THE CONTENT OF HEAVY METALS IN THE BEES BODY DEPENDING ON LOCATION AREA OF HIVES

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Abstract

The aim of this paper was to determine residues of heavy metals such as Pb, Cd and Cu in the bees' body in different areas with different human impact. For this, have been selected 4 research areas (forest area, agricultural area, transport area and industrial area) where have been placed each 12 bees families. The forest area served as control where, practically, lacked sources of pollution. In the agricultural area the main sources of pollution are vehicles from nearby auto route, the former deposit of pesticides and agricultural machinery. The transport area is located at the periphery of the city and is, one, affected by smoke gases from road transport. Also, at the periphery of the city is located the industrial area with impact of road transport, and industry. In the period 2012-2013 in May and June, from each family have been collected by 50 worker bees. The quantitative analysis of studied metals in the bees' body had been performed using Atomic Absorption Spectrometry with thermal atomization (GFASS).

It was found, that the honey bee responds to environmental changes, by variation of Pb, Cd and Cu concentrations in her body in dependence on bee families' location. Among the studied metals, the highest concentrations in all research areas in the bees body record Cu, followed by Pb and Cd. The average concentrations of Pb in the bees body from the industrial and the transport areas have been significantly higher compared with those from forest area, respectively with 0.797 mg/kg or 402.5% (t_d = 7.73; P < 0.001) and 0.666 mg/kg or 336.4% (t_d = 8.12; P < 0.001). The average content of Cd in the bees body from industrial and transport areas, have been significantly higher compared with those from the forest area, respectively with 0.157 mg/kg or 413.2 % (t_d = 6.04; P < 0.001) and 0.089 mg or 234.2% (t_d = 8.09; P < 0.001). The average content of Cu in the bees body from the agricultural areas was significantly higher than those from forest area with 3.52 mg/kg or 81.8% (t_d = 10.66; P < 0.001). In all research areas, the heavy metals concentrations in the bees' body have been far away and much lower than the noxious dose for bees.

Key words: bees body, heavy metals, lead, cadmium, copper

INTRODUCTION

Honey bee *(Apis mellifera* L.) has a enormous significance, both to maintaining balance of natural and atrophic ecosystems, and for his economic and social role expressed by directed benefits of pollination crop and valorization of offered bee products (pollen, honey, propolis, wax).

These insects, participate to pollination of 250,000 species of plants, ensure pollination of about 150 of agricultural crops and increase their productivity by about 30%. Of the total pollinating insects, the honey bees represent 80%. Indirectly, many animal species lives on plants pollinated by bees [12].

Along the years, numerous personalities, have been evoked the importance of bees and beekeeping. Thus, Albert Einstein said that "If the bee disappeared off the surface of the globe, then man would have only four years of life left. No more bees, no more pollination, no more plants, no more animals, no more man."

A bee family count to 60 thousand worker bees, of which up to 30 thousand searches territory around the hive. In picking a flight bee visits from 50 to 100 flowers. The productive flight of honeybee is of 3 km from the apiary; in searching of food it can cover an area of 12 km², thus, giving us an overall picture of the state of location and environmental conditions from the collection area. By this, the bees are continuously

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exposed at the contaminants present in the area around the apiary, during their foraging activity.

Pollutants such as heavy metals are emitted in air, water and soil by anthropogenic polluting sources and natural phenomena geological-climatic, being accumulated, subsequently, by flora and fauna of those ecosystems. However, some heavy metals from environmental components, containing below the maximum allowable concentrations, have a beneficial role of catalyst and constant maintenance of multiple physiological processes of plants, animals and man.

In this context, monitoring of heavy metals concentration in biocenosis components represents a major issue, because it allows assessment environmental quality.

