Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 54(2) (2016), 371–380 **http://annagricmoshj.com**

Determination of certain metals in beebread from different districts in Alexandria and El-Beheira governorates

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Abstract

The present study was carried out to determine the contents of 8 metals (iron, zinc, manganese, copper, lead, chromium, arsenic and cadmium) in 12 beebread samples collected from different districts in Alexandria and El-Behaiera governorates throughout the period from March to September 2013. The obtained data showed that each of chromium (Cr) and arsenic (As) are not detected. In all districts elements could be ranked in the following descending order: Fe > Zn > Mn > Cu > Pb > Cd.

The highest and lowest levels of the studied metals in beebread samples were recorded (as ppm.) in different districts as follows:

- For iron the highest was in Etay- El baroud (167.36) and the lowest was in Al-Muntazah (61.06).
- For zinc the highest was in Abu-Hommoss (124.93) and the lowest was in El-Tabya (21.95).
- For copper the highest was in Kafr-El dawar (21.15) and the lowest was Al-Muntazah (3.12).
- For Manganese the highest was in Edko (44.87) and the lowest was was in Kafr-El dawar (13.02).
- For lead the highest was in Kafr El-Dawar (3.57) and the lowest in Etay- El baroud (0.95).
- For Cadmium the highest was in Kafr-El Dawar (1.12) the lowest in Khorshid (0.120).

All the recorded values of lead exceeded the Polish permissible slandered (0.4/0.51 ppm), the Finland accepted levels of lead in food (0.3 ppm) and also, the national accepted limits in similar products (1.0 ppm) in Romania except the samples from Abu-Hommoss, Al-Muntazah and al-Delengat which recorded mean values 0.22, 0.23 and 0.25 pp, respectively. also, the mean value of cadmium was 0.51 ppm that exceeded the Polish permissible slandered and Trade Norms (0.05 ppm) and also, the Finland Norms and Kenya Bureau of Standard limits in food products (0.1 ppm).

Significant positive correlations had detected between cadmium and lead on pollen (r = 0.387), cadmium and copper (r = 0.442), lead and copper (r = 0.354) and zinc and iron (r = 0.041). Negative unsignificant correlations had detected between cadmium and manganese (r = – 0.219), cadmium and Iron (r = – 0.150), lead and each of zink, manganese and iron (r = – 0.301, – 0.253 and – 0.150, respectively). In addition, negative insignificant correlations had detected between manganese and copper (r = – 0.316) and between iron and copper (r = – 0.114).

The Daily Intake metals (DIM) of Iron (10 - 60 mg/day), zinc (15 mg/kg/ day), copper (2 – 3 mg/kg/day) and Manganese (0.5 – 5 mg/day) in the recommended human pollen diet (3.66 mg/diet). These values are lower than the permissible limit which recommended by World Health Organization (WHO, 1994).

Keywords: Beebread, heavy metals, Apis mellifera, Honeybee

Introduction

Pollution is the contamination of the environment by any agent (chemical, biological or physical) that modifies the natural characteristics of the atmosphere. In Egypt and most countries, pollution by chemicals and heavy metals were known as a distinct problems only in 10 to 20 years ago (**Moussa and Abdelkhalek, 2007**). One of the alternative approaches is the use of biological indicators to demonstrate the environmental pollution. Insects were used as bio-indicator in terrestrial ecosystem pollution (**Iqra *et. al*.2015; Osman *et. al*.2015**). Honeybee *Apis mellifera* L. seems to be a good test insect of pesticide and heavy metal pollution **(Celli, 1984; Jones, 1987; Rashed *et. al*., 2009; Roman, 2010** and **Van der Steean *et. al*., 2012)**. Also, honey bee products used as indicators for environmental pollution in many countries **(Jablonski *et al*., 1995**; **Leita *et al*., 1996; Conti, 2000; Fakhimzadeh and Lodenius, 2000 a & b; Przybyłowski and Wilczyńska, 2001; Conti and Botrè, 2001; Demirezen and Aksoy, 2005; Erbilir and Erdoĝrul, 2005; Freds &Montenegro, 2006; Frias *et. al*., 2008; Mbir *et. al*., 2011**; **Perugini *et. al.* 2011**and **Shair & Taher, 2012)**. Beebreed as honey bee product is a useful indicator of pollution, so many investigations on determination of the heavy metals (e.g Fe – Zn – Cu- As – Cd- Cr- Pb- Mn-Se) in beebread were carried out (**Bahreyni *et al*., 2006; Harmanescu *et al*., 2007; Szczêsna, 2007; Campos**

***et al.*, 2008; Roman, 2009; Morgano *et al.*, 2010; Popescu *et al.*, 2010; Moroń *et al.*, 2011; Anđelković *et al.*, 2012; Dima *et al.*, 2012; Iannotti *et al.*, 2012; Lambert *et al.*, 2012; Marco *et al.*, 2012; Morgano *et al.*, 2012; Silva *et al.*, 2012; Vincevica-Gaile *et al.*, 2012; Al Naggar *et al.*, 2013** and **Formicki *et al.*, 2013).**

The aim of this study is to analyze the beebread (stored pollen) samples, collected from 12 apiaries situated at different districts in Alexandria and El-Beheira governorates from March – September 2013; for determination of the environmental pollution with certain metals (Fe, Zn, Cu, Mn, Pb, Cd, As and Cr).

