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The Bee honey as an Indicator to Environmental pollutions by Heavy metals in South Egypt, Egypt.

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ABSTRACT

The present study aims to estimate the distribution and speciation of heavy metals in bee honey that is useful in assessing possible chemical pollution and perceiving pollution sources. The fine fraction was analyzed for the metal content (Co, Ni, Cu, Pb, Cd, Fe, Mn and Zn). To study the importance of honey as indicator of environmental pollution and the relationship between pollution and contamination of the environment honey samples and soil heavy metals. Were collected 47 samples (30 honey and 17 soil samples) from 22 areas near sources of pollution such as factories and roads and gathering population and sanitation has been analysis of soil samples near the apiary from which the honey samples, represents five governorates: Sohag, Qena, Luxor, Aswan and the New Valley in the period from 2011-2012, these samples were collected during the flow and before the trading and marketing to ensure the quality of honey samples by compiled and vowed not to cheat, then was estimated concentrations of eight heavy metals using atomic absorption spectrophotometer. Mean values of heavy metals in honey were 0.437, 0.166, 0.225, 0.104, 0.411, 0.525, 0.209 and 0.253 mg/kg. for Zn, Mn, Fe, Pb, Cd, Cu, Ni, and Co respectively. Mean higher values each of Cd (0.908) and Ni (0.657) in Qena, Zn (0.838), Co (0.694) in New valley, Higher values of Zn, Mn, Fe, Pb, Cd, Cu, Ni and Co in soil samples were 4.400, 4.650, 4.800, 2.400, 1.965, 3.400, 1.600 and 2.200 mg / kg, respectively. The correlation between the presence of elements of honey and soil, found low correlation with each of, Fe, Pb, Cd and Cu elements, while a negative correlation with both Zn, Mn, Ni and Co, Because of the diversity areas from which the samples and the different environment in each sample in terms of the source plant, geographical location, topography and climate of each region, and also this study showed that the concentration of elements in bee honey were within the acceptable limits by comparison with other study in other countries in the world. Through the results obtained by this study confirms the possibility of using honey as an indicator of environmental contamination of toxic heavy elements.

INTRODUCTION

The bee honey is the natural sweet substance produced by honey bees from the nectar of plants, which bees collect, transform by combining with specific substances of either own , deposit, hydrate , store and leave in honeycombs to ripen and nature . Honey is essentially a highly concentrated water solution of two sugars, dextrose and levulose, with small amount of at least 22 other more complex sugars. Many other substances also occur in honey, but the sugars are by for the major components. Honey is a remarkably complex natural liquid that is reported to contain at least 181 substances (Nele Gheld *et al.* 2002). Honey is perhaps one of the most complex foodstuffs produced by nature and certainly the only sweeting agent that can be used by humans without any processing. These properties result from its chemical composition, the composition of honey depends highly on the type of flowers utilized by bees, climatic conditions in which the plant grow and maturation, it is a quick, safe and natural energy giver because its simple sugar is quickly absorbed into the blood stream, honey is an easily digestible foodstuff containing a rang of nutritiously important complementary elements, besides a high content of a range of saccharides , there are also organic acids , amino acids , mineral matters, colors, aromatic substances and a trace amount of flats (Bogdanov *et al.* , 1999) . Besides these, honey contains very valuable but unstable compounds, such as enzymes substance of hormonal (Yilmaz and Yavuz, 1999). Honey contamination by heavy metals (especially Pd, Cd and Cu) that are widely spread in our environmental. Several authors have indicated that bees and their products may be used as biological indicators of the environmental pollution present in the area where they fly (Fernández *et al*. 1994; Sanna *et al*., 2000; Buldini *et al*., 2001; Bogdanov *et al*., 2003; Porrini *et al*., 2003; Celli and Maccagnani, 2003; Fredes and Montenegro, 2006). However, Jones (1987) indicates that there is no correlation between the content of elements such as Ag, Cd and Pb present in the soil and in the honey. Furthermore, the exact way in which honey reflects the mineral components of, plants visited by bees, soil, or atmosphere is unknown. Additionally, honey is not the most sensitive tool to evaluate environmental contamination with heavy metals, due to the low concentration present in this product and the great variability caused by factors like origin source, floral density, season of the year and rainfall, among others. Therefore, it is important to take in account the type of equipment used to produce honey as well as the quality of the equipment used to store honey after harvesting as possible sources of honey contamination with heavy metals. Contact with stainless steel surfaces during harvesting, processing and/or preparation of honey for the market, can generate high Cr content, due to the corrosive effect of honey acidity. Likewise, storing honey in galvanized containers can be a source of Zn contamination (González *et al*., 2000; Bogdanov *et al*., 2003). Also, many researchers have indicated that bees and their products may be used as biological indicators of the environmental pollution present in the area where they fly Bee honey can be a good source of major and trace elements needed by humans, where it contains metals up to 0.17 %. Metals such as Cr, Co, Cu, Fe, Mn, and Zn or essential for humans, and they may play an important role in a number of biochemical processes. Some of them are present at the trace level and being toxic if they exceed safety levels. As a foodstuff used for healing purposes, honey must be free of any objection able content, and should contain only small amounts of pollutants, such as heavy metals. In the past several years, there has been increasing evidence of the heavy metals capacity of the honey. In order to have a beneficial effect honey must be free of any contaminating agents. Any heavy metals present in honey above the admitted levels by pollution standards, are threats to human body through the possible negative effect of the contaminants. Although the characteristics of honey through the total chemical analysis has received an increasing attention due to the possibility of its discrimination, useful classification according to the origin as well as practical indication of a potential product fraud, a more comprehensive understanding of the measurements of a much wider variety of its properties. It is especially beneficial to take nourishment of the honey into consideration. Not only is the total metal content important but metal bioavailability is of prim importance in the evaluation of the quality and the dietary value of food and beverages. Since honey is a nutritional resource that depends on biotic factors around the beehives, the presence of heavy metals could be related to its geographical and botanical origin. The aim of this study was to find a link between the amount of heavy metals found in the samples from the possibly contamination soil and the samples from pollution honey of heavy metals which obtained from various sources of city and villages in Upper Egypt.