More than 20 years, bees are used as indicators of pollution of ecosystems. After some information [17] special bees are recognized, by most authors, as indicators of environmental pollution with heavy metals. During active movement, the heavy metals enter in the bees' body through the air spray and are absorbed by both through the surface of the porous body, and breathing [1]. After Скребнева Л. et al. (2012) [17], most objectively, reflects the level of pollution area, in particular, working bees flying in summer time, due to their active contact with the atmosphere and environment components. There are a number of works [12, 10, 6] demonstrates the accumulation of heavy metals in the bees fat and rectum. The heavy metals presents in the atmosphere can be stored on the bees' body brushes, in pollen or can be absorb with nectar, mildew or water. The norms regarding the maximum permissible concentrations of heavy metals (Pb, Cd, Cu) in the bees' body are not established.

However, it is clear, that excessive levels of Pb (12-50 mg per head/bee), Cd (6-30 mg per head/bee) and Cu (50 to 250 mg per head/bee) are important reasons of regression and even the disappearance of species *Apis mellifera* (Enczuk W., et al, 2002) [2].

The content of heavy metals in the bees body depending on a large number of factors: the extent and the location of the apiary, type of soil and nectar plant from area, the ecological status of the area, the methodology of increasing bee families (including food stimulation supplements), working bees age, physiological status and health of bee colonies etc. [12, 10, 4, 7].

MATERIAL AND METHODS

The research was conducted in 4 areas in the centre of the country, with different anthropogenic impact (forest area, agricultural area, transport area and industrial area), where have been placed each 12 bees families. As a control zone has been taken forest area, where is situated experimental apiary of Institute of Zoology (Forest Sector no. 21, No. 9 Canton. Ghidighici town, Chisinau municipality). In radius of 3 km (bee productive flight) from the apiary there is not source of pollution, the forest land from this area constituted - 32.2%, where melliferous plants, linden and acacia are predominant, also, various herbaceous forest plants. The agricultural area is located at the edge of the Braviceni village (Orhei district). The potential sources of pollution in this area are auto vehicles on the route Chisinau - Balti, former pesticide deposit which was evacuated by 2.5 decades ago, agricultural machinery etc. In this area, arable lands prevails with - 62.8%, followed by pastures and meadows with - 22.2%. The transport area is located at the periphery of Chisinau city, (road Balkans). The potential sources of pollution in this area are: heavy traffic with over 1200 cars in one hour, 12 fuel supply stations, car market, asphalt factory "Edilitate", auto industry, pollution coming from city etc. In mentioned area prevails urban land with - 45.3% followed by forest lands with - 24.9% (parks). The industrial area, is also situated at the periphery of Chisinau city (Industriala Street 14/2), meeting the most polluting sources. Potential polluters in this area are: road transport, over 20 fuelling stations, exhaust smoke coming from the city, "Glass factory", Factory of chemicals household products "Agurdino", the brick factory "Macon" etc. This area is dominated by urban land with - 63.1% and forest land (smaller parks) constitutes only - 13.9%. From each families throughout 2012-2013 years (May and June), have been collected each 50 worker bees. Bee samples

were taken from the front of the hive, and stored in the plastic perforated containers, according to sanitary norms [3] and transported to the laboratory for analysis. Sample preparation have been consisted in dry calcinations and transfer gray matter in solution by extraction with acid. For determination of Pb, Cd and Cu metals in the bees body from the ash solution have been used the atomic absorption spectrometry of the elements in graphite atomizer (AAnalist 800, f. Perkin Elmer 2001) [8, 9]. The method is based on measuring the wavelength of resonance absorption of the element atomic vapor, generated as a result of the electro thermal atomization of the sample in graphite furnace.

The data obtained in experiences have been processed statistically using the "Statistics 7". It had been calculated arithmetic mean (M), average error (m) and standard deviation (σ). The research results obtained have been statistic - biometric analyzed after methods by Плохинский Н.А., 1969 [16]. Also, it had been calculated certainty coefficient (t_d) of obtained differences between of research areas after formula:

$$t_d = \frac{d}{\sqrt{m1^2 + m2^2}}$$

where: $d = M_1 - M_2$; (the difference between arithmetic averages); m_1 - average error M_1; m_2 - average error M_2.