Materials and methods

1- Sample collecting:

The pollen samples (beebread) were collected during the period from March to September 2013 from 12 apiaries situated at different districts of Alexandria and El-Beheira governorates (Edko, El-Delengat, Abu-Hommoss, Etay El-Barud, Kafr El-Dawwar, Hawsh-Eissa, El-Tarh, Abis, Al-Tabia, Khorshid, Al-Muntazah, Ezbet-Mohsen.

Pollen samples were collected by cutting a piece of comb approximately 4 inch containing beebread (in both sides of the comb) by a disposable plastic knife. The samples were stored in plastic bags and kept in freezer at -20°C until use. The pollen were removed by breaking the comb, and then dried overnight at 105°C. After that the samples stored at refrigerator temperature till the time of analysis.

2- Sample preparation:

The certain metals analysis of beebread samples were carried out in Water and Soil Department, Faculty of Agriculture, El-Shatby, Alexandria University by using dry ashing technique (**Issac and Kerber, 1971**). One gram of sample in a crucible was placed in a preheated muffle furnace at 200 – 250 °C for 30 min, and then cooled down. Five ml of 6N HNO3 (nitric acid solution) was added. The solution was filtered through Whatman filter paper No.42 and <0.45 μm Millipore filter paper, then transferred quantitatively to a 25 ml volumetric flask by adding distilled water. The total heavy metals contents determination by flame atomic absorption spectrometry (F-AAS) with high-resolution continuum source. Eight metals were quantified for each pollen samples: Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Lead (Pb), Cadmium (Cd), Arsenic (As) and Chromium (Cr).

3- Instrument:

Analysis of heavy metals was made with Varian, spectra AA-220, Analytic-Jena device, by Flame atomic absorption spectrometry (F-AAS) in air/acetylene flame. The device working parameters (air, acetylene, optics and electronics) were adjusted for maximum absorption for each element. Acetylene was of 99.99 % purity. Under the optimum established parameters, standard calibration curves for metals were constructed by plotting absorbency against concentration.

In Egypt, there were not established maximum permitted levels for the elements featuring toxic properties (heavy metals) in bee pollen. Data of all pollen samples were evaluated and compared with those of Polish Norm PN-R-78893:1997, Trade Norm BN-89/9161-06:1989, Finland Norm (Fakhimzadeh and Lodenius, 2000b). Pollen composition and standardization by Campos et al. (2008), maximum national limits accepted for heavy metals in similar product excerpt Romanian Ministry of Public Health Ordinance No. 975/1998 (Ordinance no. 975, 1998), Kenya Bureau Standards (1996),

4- Calculation of daily oral intake of metals from pollen:

The estimation of daily oral intake was calculated according to **Cui *et al.*** (**2004**) as follow:

Daily Intake of Metals (DIM) = daily pollen consumption × average of metal concentration in pollen (mg/day).

The recommended amount of bee pollen in human daily diet must be about 36g (about 4 teaspoons); it would supply the body with every vital nutrient necessary to sustain life. Pollen is usually taken 3 times a day before eating (**Komosinska-Vassev, 2015**). The calculated values were compared with the standards of **WHO, 1994 and USAEPA, 2010**.

5- Statistical analysis:

The F-test was applied for analysis of variance. L.S.D., was used to determine the significant differences. Data were analyzed according to analysis of variance procedure using **Mstat-C Package (1989)**.

Results and Discussion

The purpose of investigation was to estimate the levels of contamination with heavy metals, i.e (Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Lead (Pb), Cadmium (Cd), Arsenic (As) and Chromium (Cr)) in beebread samples collected from 12 apiaries from Alexandria and El- Behaira Governorates during the period from March to September 2013.