MATERIALS AND METHODS

1. Samples collection:

a. honey samples: - Thirty natural bee honey samples collected from 22 regions represents different kinds from honey and seventeen soil samples represent some studying sites were collected by the author and beekeepers from Sohag, Qena, Luxor, Aswan and New-valley governorates (Fig1 and Table1), south Egypt during 2008 and 2009. The samples had been stored sealed sine that time and were well preserved clean and dry class bottle limited in formation was available regarding the origin of each sample The samples analysis has followed standard methods for detecting heavy metals Zn, Mn, Fe, Pb, Cd, Cu, Ni and Co.

b. Soil samples: - Seven teen soil samples were collected from the agricultural farms in the studied areas. Soil samples were crushed, and the representative samples were prepared by coning and quartering method. Each representative sample was dried in an electric furnace at 105°C for 5 h, and then powdered by agate mortar up to 100 µm. The powdered samples were kept in clean plastic pockets.

2. Samples preparation for analysis:

a. Honey sample: Samples preparation was carried out according to Rashed and Soltan (2004). Two grams of honey dried sample was digested in a covered Teflon beaker using 20 mL HNO3: HCl (3: 1) acid mixture then heated to a clear solution for 3 h and continued near dryness. The content after cooling was extracted using 1N HCl and completed to 50 mL by deionized water.

b. Soil samples: A total of 0.5 g dried sample was added to 20 mL HNO_3 : HCl (1 : 3) acid mixture in a covered Teflon beaker, then heated to a clear solution for 1 h and continued near dryness; the cooled residue was dissolved in 10 mL 0.1N HCl and filtered through Whatman number 42 filter paper into 50 mL measuring flask and filled to the mark with deionized water (AOAC 1984).

3. Analytical measurements

a. Standard solution for atomic absorption spectroscopy (AAS): All metal standard solutions for atomic absorption spectroscopy were prepared from certified atomic absorption metal stock (mg/kg.) of Cd Zn, Mn, Fe, Pb, Cd, Cu, Ni, And Co (BDH chemical, Ltd., England). Working solution was prepared by diluting the stock. Blank samples were prepared according to honey and soil sample preparations. Quantitative determination of heavy metals above was performed by an atomic absorption spectrophotometer (Solar 969) and air compressor Pu 6003 at respective metal characteristic wavelengths using their hollow cathode lamps. The atomic absorption data were acquired by aspirating aqueous single element standard solution blank and samples with two deionized water rinses between every two readings. Each sample was analyzed in triplicate.

b. Contamination control and method validation: For accurate determination of heavy metals in honey and soil samples, contamination control was kept under certain conditions; all stages of sample preparation and analysis were carried out in clean equipments and tools free from the studied metals. This accuracy was maintained by using laboratory equipments and tools from pyrex, washed with 1% HNO3 acid, rinsed twice with deionized water, oven dried at 105°C and placed in a clean environment until analysis. All reagents used were of analytical grade (BDH, Merck). The precision of the analytical method was evaluated by repeating the measurement of the absorbance signals for each element on the same sample. The relative standard deviation (RSD) was in the same order of 2.5% for each analyte.

4. Data analysis:

a.Simple correlation: After analysis heavy metals in each of soil and honey were calculated the simple correlation, as the relationship between the heavy metal in soil and honey.

b. Simple lineal regression was performed between the elements obtained in bee honey and soil samples. The gradient and intercept were established for each regression equation, and set the values of regression, which reflects the dispersion of values around the straight line.

Fig. (1): Egypt map show sampling sites:

Sohag sites Qena sites Luxor site Aswan site New valley

Table (2): Sites, geographic sources, botanical organ, honey kind and extract number of seventeen areas in five governorates in south Egypt during 2008 – 2009.

Sample No.	Geog. sources	Botanical organ		Honey kind	Extract No.	Year
		Dominant plant	Scientific name			
S1	El-Kawsar	Clover	*Triflium alexandrium*	Clover honey	1st extract	2008
S2	El-Kawsar	Nabk	*Ziziphus lotus* (L.)(Lam.)	Nabk honey	2nd extract	2008
S3	Sohag	Clover	*Triflium alexandrium*	Clover honey	1st extract	2008
S4	Gehana	Sesame	*Sesamum indicum*	Sesame honey	2nd extract	2008
S5	Gehana	Cotton	*Gossypium SPP*	Cotton honey	2nd extract	2009
S6	El-kawsar	Nabk	*Ziziphus lotus* (L.)(Lam.)	Nabk honey	2nd extract	2009
S7	El-kawsar	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
S8	Sakoulta	Nabk	*Ziziphus lotus* (L.)(Lam.)	Nabk honey	2nd extract	2009
S9	Maragha	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
S10	Tema	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
S11	Gohina	Nabk	*Ziziphus spina-christi*	Nabk honey	2nd extract	2009
S12	El-kawamel	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
S13	Gohina	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
S14	Dar El-Salam	Sesame	*Sesamum indicum*	Sesame honey	2ndextract	2009
Q1	Qena	Latency	*Cuminum cyminum*	Latency honey	2nd extract	2008
Q2	El-Rafaha	Clover	*Triflium alexandrium*	Clover honey	1st extract	2008
Q3	El-wakf	Alfalfa	*Medicago sativa*	Alfalfa honey	2nd extract	2009
Q4	Qena	Fennel	*Foeniculum vulgare Mill*	Fennel honey	1st extract	2009
Q5	Nkada	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
Q6	El-Rafasha	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
L1	El-Karnak	Clover	*Triflium alexandrium*	Clover honey	1st extract	2008
L2	Esna	Citrus	*Citrus sp.*	Citrus honey	1st extract	2009
A1	Aswan	Clover	*Triflium alexandrium*	Clover honey	1st extract	2008
A2	Komombo	Citrus	*Citrus sp.*	Citrus honey	1st extract	2009
N1	El-Hendaw	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
N2	El-Rashda	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
N3	El-Mawhop	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
N4	El-Sheikwaly	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
N5	El-Moshyia	Clover	*Triflium alexandrium*	Clover honey	1st extract	2009
N6	Bed-kholo	Citrus	*Citrus sp.*	Citrus honey	1st extract	2009

S = Sohag *Q = Qena* *L = Luxor* *A = Aswan* *N= New valley*

RESULTS AND DISCUSSION

Comparison between concentrations of heavy metals in bee honey from different areas:

Air and water contain heavy metals from industrial activities and traffic, which can also contaminate the bee cologne and its products. The air can contain heavy metals that are transported via water and soil to the plants to reach the nectar and the end to bee honey. The results reveal that the concentrations of the studied metals (Zn, Mn, Fe, Pb, Cd, Cu, Ni, and Co) in bee honey were from 0.070 to 0.930 mg/kg^{-1} for Zn, from 0.000 to 0.800 mg/kg^{-1} for Mn, from 0.000 to 0.720 mg/kg^{-1} for Fe, from 0.001 to 0.800 mg/kg^{-1} for Pb, from 0.017 to 2.230 mg/kg^{-1} for Cd, from 0.000 to 2.120 mg/kg^{-1} for Cu, from 0.000 to 0.900 mg/kg^{-1} for Ni and from 0.000 to 0.912 mg/kg^{-1} for Co (table 2). Presented data in above table show that heavy metals under study (Zn, Mn, Fe, Pb, Cd, Cu, Ni, and Co) concentrations in bee honey of different governorates in Sohag governorate were from 0.100 to 0.700 mg/kg^{-1} for Zn, from 0.000 to 0.800 mg/kg^{-1} for Mn, from 0.000 to 0.720 mg/kg^{-1} for Fe, from 0.001 to 0.800 mg/kg^{-1} for Pb, from 0.017 to 0.825 mg/kg^{-1} for Cd, from 0.000 to 2.120 mg/kg^{-1} for Cu, from 0.000 to 0.800 mg/kg^{-1} for Ni and from 0.000 to 0.912 mg/kg^{-1} for Co. In Qena governorate were from 0.070 to 0.460 mg/kg^{-1} for Zn, from 0.002 to 0.410 mg/kg^{-1} for Mn, from 0.000 to 0.610 mg/kg^{-1} for Fe, from 0.005 to 0.210 mg/kg^{-1} for Pb, from 0.098 to .2230 mg/kg^{-1} for Cd, from 0.184 to 0.912 mg/kg^{-1} for Cu, from 0.140 to 0.900 mg/kg^{-1} for Ni and from 0.000 to 0.600 mg/kg^{-1} for Co. In Luxor governorate were from 0.150 to 0.160 mg/kg^{-1} for Zn, from 0.003 to 0.007 mg/kg^{-1} for Mn, from 0.014 to 0.034 mg/kg^{-1} for Fe, from 0.100 to 0.022 mg/kg^{-1} for Pb, from 0.346 to 0.430 mg/kg^{-1} for Cd, from 00.190 to 0.800 mg/kg^{-1} for Cu, from 0.000 to 0.003 mg/kg^{-1} for Ni and from 0.000 to 0.001 mg/kg^{-1} for Co. In Aswan governorate were from 0.006 to 0.006 mg/kg^{-1} for Zn, from 0.001 to 0.300 mg/kg^{-1} for Mn, from 0.054 to 0.180 mg/kg^{-1} for Fe, from 0.001 to 0.002 mg/kg^{-1} for Pb, from 0.001 to 0.456 mg/kg^{-1} for Cd, from 0.002 to 0.400 mg/kg^{-1} for Cu, from 0.040 to 0.400 mg/kg^{-1} for Ni and from 0.001 to 0.220 mg/kg^{-1} for Co. In New valley governorate were from 0.340 to 1.320 mg/kg^{-1} for Zn, from 0.110 to 0.280 mg/kg^{-1} for Mn, from 0.129 to 0.440 mg/kg^{-1} for Fe, from 0.003 to 0.014 mg/kg^{-1} for Pb, from 0.045 to 0.874 mg/kg^{-1} for Cd, from 0.000 to 0.900 mg/kg^{-1} for Cu, from 0.140 to 0.600 mg/kg^{-1} for Ni and from 0.040 to 0.912 mg/kg^{-1} for Co. Comparing heavy metal (Zn, Mn, Fe, Pb, Cd, Cu, Ni, and Co) concentrations in bee honey of different governorates under study (Table 2), the analyses of bee honey shows high concentrations of Zn in Sesame honey at Dar El-Salam district , Clover honey at El-Hendaw , El-Rashda and El-Mawhop villages in New valley, Mn in Clover honey at Maragha district , Fe in Nabk honey at Sakoulta district , Pb in Nabk honey at El-Kawsar city , Cotton honey at Gehana district , Cd in Clover honey at Maragha district, in Latency honey at Qena city , in Clover honey at El-Rafaha village , in Alfalfa honey at El-wakf district and in Clover honey at El-Moshyia village, Cu in Clover honey at El-Kawsar city, in Sesame honey at Gehana district, in clover honey at El-Rafasha village , Ni in clover honey at Sohag city, in Latency honey, Fennel honey at Qena city and in clover honey at Nkada district and Co in clover honey at El-kawsar city, in clover honey at El-kawamel village and Citrus honey at Bed-kholo village. This last behavior is attributed to the geology of the area, which contains rocks and ores rich with Mn (Magnard 1983), while the presences of Cd in Qena honey are related to the timber plantations that are irrigated from sewage, Cu, Pb, and Zn in El-Kawsar honey and Dar

El-Salam district are related to the industrial activity emission input and bridges in these regions. The presence of Fe and Cu Sakoulta samples are resulted from the pollution input near from motorways in the area. If we compare the honey in all the studied areas, Aswan honey shows lower Zn, Cd and Cu concentrations, Luxor honey shows lower Mn , Fe, Ni and Co and Sohag recorded lower Pb. El-Kawsar honey (Nabak) Show lower Zn followed by Qena honey (Fennel). Dar El-Salam honey (sesame) recorded lower concentration in Mn metal. El-kawamel clover honey and Qena honey (Latency) shows lower in iron metal, while Pb Luxor, Qena , Sohag and New valley. than in Esna (A), Esna (B), Edfu, and Kom Ombo honey. This reveals that the Aneeba area is free from pollution. From the data obtained during this survey it appears that, honey may reflect spatial variability in the levels of heavy metal concentration to some extent. For example, honey obtained from El- Rashda village, New valley Governorate contained elevated concentration of Mn, and Cu and it was elevated too of Zn,Ni, Co, Pb and Cu, the concentration were 1.32, 0.400,0.40,0.30 and 0.020 Mg/L ppm. respectively in the sample number 11 (Bedkholo , New valley Governorate). Of particular in interest in this respect are the samples from El- Rashda village, New valley Governorate (sample number 7) which contain the highest concentration of Zn and Cu elements recorded in this study, these honey may have been influenced by deposition of sample location, as for as sample number (1) of Cu and Ni elements, number (2) for Fe element and number (3) for Pb and Cu elements.In addition levels of metals in honeys from the Dar-el salam, Sohag Governorate are among the lowest recorded in this survey of the metals Co, Mn, Ni, Fe and Pb elements.The previous examples of elevated heavy metal contents are from locations where increased atmospheric deposition of Zn, Ni and Pb minerals could reasonably by expected. Despite this, samples adjudged to be influenced by the proximity of major roods, factories of and soils treated with pesticides.

