CORELATION BETWEEN THE CONTENT OF HEAVY METALS (Pb, Cd and Cu) IN THE BEE PRODUCTS AND THE BEES BODY

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Abstract

The aim of this paper is to determine the correlation between the content of heavy metals (Pb, Cd, Cu) in the bees body and bee products. The study was conducted by placing 12 hives with bees' families in four research areas (the forest area, the agricultural area, the transport area and industrial area) with different human impact. 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 and is one affected by smoke gases from road transport undertaking of producing asphalt automobile market, vulcanization etc. At the periphery of the city is located the industrial area with impact of road transport, glass factory, factory, household chemical products, thermal and electrical station and other small enterprises. From every experimental family were taken bee pollen, honey and propolis samples, according to current regulations and transported to the laboratory for analysis. The content of heavy metals in the samples was determined using Atomic Absorption Spectrometry with thermal atomization (GFASS).

Research results have shown that, between the concentration of heavy metals in pollen, honey bees and their content in the bee body exist a quite close positive correlative link. The highest values of correlation coefficients have been recorded between Cd content in pollen and bees (r_{xy} = 0.77; P < 0.001), between Pb content in pollen and bees (r_{xy} = 0.77; P < 0.001), pollen and honey (r_{xy} = 0.71; P < 0.001), honey and bees (r_{xy} = 0.68; P < 0.01) and between Cu content in pollen and bees (r_{xy} = 0.71; P < 0.001), pollen and honey (r_{xy} = 0.67; P < 0.01), honey and bees (r_{xy} = 0.62; P < 0.01). Someone correlative links between the concentration of heavy metals in propolis and other apiarian products (pollen, honey) and bees body, have not been identified.

Key words: bees, pollen, honey, propolis, heavy metals, correlation

INTRODUCTION

Excessive growth of air emissions from transport, industries, agriculture, etc., negatively influence the environment. The main sources of environmental components pollution in the Republic of Moldova are emissions from transport, industry and transboundary pollution [6, 12]. A first result of these human activities is increasing the content of this pollutants in soil, water, plants and other components of the environment. Also, being evacuated in the environment, pollutants not only remain at the its evacuation zone, but with the air masses can be transported at the long distances. Heavy metals (MG) such as: Cd, Pb, Hg, Co, Cr, Cu, Ni and Zn with negative impact on environment, are emitted into the atmosphere as dust, and to high temperatures at the form of gases as a result of combustion and from different industrial processes [4].

Almost all components of the environment (soil, water, air, vegetation) are visited by the honey bee, as a result in a wide range of products (pollen, nectar, propolis, water) are brought into the hive and, with these, and pollutants. Some of these pollutants accumulate in the bee body (due vital activity) and, mostly, in bee products (honey, pollen, propolis). So, honey bees absorb pollutants directly from water and air and indirectly through pollen and nectar collected from flowers [7].

There are multiple research on heavy metals content in the bees body and their products [1, 7, 3, 2], instead, information on

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their correlation in the bees body and their products are not sufficient. In Europe there are very few studies [9] describe the correlations between the content of heavy metals in the bees body and bee products. In the Republic of Moldova, the researches regarding heavy metals content in the bees body and bee products have been conducted sporadically by some researchers [5], without an analysis of correlations of these concentrations in bee components.

In this context, the aim of present research, it was to determine the content of heavy metals such as Pb, Cd and Cu in bee products and bees body and elucidating the correlations between the mentioned elements in the bees body and bee products.

MATERIALS AND METHODS

The research was conducted in 4 areas from the centre of the country, with different anthropogenic impact: forest area, agricultural area, transport area and industrial area. 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 no source of pollution, the forest land from this area constituted - 32.2%, where meliferous 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, 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%.

