with their content in the soil. At the soil-plant stage, a decrease in copper concentration by 2.8 times, zinc by 1.6 times, cadmium by 1.3 times, and lead by more than 20 times has been found. Differences in all cases are highly reliable (Table 2).



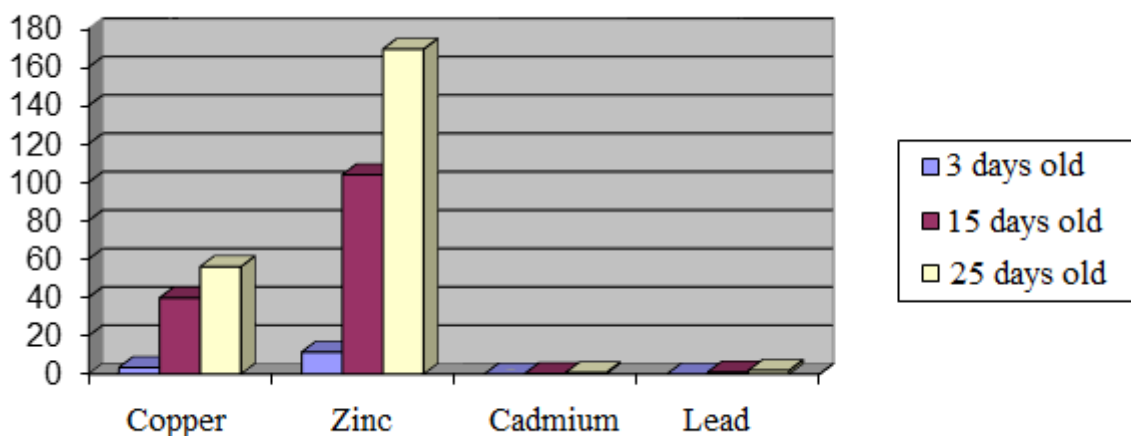
**Table 2.** The content of heavy metals in honey products, mg/kg

Object to be examined	Zinc	Copper	Lead	Cadmium	Arsenic	Mercury
Soil	29.5	12.2	9.81	2.40	0.18	-
Plants	18.75	4.33	0.45	1.91	-	-
Bee's body	49.1	15.0	0.47	0.19	-	-
Pollen	18.9	5.2	0.48	0.09	0.34	-
Propolis	59.1	5.0	2.26	0.78	-	-
Bee bread	14.6	4.3	0.25	0.07	-	-
Royal jelly	6.38	3.10	2.8	-	0.02	-
Wax	0.78	0.57	0.18	0.007	-	-
Honey	0.32	0.35	0.09	0.04	-	-

Based on the data in Table 2, it is possible to judge the dependence of the content of toxic elements in honey products on their presence in environmental objects. The most intensive migration from soil to plants was observed in zinc and copper.

When clarifying the mechanism of accumulation of heavy metals in the body of bees, three groups of 5 nucleus families each were formed, which were placed in separate sections of the ward. Young laying queens, derived from the same maternal family, were introduced into the formed families. The nucleus families were formed from an equal number of young bees (3-5 days old, obtained in wards and mixed together), that is, equalized in physiological state and origin.

In the course of investigations, it was found that the content of heavy metals in the body of bees increases quite naturally with an increase in their age (Figure 1), that is, they accumulate during the life of bees in their bodies. No lead, mercury, cadmium, and arsenic were found in the body of young (three-days old) bees, while the zinc and copper content was significantly lower than in plants (11.45 and 3.41 mg/kg, respectively).



**Figure 1.** The accumulation of heavy metals in the body of bees, depending on their age, mg / kg

A rather sharp increase in the content of heavy metals occurs in the body of bees in the first two weeks of life, that is when they perform a cycle of intra-hive work (mainly broods are grown and honeycombs are built). At this age, the zinc content is 9.1 and 11.6 times more than in the body of young bees. At the same age, lead and cadmium are found in the bees. The transition to the function of collecting nectar and pollen leads to an increase in the content of heavy metals in the body of bees, but

not so dramatically. Thus, the content of zinc and copper increases in bees at the age of 25 days, but only by 63 and 41 %, respectively, compared to the content of these elements in bees from the same families at the age of 15 days. At the same age, bees have the maximum content of lead and cadmium in their bodies (1.9 and 0.73 mg/kg, respectively).