After which has been established Pearson (P) certainty threshold by Student

RESULTS AND DISCUSSION

The content of Pb in the bees' body. Following the analysis carried out in the year 2012, we observe that the concentration of Pb in the bees body varies depending on the research area (Table 1).

Table 1 The Pb content in the bees body, mg/kg

Experimental areas	N	Average concentration, M ± m	Min - max	Difference from forest area td		t_d	P
				d	%		
year 2012							
Forest area	12	0.198 ± 0.020	0.093 - 0.331	-	-	-	-
Agricultural area	12	0.305 ± 0.025	0.192 - 0.420	0.107	54.0	3.38	< 0.01
Transport area	12	0.864 ± 0.080	0.418 - 1.250	0.666	336.4	8.12	< 0.001
Industrial area	12	0.995 ± 0.100	0.553 - 1.580	0.797	402.5	7.73	< 0.001
year 2013							
Forest area	12	0.142 ± 0.016	0.085 - 0.283	-	-	-	-
Agricultural area	12	0.215 ± 0.022	0.108 - 0.350	0.073	51.4	2.68	< 0.01
Transport area	12	0.809 ± 0.072	0.511 - 1.22	0.667	469.7	9.14	< 0.001
Noxious dose for bees*	-	109	-	-	-	-	-

Remark: * The noxious dose for bees by Enczuk W., et al., 2002 [2]

For example, the lowest concentrations of Pb in the bees body have been found in the forest area with values of 0.198 ± 0.020 mg/kg, followed by agricultural area with 0.305 ± 0.025 mg/kg which is higher with 0.107 mg/kg or 54.0 % (t_d = 3.8; P < 0.01) than forest area. The Pb concentrations in the bees body from the transport and the industrial areas are significantly higher than those from the forest area, respectively with 0.666 and 0.797 mg/kg or 336.4 and 402.5 % (t_d = 8.12 and 7.73; P < 0.001).

In the year 2013, in all research areas have been detected lower concentrations of Pb in the bees' body, unlike the year 2012. In all study areas the Pb concentration in the bees' body has ranged from minimum of 0.085 mg/kg (forest area) to maximum of 1.22 mg/kg (transport area). The lowest average of Pb concentrations in the bees body have been detected in the forest area with values of 0.142 ± 0.016 mg/kg and the highest concentrations in the transport area with value of 0.809 ± 0.072 mg/kg, the last has been with 0.667 mg/kg or 469.7% higher than the control (t_d = 9.14; P < 0.001). The Pb concentration in the bees body from agricultural area occupy intermediate levels

between the forest and the transport areas and being higher that the forest area with 0.073 mg/kg or 51.4 % (t_d = 2.68; P < 0.001).

Researchers from many countries [11, 13, 14, 17] have been studied the Pb accumulation in the bees body in different areas, with different human impact. Similar results have been recorded by Russian researcher, Ефименко А. (2012) in Krasnodar Region [13]. In his studies the Pb concentration in the bees body vary from 0.36 ± 0.02 mg/kg in polluted areas to 1.00 ± 0.02 mg/kg in unpolluted areas.

Following the analysis we have found, that high levels of Pb in the bees body from transport and industrial areas, can inform to some measure about the environment quality (soil, air, water) in the research areas, which is influenced by: heavy transport, being the main source of Pb emitting and another potential source being the industrial pollution, as are factory household chemicals production, glass factory, smoke gases coming from the city, also, transboundary pollution.

The content of Cd in the bees' body. In both research years (2012 - 2013), has been detected the presence of cadmium in the bees body. The lowest concentration of Cd has been recorded in the bees body collected from the forest area, and the highest concentration of this heavy metal has been found in the bees body collected from industrial and transport areas (Table 2).