In each of these areas have been placed 12 experimental bees families, from which, during 2 years of research 2012 - 2013 during the months of May-June, have been collected samples of bees, pollen, honey and propolis. Collection of samples was carried out under the applicable rules. From the entrance of the hive have been taken at 50 worker bees, placed in sterile plastic containers and transported to the laboratory. Also, have been taken, each 10 g of fresh pollen, honey and propolis, stored in sterile containers 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, according to the standards ГОST R 52097 - 2003 [13], ГОСТ 30178-96 [14], ГОСТ 26929-94 [15]. After carbonization mineralized samples have been oven, gradually increasing the temperature up to 450ºC, to yield a gray ash. Then occurred the extraction and separation of heavy metals from the ash by boiling with nitric acid and hydrochloric acid. Determination of heavy metals in apiarian components was performed by analysis of the ash solution using the atomic absorption spectrometry of the elements in graphite atomizer [10, 11] (AAnalist 800, f. Perkin Elmer 2001). 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.

Using the "Statistics 7" we have been determined the correlation coefficients (r_{xy}) which demonstrated traceability of heavy metals in the food chain between heavy metal content in the apiarian components and bee body by Pearson. To calculate the certainty of the obtained results, has been calculated

criterion of certainty (t_r) of the correlation coefficient after formula:

$t_r = \frac{rxy}{mr}$; where: $m_r = \sqrt{\frac{1-r2}{N-2}}$ (the error of correlation coefficient).

The correlation coefficient value was compared with Pearson (P) certainty threshold by Student: the threshold 0 (zero) - P < 0,1; the threshold 1 - P < 0,05; the threshold 2 - P > 0,01; the threshold 3 - P > 0,001 according methods by Плохинский H.A., 1969 [16].

RESULTS AND DISCUSSION

The research results demonstrate, that the concentration of heavy metals in bee products is in different correlative relation with its concentration in bee body. The heavy metals content correlates, also, between different apiarian products (Table 1).

Table 1 The correlation coefficient of Pb concentration in the main apiarian products and bees body

Corelative components	Research areas							
	Forest area		Agricultural area		Transport area		Industrial area	
	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r
year 2012								
Pollen - bees	0.32±0.29	1.10	0.59±0.26	2.27*	0.40±0.28	1.43	0.66±0.24	2.75**
Pollen - honey	0.68±0.23	2.95**	0.65±0.24	2.71**	0.71±0.22	3.23***	0.51±0.27	1.89
Honey - bees	0.22±0.30	0.73	0.50±0.27	1.85	0.45±0.28	1.61	0.53±0.26	2.04*
Pollen-propolis	-0.09±0.31	0.29	-0.01±0.32	0.03	0.16±0.31	0.52	0.23±0.31	0.74
Propolis- bees	0.07±0.32	0.22	-0.45±0.28	1.61	0.09±0.31	0.29	-0.00±0.31	0
Honey- propolis	-0.10±0.31	0.32	0.15±0.31	0.48	0.09±0.31	0.29	0.17±0.26	0.65
year 2013								
Pollen - bee	0.59±0.26	2.27*	0.77±0.20	3.85***	0.72±0.22	3.27**	-	-
Pollen - miere	0.68±0.23	2.95**	0.57±0.26	2.19*	0.63±0.24	2.65*	-	-
Honey - bees	0.47±0.27	1.74	0.44±0.28	1.57	0.68±0.23	2.95**	-	-
Pollen - propolis	-0.01±0.32	0.03	0.28±0.30	0.93	0.44±0.28	1.57	-	-
Propolis - bees	0.06±0.32	0.18	0.14±0.31	0.45	0.11±0.31	0.35	-	-
Honey - propolis	-0.36±0.29	1.24	-0.01±0.32	0.03	0.01±0.32	0.32	-	-

Remark: *P < 0.05; **P < 0.01; ***P < 0.001

Thus, between content of Pb in pollen and its concentration in bees body has been found a positive correlation.

Both in the 2012, and in the 2013 years of study, the most essential correlations, quite high of this metal in pollen and bees have been established in agricultural area (0.77 ± 0.20 r_{xy} = mg/kg; tr = 3.85; P <0.001) and in the transport area (r_{xy} = 0.72 ± 0.22 mg/kg; t_r = 3.27; P <0.001).