The concentration of Zn in honey varied from a minimum of 0.070 mg/kg to maximum of 0.930 mg/kg, the minimum value was determined in Fennel honey collected from Qena region in 2009, while the maximum level was determined in clover honey El-Mawhop region. In similar study in Lithuanian, the average concentration of Zn honey reached from 514.0 to 5639.0 μg/kg. Whatever the case, Zn are very important microelements to ensure a natural functioning of the human body. They both constitute the structure of proteins and enzymes and take part in the synthesis of proteins and metabolism. The lack of Zn could result in a weekend immunity system; longer wound healing periods, depression, impaired vision, taste and smell disorders, impotence. However, big quantities of these microelements are harmful to the human body and can result in a cellular metabolism disorders **Birutė**, *et al* (2006). The amount of Zn in honey depends on the geographic location of the apiary, acidity of the ground and particularly on the instruments used in apiaries, centrifugation and storage of honey, transport utilities and technological process. The average concentration of Zn exceeds 1235 μg/kg, while the maximum concentration of Zn is 5639 μg/kg. The content of Zn in the honey samples, according to other authors, there are cases when the concentration of Zn in honey varies within larger limits – from 500 to 19500 μg/kg.

2. Mn The higher manganese level was found as 0.800 in clover honey sample from Maragha region. And sesame honey sample was collected from Dar El-Salaam region 2009, the analysis showed it free of manganese. The reported some manganese values in the literature for honey were 0.32~1.70 μg/g(17), 0.9~10.2 μg/ g(29), respectively. Manganese values found in the present study are in agreement with the manganese levels of honey samples from Black sea region-Turkey(17) and Ireland(29).

3. The lower and higher Fe concentrations were found as 0.720 mg/kg. in clover honey sample was collected from El-Rafasha region 2008. Yozgat Sorgun Kulhuyuk and 5.2 μg/g in honey sample from Yozgat Kale Koyu, respectively. Iron values in honey samples have been reported in the range of 0.40~52.51 μg/g(14), 3.45~8.94 μg/g(17), 0.97~1.91 μg/g(28), respectively. Data reported for iron of honey samples from Canary Islands(14) are generally higher than those reported for middle Anatolia. On the other hand, the levels of iron in hones from Black sea region-Turkey(17) and from Saudi Arabia(28) are generally at the same level of our samples. The lower manganese level was found 0.18 μg/g in honey sample from Yozgat Gevrek The dispersion of Pb in honey samples is presented in Table 2 and Fig. 2. The maximum amount of this metal 0.9000 mg/kg (ppm) was found in the Nabak and clover honey collected at Gohina city, Sohag governorate, however, it did not exceed MTL. This volume of Pb depended on the locations of apiaries – the village – the suburb - the city – the governorate, or between the roads of the sites and highways, whereas it is obvious that the internal combustion engines are the main source of contamination with Pb. The minimum amounts of Pb were 0.1000

found in the clover honey collected at El-karnak area, Luxor governorate, in the upper Egypt, which is far away from highways and has no industrial giants or thermoelectric stations: in Lithuanian (2.9 to 22.1 µg/kg). For comparison, a weekly standard of Pb per person (weight 60 kg) in Germany is 1500 µg. The results of this research were compared with data of other researchers. It should be noted that the concentrations of Pb in honey established by other investigators were bigger; for example, Polish – 396– 1730 µg/kg , Hungarian – 3.2–186 µg/kg , German – 40–273 µg/kg, **Birutė**, *et al* (2006). After all that the focus of Pb in all honey samples that were analyzed was less than the world rate.

6. Cd The honey samples contained insignificant amounts of lead and cadmium. Therefore, it is appropriate to analyze concentrations of these metals in honeys to test the contamination of the environment by trace heavy metals(15). The lower lead content was found as 17.6 µg/kg in Sivas Zara honey. The higher lead content was found as 32.1 µg/kg in honey sample from Yozgat Kale Koyu. Lead contents of some honey samples around the world have been reported as 48 µg/kg(15), 30.3~58.6 µg/kg(17), 3.3~45.0 µg/kg(31) and 30~240 µg/kg(28), respectively. Lead data of honey samples from Saudi Arabia is much higher than that of our samples. The lower and higher cadmium concentrations were found as 10.9 µg/kg in Yozgat Sorgun Dişli honey and 21.2 µg/kg in Yozgat Kale Koyu honey, respectively. C admium contents of honey samples in the literature have been reported as 15 µg/kg(15), 5.23~9.82 µg/kg(17), 8 µg/kg(28), <2~63.0 µg/kg(31), respectively. The level of cadmium of our samples was higher than some of the previous data(15,17,28,32).The concentrations of Cu in honey samples vary from 0.040 to 2.120 mg/kg, the high concentration was in El- kawsar suburb, Sohag governorat while the low concentration of this microelement in honey was in El-Rashda village , New valley governorate (table2). However, there are data from other researchers stating that the concentrations of Cu in honey exceed the level obtained in the present research 4–8 [15] to 5–14 [17] times. The content of Cu and Zn in the honey samples is presented in Fig. 7. According to other authors, there are cases when the concentration of Zn in honey varies within larger limits – from 500 to 19500 µg/kg. The minimum and maximum copper levels observed were 0.25 µg/g in honey sample from Yozgat Yudan and 1.10 µg/g in Yozgat, respectively. C opper values in the literature have been reported as 0.25~1.30 µg/g(17) for honey samples from Black sea-Turkey, 1.8 µg/g(18) for samples from southeastern Anatolia of Turkey, 0.31 µg/g(30) for Lazio region (central Italy) honeys, respectively. The values for the copper contents of our samples were generally are at the same level of the literature values(17,18,28,30). The valuable characteristics of honey as a natural bio-product depend on its sort and origin. The quantitative and qualitative ratio of elements is characteristic of each blossom of the plant from each region of the governorates under studding, so the total quantity of mineral materials depends on a location. It is possible to determine the origin of specific samples of honey and the environmental pollution of a region from the quantitative and qualitative ratio of heavy metals and rare metals in honey.