Table 2 The Cd content in the bees body, mg/kg

Experimental areas	N	Average concentration, M ± m	Min - max	Difference from forest area		t_d	P
				d	%		
year 2012							
Forest area	12	0.038 ± 0.005	0.011 - 0.071	-	-	-	
Agricultural area	12	0.070 ± 0.010	0.027 - 0.128	0.032	84.2	2.71	< 0.05
Transport area	12	0.127 ± 0.010	0.084 - 0.213	0.089	234.2	8.09	< 0.001
Industrial area	12	0.195 ± 0.026	0.084 - 0.375	0.157	413.2	6.04	< 0.001
year 2013							
Forest area	12	0.029 ± 0.002	0.017 - 0.038	-	-	-	-
Agricultural area	12	0.051 ± 0.009	0.010 - 0.105	0.022	75.8	2.39	< 0.01
Transport area	12	0.119 ± 0.008	0.068 - 0.181	0.090	310.3	10.92	< 0.001
Noxious dose for bees	-	55	-	-	-	-	

Thus, in the research year 2012, Cd concentration in the bees body from the past two areas exceeded the level in the bees body collected from the forest area, respectively, with 0.157 and 0.089 mg/kg or 413.2 % and 234.2 % (t_d = 6.04 and 8.09; P < 0.001). The Cd concentrations in the bees body collected from agricultural areas, also, exceeded significantly the level of this metal in the bees body from the forest area with 0.032 mg/kg or 84.2 % (t_d = 2.71; P < 0.05).

A similar situation, has been found in the year 2013, in which the Cd concentration in the bees body from the transport area was significantly higher, compared, with those collected from the forest and agricultural areas, respectively, with 0.090 and 0.068 mg/kg or 310.3 and 133.3 % (t_d = 10.92 and 5.67; P < 0.001) registering concentration from minimum of 0.017 mg/kg to maximum of 0.181 mg/kg. In the bees body from the forest area have been detected Cd concentrations at the level of 0.029 ± 0.002 mg/kg, with a difference of 0.022 mg/kg or 75.8% lower than those from the agricultural area (t_d = 2.39; P < 0.01). Such researches have been conducted by many researchers [15, 17, 14, 10]. For example, Скребнева Л., (2012) [17], in his research on Cd accumulation in the bees body from different regions, have been detected concentrations of 0.12 mg/kg in the bees body from unpolluted area and 0.23 mg/kg in those from the polluted area.

The presence of more increased of Pb and Cd concentrations in the bees body collected from transport and industrial areas due to heavy traffic in these areas, vulcanizing, industry, also, transboundary pollution.

The content of Cu in the bees' body. In all research areas, both in the years 2012 and 2013, has been found the presence of copper in the bees body varying from minim - 2.81 mg/kg (forest area) to maximum - 9.11 mg/kg (agricultural area) (Table 3).

Table 3 The Cu content in the bees body, mg/kg

Experimental areas	N	Average concentration, M ± m	Min - max	Difference from forest area		t_d	P
				d	%		
year 2012							
Forest area	12	4.30 ± 0.14	3.79 - 5.12	-	-	-	-
Agricultural area	12	7.82 ± 0.30	5.89 - 9.11	3.52	81.8	10.66	< 0.001
Transport area	12	5.51 ± 0.23	4.02 - 6.96	1.21	28.1	4.53	< 0.001
Industrial area	12	6.67 ± 0.45	4.04 - 9.05	2.37	55.1	5.03	< 0.001
year 2013							
Forest area	12	3.80 ± 0.16	2.81 - 4.94	-	-	-	-
Agricultural area	12	6.70 ± 0.31	4.82 - 8.09	2.90	76.3	8.31	< 0.001
Transport area	12	4.94 ± 0.33	3.12 - 6.29	1.14	30.0	3.10	< 0.001
Noxious dose for bees		455	-	-	-	-	-

In the year 2012 of study, comparing areas of research, we have been found that the highest concentration of Cu in the bees' body had been recorded in the agricultural area. The concentration level of this metal in the bees body from agricultural area was higher than those from the forest area with 3.52 mg/kg or 81.8 % (t_d = 10.66; P < 0.001).