The significance of correlation coefficients of Pb concentration in pollen and bees in research area is the highest threshold of certainty by Student. In industrial zone was, also, registered a high correlation of Pb concentration between pollen - bee (r_{xy} = 0.66 ± 0.24 mg/kg; t_r = 2.75; P < 0.01) with 2 threshold certainty, and in the forest and agricultural areas have been established a *correlation* at the *medium level* (r_{xy} = 0.59 ± 0.26 mg/kg; t_r = 2.27; P< 0.05). The existence in all areas of significant correlations between Pb content in pollen and bees, is the fact, that bees direct contact with pollen grains from flowers by searching and transporting this, also, being the main protein source for their food.

Between Pb concentration in pollen and honey have been found, also, *quite high correlations,* in both 2012 and 2013, in all studied areas. The highest correlation coefficient of Pb content in these components has been registered in the year 2012, in the transport area (r_{xy} = 0.71 ± 0.22 mg/kg; t_r = 3.23; P < 0.001), which corresponds to the highest threshold of certainty. *High correlations* of this metal concentrations in pollen and honey have been also obtained in forest (r_{xy} = 0.68 ± 0.23 mg/kg; t_r = 2.95; P < 0.01) and agricultural areas (r_{xy} = 0.65 ± 0.24 mg/kg; t_r = 2.71; P < 0.01), which correspond to two threshold of certainty by Student. A medium correlation of Pb content

in pollen and honey was recorded in the industrial area.

Also, *very high correlations* between Pb concentration in pollen and honey have been found in forest (r_{xy}=0.68 ± 0.23; t_r = 2.95; P <0.01) and transport areas (r_{xy} = 0.63 ± 0.24; t_r=2.65; P <0.01), in 2013 year. In agricultural area, in the same year, has been recorded a *medium correlation* between content of Pb in pollen and honey (r_{xy} = 0.57 ± 0.26; t_r = 2.19; P <0.05). These data being significant with one threshold of certainty by Student. This is explained by the fact, that pollen gathered by bees and stored in the cells, has been a direct connection with the nectar of flowers, of which after the transformation process, it is made honey itself.

Also, a significant *positive correlation* has been established between concentrations of Pb in honey and bees. For example, in the 2012 year the correlation coefficient of these concentrations in the industrial area has been average value (r_{xy} =0.53 ± 0.26; t_r = 2.04; P < 0.05). In the 2013, such correlation have been established in the transport area, the value of this coefficient being rather high (r_{xy} = 0.68 ± 0.23; t_r = 2.95; P < 0.01). In the other areas (forest and agricultural), has been found a obvious tend of *medium correlation* of Pb concentration in these two components (r_{xy} = 0.47 ± 0.27 and 0.44 ± 0.28).

The correlation between Pb concentration in the different apiaries products can be viewed in Figure 1.