Methods regression. Regression equations (y = ax+b) for Method 1 (AD) and the Method 2 (HTDO) for the elements analyzed are showed in Table 5, where Cr had a well adjusted regression curve. The regression curves for the other elements analyzed did not have good adjustments, possibly due to interference of masses in the resultant matrixes in both methods and to the heterogeneity of the honey samples.

Principal components analysis. The multivariable analysis performed from the results of Method 1 (AD), indicated 11 principal components that correspond to the 11 variables analyzed, which explain the 100% data variability, on which were based the maps of Figure 2 and 3. Distribution along PC1 would be mainly dependent on the concentrations of Fe, Mn, Cu and Zn, considered essential elements or micronutrients for superior plants (Adriano, 2001), while distribution along PC2 would be mainly dependent on Cd and Pb concentrations, considered non essential elements for plants (González, 1994; Adriano, 2001). The factorial map PC1-PC2 explains 43.7% of total data variability (28.6% and 15.1%, respectively). A main honey group close to the axis of interception of both components, which is characterized by a low content of heavy metals and trace elements is showed in Figure 2. Honey samples 21, 81, 35 and 84, had the highest Cd content (0.05 mg·kg-1 for honey samples 81 and 84) and Pb content (0.1 mg·kg-1 for honey samples 81 and 35). These honey samples come from beehives that are close to roads and highways. Regarding honey samples with high micronutrient content seen along the PC1 gradient, honey sample 223 had the highest Mn content (3.1 mg·kg-1); sample 8, the highest Cu and Zn contents (2.0 and 4.7 mg kg-1, respectively). In spite of honey sample 150 had the highest Fe (6,4 mg·kg-1) content, it appears closer to the interception axis of both components, due to the lowest Mn and Zn contents (0.8 and 1.2 mg·kg-1) and the non Cu detection. Regarding the botanical origin of this honey group, hualputra specie (uniflora honey 47 and 52) prevails, which could be considered a wetland plant, characterized by growing in soil with sediment and having important Zn accumulations (Schonoor, 1997). The rest of the samples correspond to multiflora honeys (8, 223, 16 and 174), with important participation of herbaceous plants. With respect to geographical origin, these six honey samples were collected in the V (8 and 16) and X Administrative Regions (47, 52, 223, and 174). To complete the results analysis, a second factorial PC3-PC5 map was made (Figure 3). Distribution along PC3 would be mainly dependent on the Ni, Al, and Cr concentrations, which could be related to the type of storage container used after honey harvest. Distribution along PC5 would be mainly dependent on the Sr concentrations, a trace element found in most honey samples analyzed. In this work, this element was found in higher concentration than those concentrations detected in a Spanish research (Terrab et al., 2004). The PC3-PC5 factorial map explains 24.3% of the total data variability (14.3% and 9.4%, respectively). A honey group close to the interception axis of PC3 and PC5 that were characterized by honeys having low Ni, Al, Cr and Sr content is showed in Figure 3. Honey 74 had the greatest Al content (14.3 mg kg-1), higher than those described for France (Devillers et al., 2002). Similar results – high Al concentrations- were found in honey samples 47, 76 and 52 with contents of 9.1, 14.3, and 9.1 mg·kg-1, respectively. These honey samples were obtained from the center-south region of Chile (VIII to X Administrative Regions), that has trumaos soil rich in Al with soft textures whose particles could deposit on flowers or reach the beehives. This could explain the presence of this element in the honey, but the higher content could be due to the use of aluminum containers. Furthermore these honey samples had lower pH values (4.2) than the pH mean found in this study. Honey 74 and 76 had the lowest pH than the other samples analyzed (3.8 and 3.9 respectively). Even though to date no maximum limits for Al has been established internationally, it would be important to report this type of information to the beekeepers, in order to handle better the possible sources of contamination with this element, since it is possible relation with Alzheimer's disease (Flaten, 2001).

Table (3) Concentration of heavy metals in honey samples collected from within the forage area during 2008 and 2009

Sample No.	***Heavy metals in honey mg / kg. (ppm.)***							
	Zn	**Mn**	**Fe**	**Pb**	**Cd**	**Cu**	**Ni**	**Co**
S1	0.100	0.007	0.006	0.180	0.025	0.044	0.100	0.000
S2	0.650	0.010	0.450	0.800	0.017	2.120	0.110	0.000
S3	0.350	0.005	0.600	0.110	0.025	0.800	0.800	0.000
S4	0.115	0.004	0.003	0.110	0.024	1.200	0.110	0.000
S5	0.245	0.005	0.500	0.800	0.080	0.536	0.000	0.000
S6	0.305	0.003	0.003	0.120	0.066	0.480	0.000	0.000
S7	0.135	0.110	0.150	0.003	0.048	0.000	0.600	0.912
S8	0.135	0.190	0.720	0.001	0.235	0.000	0.120	0.310
S9	0.700	0.800	0.210	0.001	0.124	0.000	0.000	0.750
S10	0.103	0.400	0.300	0.004	0.645	0.000	0.000	0.210
S11	0.150	0.200	0.210	0.002	0.067	0.700	0.000	0.150
S12	0.350	0.700	0.000	0.001	0.345	0.400	0.000	0.900
S13	0.190	0.120	0.110	0.002	0.825	0.170	0.000	0.420
S14	1.560	0.000	0.270	0.002	0.234	0.400	0.000	0.100
Rang	**0.100 - 0.700**	**0.000 - 0.800**	**0.000 - 0.720**	**0.001- 0.800**	**0.017- 0.825**	**0.000- 2.120**	**0.000 - 0.800**	**0.000 - 0.912**
Mean	**0.363**	**0.182**	**0.252**	**0.153**	**0.197**	**0.489**	**0.131**	**0.536**
Q1	0.460	0.002	0.000	0.110	.2230	0.560	0.800	0.000
Q2	0.310	0.005	0.002	0.200	.1347	0.184	0.140	0.600
Q3	0.135	0.003	0.150	0.110	.1450	0.912	0.600	0.000
Q4	0.070	0.410	0.320	0.005	0.098	0.800	0.800	0.320
Q5	0.345	0.270	0.610	0.009	0.124	0.800	0.900	0.120
Q6	0.230	0.002	0.150	0.210	0.200	0.900	0.700	0.000
Rang	**0.070 - 0.460**	**0.002 - 0.410**	**0.000 - 0.610**	**0.005 - 0.210**	**0.098 - .2230**	**0.184 - 0.912**	**0.140 - 0.900**	**0.000 - 0.600**
Mean	**0.258**	**0.155**	**0.205**	**0.107**	**0.908**	**0.693**	**0.657**	**0.347**
L1	0.150	0.007	0.014	0.100	0.346	0.800	0.000	0.000
L2	0.160	0.003	0.034	0.022	0.430	0.190	0.003	0.001
Rang	**0.150 - 0.160**	**0.003 - 0.007**	**0.014 - 0.034**	**0.100 - 0.022**	**0.346 - 0.430**	**0.190 - 0.800**	**0.000 - 0.003**	**0.000 - 0.001**
Mean	**0.155**	**0.005**	**0.024**	**0.061**	**0.388**	**0.495**	**0.002**	**0.001**
A1	0.405	0.300	0.180	0.002	0.456	0.400	0.400	0.220
A2	0.006	0.001	0.054	0.001	0.001	0.002	0.040	0.001
Rang	**0.006 - 0.006**	**0.001 - 0.300**	**0.054 - 0.180**	**0.001- 0.002**	**0.001- 0.456**	**0.002 - 0.400**	**0.040 - 0.400**	**0.001 - 0.220**
Mean	**0.006**	**0.151**	**0.117**	**0.002**	**0.229**	**0.201**	**0.220**	**0.111**
N1	1.320	0.280	0.280	0.014	0.562	0.600	0.230	0.040
N2	1.120	0.160	0.200	0.004	0.230	0.400	0.140	0.230
N3	0.930	0.190	0.129	0.003	0.095	0.000	0.140	0.340
N4	0.710	0.190	0.440	0.005	0.688	0.900	0.200	0.230
N5	0.340	0.160	0.150	0.004	0.045	0.600	0.210	0.330
N6	0.610	0.110	0.150	0.003	0.874	0.000	0.600	0.912
Rang	**0.340 - 1.320**	**0.110 - 0.280**	**0.129 - 0.440**	**0.003 - 0.014**	**0.045 - 0.874**	**0.000 - 0.900**	**0.140 - 0.600**	**0.040 – 0.912**
Mean	**0.838**	**0.182**	**0.225**	**0.006**	**0.416**	**0.417**	**0.507**	**0.694**
Rang	**0.070 – 0.930**	**0.000 – 0.800**	**0.000 – 0.720**	**0.001 – 0.800**	**0.017 – 2.230**	**0.000 – 2.120**	**0.000 – 0.900**	**0.000 – 0.912**
Mean	**0.413**	**0.026**	**0.213**	**0.098**	**0.398**	**0.673**	**0.197**	**0.236**