Feeding bees for 20 days with sugar syrup did not lead to significant changes in the content of the studied metals in their bodies. All fluctuations were within the accuracy of the measurement of these parameters. Keeping bees on a protein-free diet for twenty days led to their almost complete death. During this period, they grew 14 times less brood than bees from families that had honey and pollen.

There was no significant increase in the content of heavy metals in the body of the bees, fed with pure honey for 20 days. The lifetime of bees in this group of nuclei was significantly higher than that of bees in sugar syrup, but at the same time, they grew brood 4.3 times less during this time than bees from families that ate both honey and pollen.

When young bees consumed a larger amount of protein feed (bee bread), there was a significant increase in heavy metals in their bodies. Thus, the zinc content for 20 days of feeding bees with bee bread increased by 12.3 times and that of copper by 14.2 times, compared with the amount of the same metals in bodies of 3-5-days old bees. Arsenic, cadmium and mercury were not found in bees, and the lead content increased from zero to 1.5 mg/kg for 20 days of feeding the bees with bee bread.

From the studied honey products: honey, wax, pollen, bee bread, propolis, and royal jelly, honey and wax contained the smallest amount of the heavy metals (Table 2).

Due to the fact that the requirements of SanPiN 2.3.2. 1078-01 do not have standards for the content of heavy metals for wax, we relied on their MAC in honey, when analyzing the data on the content of toxic elements in wax. All values of the content of elements for the studied period of time were within the normal range.

The content of elements in soil samples can be associated with both soil-forming rocks and the influence of anthropogenic factors. In this case, soil contamination with heavy metals, such as lead and zinc, can be triggered by being close to the apiary of the road and railway. Despite the fact that initially, the content of toxic elements in soil samples exceeded the MAC, in almost all honey products, except for propolis and pollen, the concentrations of heavy metals were within the normal range.

The maximum content of toxic elements was noted in the soil, propolis, pollen and the minimum one - in honey.

The content of zinc, copper, and lead in the honey samples was significantly less than in propolis, pollen, the body of bees, plants and soil. So, the zinc content in honey was less than in bees from the same families by almost 153 times, copper - by 42.9 times, cadmium - by 4.8 times and lead - by 5.2 times.

Significant differences in the level of heavy metals in such honey products as honey, pollen, and propolis are determined primarily by their biochemical composition, processes of formation and mechanisms of processing by bees.

Glucose, fructose, and sucrose predominate in the nectar of plants. For the secretion of nectar plants form carbohydrates, which are supplied to the nectaries and used, while absorbing carbon dioxide from the air, water from the soil, and also using solar energy. It should also be remembered that a portion of nectar collected by a bee is formed (secreted) for the very several hours.

It is the above that determines the minimum content of heavy metals in nectar as compared to

pollen, which is formed during a few days from completely different components (lipids, proteins, carbohydrates, etc.).

The content of heavy metals in the nectar selected from honey sacs was 2,500-3,000 times less than in the pollen pellet collected by bees from the same families.

Significant differences were found in the content of lead in the nectar from the honey sacs and the honey prepared from it. So the lead content in the nectar introduced by bees into the hive averaged  $0.69 \pm 0.033$  mg/kg, and its content in the honey from the same family was  $0.43 \pm 0.057$  mg/kg. The identified differences are significant ( $P = 0.99$ ). Apparently, these differences, determined by the fact that the bees processing nectar into honey, drain by the intermediate valve in the honey sac a significant amount of pollen grains from nectar, and the content of heavy metals in these pollen grains are much higher than in honey.

A significant reduction in the amount of heavy metals in mature honey is provided precisely by reducing the content of pollen grains in it.

For the two-year period of investigations according to the results of the analysis of the content of toxic elements in honey samples, there have been no cases of exceeding the MAC of toxic elements. In addition, no residual mercury has been detected in honey samples.

As a result of the analysis of propolis samples for the content of heavy metals in them, the MAC of lead is found to be 2.26 mg/kg at a rate of 1.00. The concentration of heavy metals in propolis may be the same as in soil and plants, and in some cases even higher. This is due to the fact that their entry into propolis comes from the soil in the process of extracting resinous substances by plants, which takes quite a long time. Along with this, they can accumulate volatile substances from the air.

Analysis of the results of the study on the content of heavy metals in royal jelly samples has shown that the samples revealed an excess of the allowable lead content by a factor of 1.8 mg/kg.

The remoteness of the family from the source of pollution has a strong influence on the level of pollution of honey products. To study this factor, we had a special experiment.