This is explained by the use by the farmers of agrochemicals products containing copper to treat crops. A certain difference of the Cu concentration in the bees body than the forest area, we have been found also, in the industrial area (2.37 mg/kg) and transport area (1.21 mg/kg) (**t_d** = 5.03 and 4.53; P < 0.001).

Similar results have been obtained in the year 2013 of study. For example, results of laboratory tests analysis have been showed that Cu concentrations in the bees body from agricultural and transport areas were more higher than those from forest area, respectively 2.90 and 1.14 mg/kg or 76.3 and 30.0% (**t_d** = 8.31 and 3.10; P < 0.001). The accumulation of copper in the bees' body has been studied by many researchers (Table 4). For example, the Russian researcher Назарова Н. П. (2005) [15], collecting the bees samples from different regions, detected average of Cu concentrations in the range of 5.17 ± 0.53 mg/kg, to 24.93 ± 0.35 mg/kg.

The heavy metal Cu, is also a trace mineral necessary in certain amounts for macro micro-organisms life, thus, the Cu accumulating in the bees body from researcher areas, is a natural phenomenon necessary nutrition and vital activity of bees.

More obvious, the heavy metal content in the bees body (year 2012), can be viewed in figure 1.

The researches from different regions (Table 4), regarding heavy metal content (Pb, Cd and Cu) in the bees' body, demonstrate that its concentration depends on the location of the research area. For example, the content of Pb in the bees body varies of maximum 14.70 mg/kg (polluted area), to minimum 0.2 $\mu g/kg^{-1}$ (unpolluted area), the content of Cd varies from 1.85 mg/kg (polluted area), until 0.01 $\mu g/kg^{-1}$(unpolluted area), and the concentration of Cu in the bees body contains values between 24.93 mg/kg (polluted area) and 9.0 $\mu g/kg^{-1}$(unpolluted area).

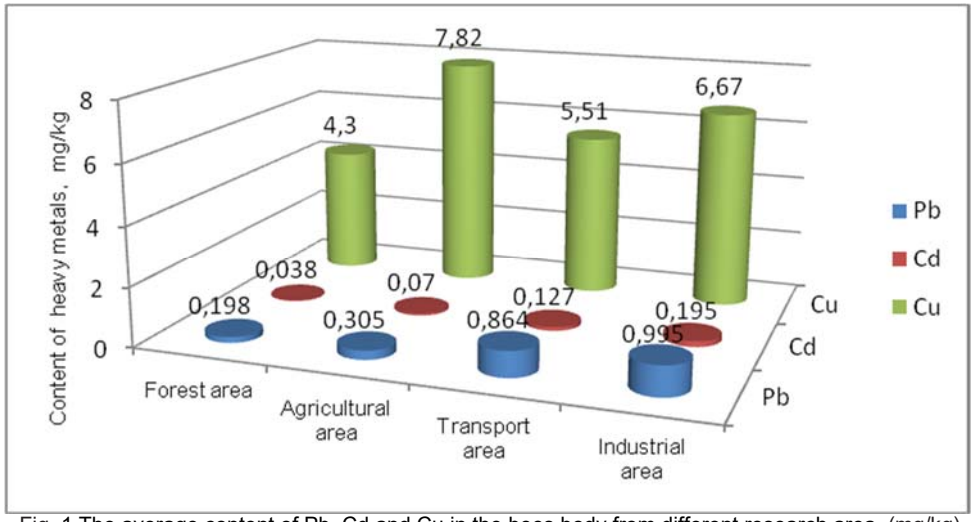

Fig. 1 The average content of Pb, Cd and Cu in the bees body from different research area, (mg/kg)