Table (4): The total heavy metals content (ppm) in the examined soil samples collected from within the forage area during 2008 and 2009.

Site No.	Heavy metals in soil samples mg / kg. (ppm.)							
	Zn	**Mn**	**Fe**	**Pb**	**Cd**	**Cu**	**Ni**	**Co**
S(1)	0.000	0.000	0.000	0.000	0.980	0.000	0.000	1.950
S(7)	0.135	3.000	3.460	1.430	1.230	2.250	0.460	0.230
S(8)	1.100	2.830	1.900	0.700	0.456	3.400	0.500	0.000
S(9)	4.100	4.650	1.800	1.200	1.965	2.300	1.500	0.000
S(10)	1.300	4.030	2.500	0.700	0.876	2.300	1.200	0.000
S(11)	0.990	0.246	1.100	1.200	1.075	0.300	0.800	0.000
S(13)	1.500	0.000	0.000	1.400	1.650	1.500	0.000	0.980
S(14)	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.540
Q(18)	0.900	3.126	4.500	0.500	0.789	2.500	1.600	0.890
L(19)	4.400	4.450	3.400	1.900	1.560	2.500	1.400	0.780
A(22)	2.800	0.000	0.000	0.600	0.987	0.800	0.000	0.320
N(23)	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.310
N(24)	1.400	0.900	2.100	0.800	0.983	0.200	0.400	0.340
N(25)	0.600	2.450	1.600	1.700	1.765	1.600	1.150	0.110
N(26)	1.400	3.060	4.800	2.400	1.568	1.600	1.400	0.000
N(27)	0.900	3.200	2.700	0.600	1.000	2.000	1.500	2.200
N(28)	2.700	5.670	2.900	0.600	0.865	3.900	2.600	1.320

Table (5): Pearson correlation coefficients of the measured. Correlations in italics are significant (individual significance level: 0.05)

Soil \ Honey	Zn	Mn	Fe	Pb	Cd	Cu	Ni	Co
Zn	-0.171							
Mn		0.140						
Fe			0.388					
Pb				-0.018				
Cd					-0.294			
Cu						-0.367		
Ni							0.424	
Co								0.424

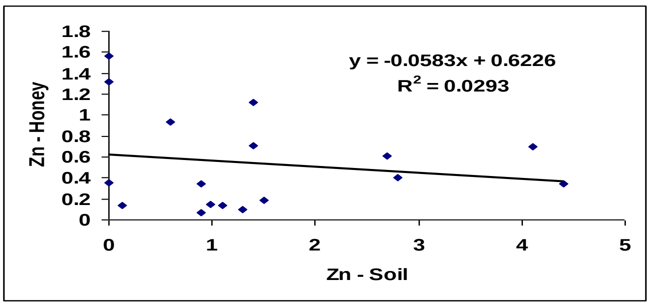

Fig.3: Regression equation between Zn concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

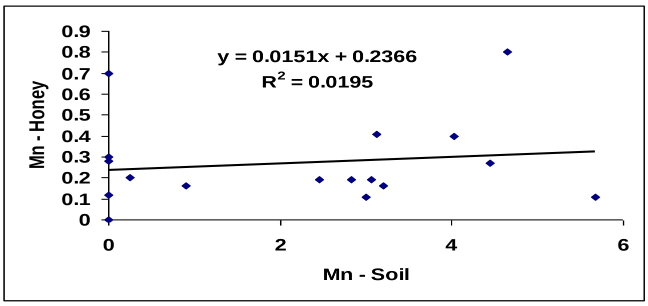

Fig.5: Regression equation between Mn concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

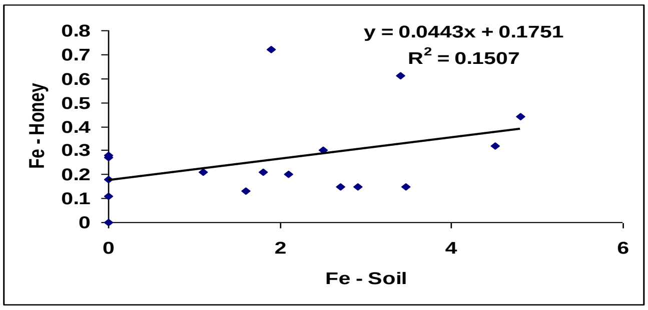

Fig.7: Regression equation between Fe concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