The pollen pellet collected by bees mainly from dandelions, growing in the area of the large busy highway of federal significance M5 "Ural" (Moscow-Chelyabinsk) was investigated. One experimental group of families was located 150 m from the highway, the other one was about 1,000 m from it. There were 5 families of analogs in each group. From selected samples of the pellet collected by bees from experimental families, the pollen was separated mechanically (by color) and analyzed for the lead content. This was the pollen from only one type of dandelion plant growing at a different distance from the highway.

It was found that the lead content in the pollen collected by bee families located 150 m from the highway averaged  $1.56 \pm 0.03$  mg/kg, and the pollen of the same plant species, but selected by bee families that were at a distance of about 1,000 m, had only  $0.03 \pm 0.0067$  mg/kg, which was 52 times less (the difference is highly reliable  $P > 0.999$ ). After 10 days of the experiment, samples of bees returning from the field with the pollen pellet were taken, and the lead content was determined.

In terms of the lead content in the body of bees from the experimental families, significant differences were established -  $3.22 \pm 0.092$  and  $0.21 \pm 0.017$  mg/kg, respectively (the differences were highly significant  $P > 0.999$ ). Thus, the lead content in the body of bees collecting pollen is highly significantly increased (accumulated), which can be clearly seen from the experimental data obtained.

Of all the honey products, honey is the most environmentally friendly product, and propolis, pollen, and bee bread are the most polluted ones. The investigations make possible to make such a conclusion.

It was established that the maximum purity of honey was determined by its biochemical composition (mainly carbohydrates, which are secreted by secretory cells of nectaries within a few hours) and by careful draining of pollen grains from nectar by an intermediate valve in a honey sac.

It was revealed that the main amount of heavy metals was accumulated in the body of bees due to intensive two-week consumption of pollen and bee bread contaminated hundreds of times more than nectar and honey, as well as when processing the nectar into honey while the pollen filtering and getting into the midgut.

It is necessary to select ecologically clean areas when receiving pollen and bee bread especially carefully. For the production of pollen and bee bread, it is impossible to place bee families closer than 1,000 m from major highways, as this will lead to an unacceptable level of lead content in these products.

The bee, pollen, and propolis can serve as objective indicators of environmental cleanliness and the content of heavy metals.

#### 4. CONCLUSION

It has been established that at the soil-plant stage, the concentration of copper decreases 2.8 times, zinc 1.6 times, cadmium 1.3 times, and lead more than 20 times. The differences in all cases were highly reliable.

It has been established that the content of heavy metals in the body of bees naturally increases with an increase in their age. No lead, mercury, cadmium, and arsenic have been found in the body of young (three-day) bees, while the zinc and copper content is significantly lower than in plants (11.45 and 3.41 mg/kg, respectively).

It has been revealed that the consumption of a greater amount of protein feed (bee bread) by young bees leads to a highly significant increase of heavy metals in their bodies. Bee bread nutrition for 20 days resulted in an increase in the zinc content by 12.3 and copper by 14.2 times as compared with the level of these metals in the body of 3-5 day bees. Arsenic, cadmium, and mercury have not been recorded in the body of the bees, and the lead content for 20 days of bee bread nutrition has increased from zero to 1.5 mg/kg.

The zinc content in honey is less than in bees from the same families by almost 153 times, copper by 42.9 times, cadmium by 4.8 times and lead by 5.2 times. Propolis and pollen contain the greatest amount of heavy metals from all honey products.

The maximum purity of honey is determined by its biochemical composition and by careful filtering of pollen grains from nectar through an intermediate valve in a honey sac. So the lead content in nectar is 1.6 times more than in honey from the same family.

The lead content of pollen collected by bee colonies 150 m from the highway is 52 times higher than that of the same plant species but selected by families about 1,000 m away. Differences in lead content in the bee body from experimental families are also significant - 15.3 times.

Thus, the use of bees and honey products as environmental indicators will improve standardization methods aimed at improving the quality of honey products. The obtained data can be taken as a guide when planning the placement of beekeeping in certain regions of the country, as well

as providing some objective evaluation of the environmental situation. Bees and their products such as pollen pellet and propolis can be recommended as objects for monitoring the cleanliness of the environment and the content of heavy metals in it.

#### 4. AVAILABILITY OF DATA AND MATERIAL

Data used or generated from this study is available upon request to the corresponding author.

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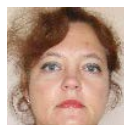
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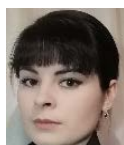
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