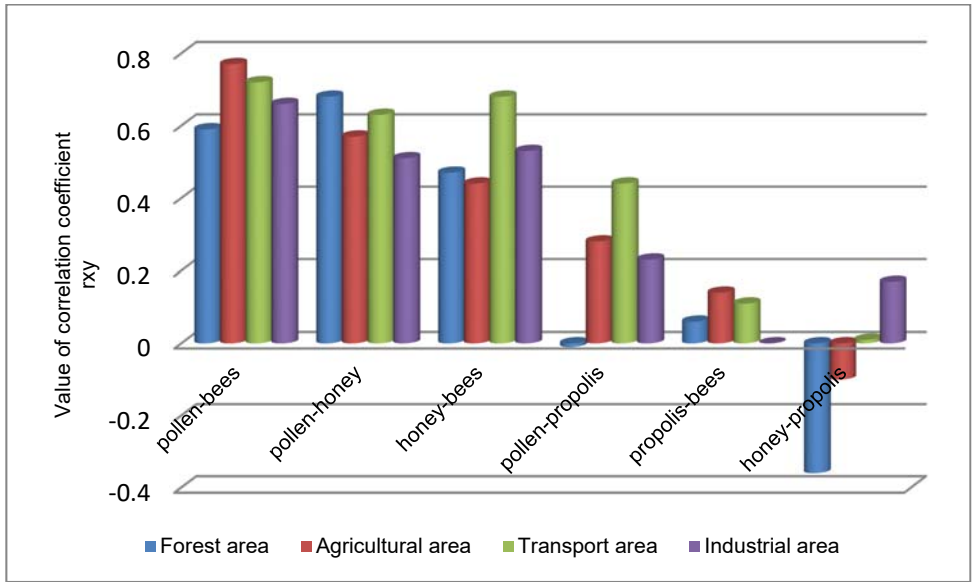

Fig. 1 Correlation between Pb content in the bees body, pollen, honey and propolis

In all study areas, in both research years, the Pb concentration of propolis - pollen and propolis - honey relationship, has been found a *very low correlations* or even complete lack. This is explained by the fact, that propolis is a resinous substance, collected especially from plant buds of a small group of bees [8] as well as other non-traditional sources such as asphalt tar [17].

From the researches carried out, it was observed, that there is a *positive correlation*, quite significant between Cd concentration in pollen and bees (Table 2.). In 2012 year of study, *quite high correlations* have been found in forest area (r_{xy} = 0.77 ± 0.20; t_r = 3.85; P < 0.001), industrial area (r_{xy} =0.70 ± 0.23; t_r = 3.04; P <0.001) and in the transport area (r_{xy} = 0.65 ± 0.24; t_r = 2.70; P <0.01).

Table 2 The correlation coefficient of Cd concentration in the main apiarian products and bees body

Corelative components	Reseach areas							
	Forest area		Agricultural area		Transport area		Industrial area	
	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r
year 2012								
Pollen- bees	0.77±0.20	3.85***	0.46±0.28	1.64	0.65±0.24	2.70**	0.70±0.23	3.04***
Pollen- honey	-	-	-	-	-	-	-	
Pollen-propolis	0.23±0.31	0.74	0.11±0.31	0.35	0.40±0.28	1.43	0.14±0.31	0.45
Propolis- bees	-0.15±0.31	0.48	-0.24±0.31	0.77	0.04±0.32	0.12	-0.25±0.31	0.81
Honey- bees	-	-	-	-	-	-	-	
Miere- propolis	-	-	-	-	-	-	-	-
year 2013								
Pollen- bees	0.58±0.26	2.23*	0.61±0.25	2.44*	0.63±0.24	2.62**	-	-
Pollen- honey	-		-		-		-	-
Pollen -propolis	-0.05±0.31	0.16	0.25±0.31	0.81	0.37±0.29	1.27	-	-
Propolis - bees	0.05±0.31	0.16	0.02±0.32	0.06	0.23±0.31	0.74	-	-
Honey - bees	-		-		-		-	-
Honey- propolis	-		-		-		-	-

Remark: *P < 0.05; **P < 0.01; ***P < 0.001

In the 2013, *high correlations* have been found between the concentration of Cd, in the same products in the transport are ($0.24 \pm r_{xy} = 0.63$; $t_r = 2.62$; P <0.01) and agricultural area ($r_{xy} = 0\ 61 \pm 0.25$; $t_r = 2.44$; P <0.01), being significant with two threshold of certainty by Student.

In the forest area these correlation have had a *medium level* ($r_{xy} = 0.58 \pm 0.26$; $t_r = 2.23$; P <0.05). It has not been possible to establish any correlation between the concentration of Cd in pollen and honey, because its concentrations in honey show undetectable levels (< 0.001 mg/kg).

The content of Cd in propolis does not correlate in any way with concentration of this metal in pollen and honey, because correlation coefficients, calculated on all bee products studied have had no significant values.

Certainty coefficient of all studied correlation between Cd content in propolis and other apiarian components have been below zero threshold by Student.

The research results demonstrate that, in both research years, exist a *positive correlation* between the concentration of Cu in pollen and bee, pollen and honey, and in some cases in honey and bees (Table 3).