Table 4 Content of Pb, Cd and Cu in the bees' body, after different authors

Element / Type of area	Concentration	Research area	Referrers
Lead Unpolluted areas Polluted areas	0.59 1.29 (mg/kg)	Республика Татарстан	Скребнева Л. и другие, 2012 [17]
	0.2- 2.4 0.3 – 15.5 (ig/kg^{-1})	Zagora region	Zhelyazcova I.,Gurgulova K., 2004 [10]
	0.36±0.02 1.00±0.02 (mg/kg)	Краснодарский Край	Ефименко А.,2012 [13]
	1.15 - (mg/kg)	Приморский Край	Кодесь Л., Бычкова Н., *2010* [14]
	0.439-2.744 (mg/kg)	Italy	Porrini C. et al, 2002 [5]
	4.94±0.54 14.70±1.21 (mg/kg)	Республика Татарстан	Назарова Н. П., 2005 [15]
Cadmium Unpolluted areas Polluted areas	0.12 0.23 (mg/kg)	Республика Татарстан	Скребнева Л. и другие, 2012 [17]
	0.06 - (mg/kg)	Приморский Край	Кодесь Л., Бычкова Н., *2010* [14]
	0.01 – 0.3 0.03 – 6.2 (ig/kg^{-1})	Zagora region	Zhelyazcova I.,Gurgulova K. 2004 [10]
	0.50±0.17 1.85±0.03 (mg/kg)	Республика Татарстан	Назарова Н. П., 2005 [15]
Copper Unpolluted areas Polluted areas	9 – 21 13 – 27 (ig/kg^{-1})	Zagora region	Zhelyazcova I., Gurgulova K. 2004 [10]
	2.40±1.40 24.93±0.35 (mg/kg)	Республика Татарстан	Назарова Н. П., 2005 [15]

Our researches, as well, and researches of other authors, regarding accumulation of heavy metals (Pb, Cd and Cu) in the bees body, demonstrates that, the Cd has been recorded the lowest concentrations and the Cu has been recorded the highest concentrations. An important factor regards the accumulation, or increasing of the heavy metal concentration in the bees body is nutrition of young honey bee with pollen (up to 19 days), after, the bees begin a period more active by participating in gathering pollen, nectar, propolis, etc. After Еськов Е.К (2006) [12], a significant amount of heavy metals that is in the nectar is eliminated

by the transformation in the honey which influences their concentration in the bees body.

Generalizing research results, we can conclude that concentrations of heavy metals (Pb, Cd and Cu) in the bees body in all research areas are far away and much lower, compared with the noxious dose for bees established by Enczuk W. et al. (2002) [2].

CONCLUSIONS

1. The honey bee responds to environmental changes, by variation of Pb, Cd and Cu concentrations in her body in dependence on bee families' location.

2. Among the studied metals, the highest concentrations in all research areas in the bees' body record Cu, followed by Pb and Cd.

3. The average concentrations of Pb in the bees body from industrial and transport areas have been significantly higher compared with those from forest area, respectively with 0.797 mg/kg or 402.5% (t_d = 7.73; P < 0.001) and 0.666 mg or 336.4% (t_d = 8.12; P < 0.001).

4. The average content of Cd in the bees body from industrial and transport area have been significantly higher compared with those from the forest area, respectively with 0.157 mg/kg or 413.2 % (t_d = 6.04; P < 0.001) and 0.089 mg/kg or 234.2% (t_d = 8.09; P < 0.001).

5. The average content of Cu in the bees body from the agricultural areas was significantly higher than those from the forest area with 3.52 mg/kg or 81.8% (t_d = 10.66; P< 0.001).

6. In all research areas, the heavy metals concentrations in the bees' body have been far away and much lower than the noxious dose for bees established by Enczuk W., et al. (2002).

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