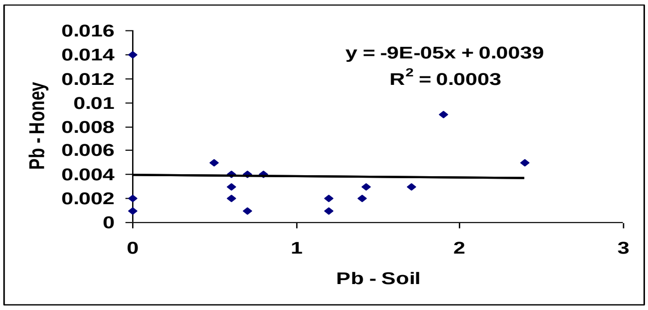

Fig.9: Regression equation between Pb concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

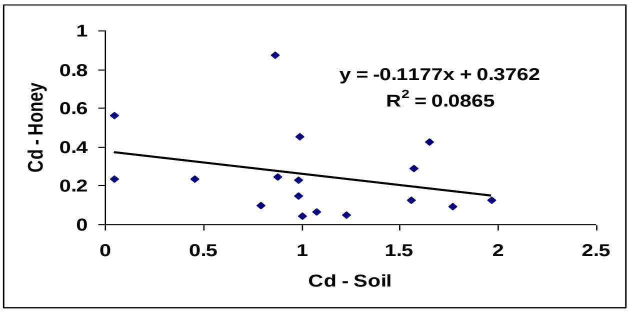

Fig.11: Regression equation between Cd concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

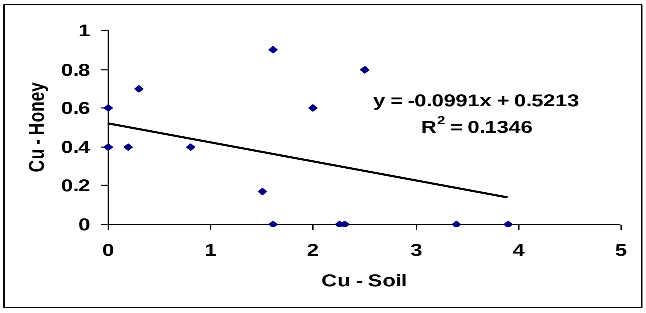

Fig.13: Regression equation between Cu concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

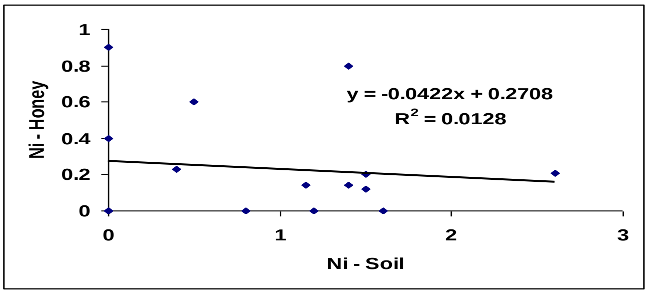

Fig.15: Regression equation between Ni concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

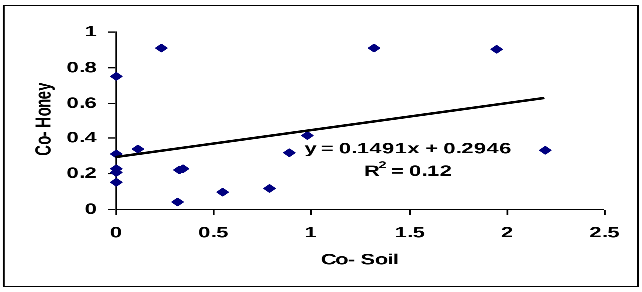

Fig.15: Regression equation between Co concentration in honey and soil analyzed. Samples collected seventeen regions during 2008 and 2009.

Table (6): Regression equation (y = ax+b) between heavy metals in bee honey samples and soil samples collected different regions during 2008 to 2009.

Element	Regression and determination coefficient	
Zn	y = -0.0583×+0.6226	R^2 = 0.0293
Mn	y = 0.0151×+0.2366	R^2 = 0.0195
Fe	y = 0.0443×+0.1751	R^2 = 0.1507
Pb	y = -9E-05×+0.0003	R^2 = 0.0003
Cd	y = -0.1177×+0.3762	R^2 = 0.0865
Cu	y = -0.0991×+0.5213	R^2 = 0.1346
Ni	y = -0.0422×+0.2708	R^2 = 0.0128
Co	y = -0.1491×+0.2946	R^2 = 0.1200

Conclusions

1. The concentration of different heavy metals in Lithuanian honey varies greatly: Pb – 2.9–22.1 μg/kg, Cd – 4.1–14.6 μg/kg, Cu – 119.6–342.9 μg/kg, Zn – 514.0–5639.0 μg/kg, but are within Maximum Tolerable Limit of Lithuanian Standards of Hygiene. The content of these microelements in Lithuanian honey are lower than in honey of other EU countries. Lithuanian honey is eco-friendlier comparing to honey collected in other EU countries and can successfully compete in the market.
2. Honey can serve as an indicator of environmental pollution with HM. The maximum content of Pb (22.1 μg/kg), high levels of Sr and Ba were found in honey collected in the Kaunas city territory, and the
maximum level of Cd (14.6 μg/kg) as well as large amounts of Pb, Cu, Sr were determined in honey collected in Elektrėnai, Šiauliai and Birštonas regions, while the lowest levels of heavy metals were estimated in honey collected in the eco-friendliest areas of Lithuania: on the seacoast, in the Varėna region and in the north of the country.
3. According to Rb content, honey can be assorted into mead and forest honey. 2446.5 μg/kg of Rb was found in honey collected in the Labanoras forest. This amount is 4÷12 times higher than in other samples.

In conclusion, Cd and Pb levels in the 47 uniflora and multiflora honey samples collected between the IV and X Administrative Region of Chile were found to be below the maximum values allowed according to the current European standards. The acid digestion (AD) method should be preferred for Cd, Ni, Pb and Zn, while for the other elements the high temperature dry oxidation (HTDO) method would be better. It would be interesting to analyze a larger number of samples and continue performing this type of research analyzing the metal content in different parts of reproductive structures of plants that use beehoneys for pollination, in known contaminated areas.