Table 3 The correlation coefficient of Cu concentration in the main apiarian products and bees body

Corelative components	Reseach areas							
	Forest area		Agricultural area		Transport area		Industrial area	
	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r	$r_{xy} \pm m_r$	t_r
2012 year								
Pollen-bees	0.53±0.26	2.04*	0.54±0.26	2.07*	0.62±0.25	2.48*	0.55±0.26	2.11
Pollen- honey	0.53±0.26	2.04*	0.67±0.23	2.91**	0.53±0.26	2.04*	0.61±0.25	2.44*
Honey- bees	0.62±0.24	2.58**	0.28±0.30	0.93	0.59±0.26	2.27*	0.49±0.27	1.81
Pollen-propolis	0.19±0.31	0.61	0.10±0.31	0.32	0.39±0.29	1.34	-0.03±0.32	0.09
Propolis-bees	0.06±0.31	0.19	0.00±0.31	0.00	-0.14±0.31	0.45	0.17±0.31	0.55
Honey- propolis	0.20±0.31	0.64	0.04±0.32	0.12	-0.03±0.32	0.09	0.05±0.32	0.15
	2013 year							
Pollen-bees	0.63±0.24	2.62**	0.63±0.24	2.62**	0.71±0.22	3.22***	-	-
Pollen- honey	0.55±0.26	2.12*	0.62±0.25	2.48*	0.60±0.25	2.40*	-	-
Honey- bees	0.28±0.30	0.93	0.58±0.26	2.23*	0.29±0.30	0.97	-	-
Pollen-propolis	0.38±0.29	1.31	0.07±0.31	0.22	0.21±0.31	0.68	-	-
Propolis-bees	0.13±0.31	0.42	-0.20±0.31	0.64	-0.03±0.32	0.09	-	-
Honey- propolis	-0.17±0.31	0.55	0.00±0.32	0.00	0.20±0.31	0.64	-	-

Remark: *P < 0.05; **P < 0.01; ***P < 0.001

In 2012, in most of the research area, between the concentration of Cu in pollen and bees, have been recorded *medium correlations,* in the forest area (r_{xy} =0.53 ± 0.26; t_r = 2.04; P <0.05), agricultural area (r_{xy} = 0.54 ± 0.26; t_r = 2.07; P< 0.05), transport area (r_{xy} = 0.62 ± 0.25; t_r = 2.11; P <0.05) and industrial areas (r_{xy} = 0.55 ± 0.26; t_r = 2.11; P< 0.05) which corresponds to one certainty threshold. Also, *medium correlations* have been registered in relation pollen - honey, excepting agricultural area, which has been recorded a *high correlation* (r_{xy} = 0.67 ± 0.23; t_r = 2.91; P < 0.01).

In the 2013 year of study, have been found a *high correlation* between the concentration of Cu in pollen and bees, especially in the transport area (r_{xy} = 0.71 ± 0.31; t_r = 3.22; P <0.001), in the forest (r_{xy} = 0.63 ± 0.24; t_r = 2.62; P <0.01) and in the agricultural areas (r_{xy} =0.63 ± 0.24; t_r = 2.62; P < 0.01).

Also, *positive correlations* have been found between the content of Cu in pollen and honey. In the year 2012, have been established *high correlations* in the agricultural area (r_{xy} = 0.67 ± 0.23; t_r = 2.91; P <0.01) and industrial area (r_{xy} = 0.61 ± 0.25; t_r = 2.44; P < 0.05).

Correlation between Cu content in the body of bees, pollen, honey and propolis can be viewed more evident in Figure 2.