REFERENCES

1. NeleGheldof, XiAo-Hong Wang, and Nikij. Engeseth (2002). Identification and Quantification of Antioxidant component of Honey from various floral sources. J. Agric. Food chem. So, 5870 – 5877.
2. Bogdanov, S., V. Kilchenmann and P. Fluri, U. Buhler and P. Lavanchy, (1999). Influence of organic acids and components of essential oils on honey taste. Am. Bee J., 139: 61-63.
3. Buldini, P. L., S. Cavalli, A. Mevoli and J. L. Sharma, (2001). Ion chromatographic and Volta metric determination of heavy and transition metal in honey – food chem., 73: 487 – 495.
4. Bogdanov, S. , M. Haldimann2,W. Luginbühl1 and P.Gallmann (2007). Minerals in honey: environmental, geographical and botanical aspects. Journal of Apicultural Research and Bee World 46(4): 269–275.
5. Bogdanov, S., A. Imdorf, J. Charriere, P. Fluri and V. Kilchenmann, (2003). The contaminants of the bee colony. Swiss Bee Research center, Bern, Zwitzer land, PP: 12.
6. Bulinski, R. , Wyszogrodzka-koma, L. and Marzec, Z. (1995) Study on some trace element contents in Polish food products. *Bromatologia-i-chemia-Toksykologiczan* **28**:2 , pp. 151-154.
7. Caroli, S. , Forte, G. , Alessandrelli, M. , Cresti, R. , Spagnoli, M. , D'llio,

S. , Pauwels, J. and Kramer, GN (2000) A pilot study for the prediction of a certified reference material for trace elements in honey. *Michrochemical Journal* **67** , pp. 227-233.

8. Carmen, A. and Cristina, M. (2001) Environmental pollution and its effects on honey quality. *The Roumanian Biotechnological Letters* **6**:5 , pp. 371-379.
9. Fernandez, M. C., E. Subra and A. Ortiz, (1994). Honey, environmental in dictator. Environmental practices for quality agriculture. Proceeding of the congress of the Spanish society of Organic Agriculture, (SSOA' 94) Toledo, Spain, PP: 37- 44.
10. Fernandez-Torres, R. , Perez-Bernal, JL , Bello-Lopez, MA , Callejon-Mochon, M. , Jimenez-Sanchez, JC and Guiraum-Perez, A. (2005) Mineral content and botanical origin of Spanish honeys. *Talanta* **65** , pp. 686-691.
11. Fredes, C. and G. Montenegro, (2002). Heavy metal and other trace elements in honey bee in Chile. Cien. Inv. Agric., 33:50-58.
12. Fredes, C. and G. Montenegro. 2006. Heavy metal and other trace elements contents in Chilean honey. Cien. Inv. Agr. (in English) 33(1):50-58.
13. Sanna, G., M. Pilo, P. Piu, A. Tapparo and R. Seeber (2000). Determination of heavy metals in honey by anodic stripping voltammeter Y at microelectrodes. Anal. Chim, Acta, 415: 165- 173.
14. Yilmaz, H. and O. Yavuz, (1999). Content of some tree metals in honey from south eastern Anatolia. Food Chem., 65: 475-476.
15. Jones, K. C., (1987). Honey as an indicator of heavy metal contamination. Water, Air and Soil Pollution 33: 179 – 189.
16. Bratu, I. and Georgeseu, C., (2005). Chemical Contamination of bee honey – ldentifrng sensor of the Environmental Pollution. J. Central European Agric. Vol. 6 No. 1(467- 470).
17. Ioannidou, M. D., G. A. Zuchariadis, A. N. Anthemidis, and J.A. Stratis, (2004). Diret determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. Talanta 65:92- 97.
18. Ahmed Hassan, Mahmoud A. A. Ghandour, Azza, M.M. Ali and Hazim, A. Mahran, (2010). Evaluation of lead, Cadmium and Copper concentrations in Bee honey and Edible Molasses. Am. J. of Applied Sciences 7(3): 315-322.
19. Rashed, M. N. , El-Haty, M. T. A. and Mohamed, S. M. (2009): Bee honey as environmental indicator for pollution with heavy metals. Toxicological & Environmental Chemistry, 91:3, 389 – 403.
20. Rashed, MN and Soltan, ME (2004) Major and trace elements in different types of Egyptian mono floral and non-floral bee honeys. Journal of Food Composition and analysis 17 , pp. 725-735.
21. Uren, A. , Serifoglu, A. and Sarikahya, Y. (1998) Distribution of elements in honeys and effect of a thermoelectric power plant on the element contents. *Food Chemistry* 61 , pp. 185-190.
22. Jivan A., Patruica S. and Popescu G.(2008): Researches concerning the heavy metal content of the rape honey originating from the Banta area in the years 2006-2007 Lucrări stiinŃifice Zootehnie si Biotehnologii,. 41 (2) 302:308 ,Timisoara
23. Maria A. L. , Juliana R., Atanassova2 and Lilyana D. Y.(2010): Botanical origin

and inorganic content of bee honey in Northeast Bulgaria (Shumen region). PHYTOLOGIA BALCANICA 16 (1): 131 – 135 Sofia

24. Fredes C. and G. Montenegro (2006). Heavy metals and other trace elements contents in Chilean honey, Cien. Inv. Agr. 33(1), 50-58.
25. Feryal Erbilir and Özlem Erdoĝrul (2005): Determination of Heavy Metals in Honey in Kahramanmaraş City, Turkey. Environmental Monitoring and Assessment 109, (3), 181-187.
26. Fredes, C. and G. Montenegro (2006): Heavy metals and other trace elements contents in Chilean honey Cien. Inv. Agr. 33(1): 50-58.
27. Osman K. A., M. A. Al-Doghairi, S. Al-Rehiayani and M. I.D. Helal (2004): Mineral contents and physicochemical properties of natural honeys produced in Al-Qassim region, Saudi Arabia. *Published in net, E-mail*: kamosman@yahoo.com.
28. Sadia B., S. Z. Husain and R. N. Malik (2008): Pollen analysis and heavy metals detection in honey samples form seven selected countries . Pak. J. Bot., 40(2): 507-516.
29. Magnard, JB (1983) *Geochemistry of sedimentary ore deposits* Springer Verlag , New York — 200-17