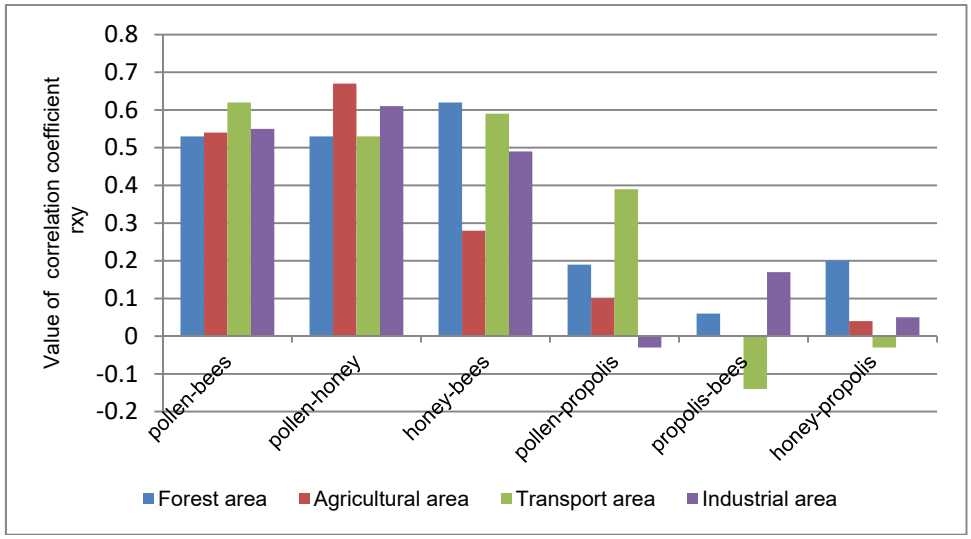

Fig. 2 Correlation between Cu content in the bees body, pollen, honey and propolis

And in the forest and transport areas have been found *medium correlation* (r_{xy} = 0.53 ± 0.26; t_r = 2.04; P < 0.05). The threshold of certainty of these correlations in most cases has been first level by Student. The *medium correlations* between Cu content in pollen and honey, also, have been recorded in the 2013 year of study: for example, in the agricultural and forest areas have been established correlation coefficients with high than medium values (r_{xy} = 0.62 ± 0.25; t_r = 2.48; P < 0.05) and (r_{xy} = 0.60 ± 0.25; t_r = 2, 04; P < 0.05) and medium values in the forest area (r_{xy} = 0.55 ± 0 26; t_r = 2.12; P < 0.05). Also, have been recorded some *positive correlation* between the concentration of Cu in honey and bees. In the 2012, the *highest correlation* of these components concentration has been recorded in the forest area (r_{xy} = 0.62 ± 0.24; t_r = 2.58; P < 0.01). In the 2013, such correlation has been recorded in agricultural area (0.26 ± r_{xy} = 0.58; t_r = 2.23; P < 0.05).

Similar research, conducted by researcher Roman A. (2010) [9] (Opole, Poland). He studied the accumulation of heavy metals in the bees body and honey in various regions, with different human impact (urban and rural). As a result of research carried out, between the

concentration of heavy metals such as Pb, Cd, Cu and Se in the bees body and honey has recorded a correlation coefficients value from 0.119 to 0.394 mg/kg-1. As a result obtaining significant differences (P<0.01) between the concentration of these metals in the bees body and their contents in honey.

Between the content of Cu in propolis and other apiarian products and bee, does not exist some correlation, because the value of correlation coefficient (r_{xy}) is very low and their meaning (t_r) is below the zero threshold by forecast probability theory without error after Student.

CONCLUSIONS

1. Between the concentration of heavy metals in pollen, honey bees and their content in the bee body exist a positive correlative link quite close.

2. The highest values of correlation coefficients have been recorded between Cd content in pollen and bees (r_{xy} = 0.77; P < 0,001), between Pb content in pollen and bees (r_{xy} = 0,77; P < 0,001), pollen and honey (r_{xy} = 0,71; P < 0,001), honey and bees (r_{xy} = 0,68; P < 0,01) and between Cu content in pollen and bees (r_{xy} = 0,71; P < 0,001), pollen and honey (r_{xy} = 0,67; P < 0,01), honey and bees (r_{xy} = 0,62; P < 0,01).

3. Some correlative links between the concentration of heavy metals in propolis and other bee products (pollen, honey) and bees body, have not been identified.

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