Data presented in Table (1) showed that both chromium (Cr) and arsenic (As) in the present study were not detected by the applied method. Regardless of collection regions, data in Table (1) illustrated that iron (Fe) recorded the highest concentrations in all tested samples. It accounted as much as 57.5% of total

content of minerals determined in the samples, its values ranged between 51.59 and 184.92 ppm, with an average content value of 101.65 ppm. The largest values of iron were comparable with **Yossef *et al*.** (**1978**), who found that pollen relatively high in iron in Giza, Egypt. Zinc (Zn) followed iron with mean percent 23.96%, its values ranged from 18.23 to 130.21 ppm with an average value of 42.36 ppm. The third largest content percentage was Manganese (Mn) (12.54%), followed by Copper (Cu) with 5%. The Mn values ranged from 10.66 ppm to 46.46 ppm with mean value of 22.18 ppm. The concentration of Cu represented a moderate values ranged between 3.05 and 21.43 ppm with mean value of 8.83 ppm. The remaining elements: Pb and Cd were observed in very low concentrations and accounted jointly for about 0.99% of the total analyzed samples. Pb values ranged between 0.16 and 4.05 ppm with an average value of 1.24 ppm. While, Cd ranged from 0.09 to 1.87 ppm with a mean value of 0.51 ppm. With respect to their content in pollen, elements could be ranked in the following descending order: Fe > Zn > Mn > Cu > Pb > Cd.

The most dispersed mineral values were those recorded for Fe with variance reached 1099.94 ppm^2, followed by zinc with variance value 738.99 ppm^2. Whereas, the least dispersed mineral content values were those for Cd (0.124 ppm^2). Other measured minerals showed much dimensioned variations. Both of Fe and Zn showed the largest mean fluctuation with standard error values of ±5.528 and ±4.531 ppm, respectively. Meanwhile, the other measured metals showed a much diminished standard error.

Table 1. Minimum, Maximum, mean variance and standard error for the measured minerals in beebread over different locations ($mg.kg^{-1}$dry mass):

Mineral	Minimum (*ppm*)	Maximum (*ppm*)	Mean (*ppm*)	Variance	Standard error
Fe	51.598	184.916	**101.650**	1099.990	± 5.528
Zn	18.230	130.214	**42.359**	738.9933	± 4.531
Cu	3.056	21.428	**8.833**	26.423	± 1.438
Mn	10.66	46.46	**22.18**	74.412	± 0.857
Pb	0.162	4.052	**1.240**	0.999	± 0.167
Cd	0.091	1.870	**0.510**	0.124	± 0.059
As	**BLD**	**BLD**	-	-	-
Cr	**BLD**	**BLD**	-	-	-

BLD., Below limit of detection

Table (2), showed that all the measured minerals were significantly varied among locations (P≥0.01). The magnitude of detected mineral levels expressed as a percentage of the mean has presented as coefficient of variability (C.V.). All the minerals showed variable values among locations reached 7.46, 9.36, 10.40, 11.29, 25.94 and 45.55% of the corresponding overall location means for Zn, Mn, Cu, Fe, Pb and Cd, respectively. **Herbert and Miller-Ihli** (**1987**) noticed that Iron and Manganese were highly variable, according to different botanical origin.

Table 2. Analysis of variance for Fe, Zn, Cu, Mn, Pb, Cd, As and Cr levels on pollen grains as affected by different locations:

Source of variation	d.f.	Mean squares							
		Fe	Zn	Cu	Mn	Pb	Cd	As	Cr
Between locations	11	5212.7**	2329.4**	82.23**	227.4**	2.952**	0.277**	-	-
Error	24	131.6	9.990	0.843	4.309	0.103	0.054	-	-
C.V.		11.29%	7.461%	10.40%	9.36%	25.94%	45.55%	-	-

C.V.; Coefficient of variability.

* - Significant differences on a level of p≤0.05 between location.

** - Highly Significant differences on a level of p≤0.01 between location.

Table (3), showed that the highest level of iron was detected from Etay- El baroud with a mean value of 167.36 ppm and the lowest in Al-Muntazah with a mean value of 61.06 ppm.

Data presented in Table (3) revealed that, the highest levels of zinc (124.93 ppm) was in beebread samples collected from apiary in Abu-Hommoss, The lowest level was recorded from El-Tabya with a mean value of 21.95 ppm .

In general, iron (Fe) occurred at the highest concentrations in all tested samples followed by zink which is comply with **Harmanescu *et al.*, (2007a &b); Popescu *et al.*,** (**2010)** and **Dima *et al.*** (**2012**) in Romania; **Grembecka and Szefer** (**2013)** in Poland**; Fakhimzadeh and Lodenius** (**2000b**) in Finland; **Szczesna** (**2007**)in Korea; **Zhelyazkova *et al.*** (**2010**) in China and **Al Naggar *et al*.** (**2013**) in Egypt.

The highest concentration of copper (Cu) was detected from Kafr-El Dawar samples, with a mean value of 21.15 ppm and the lowest from Al-Muntazah, which had 3.12 ppm (Table, 3). The latter results are comparable with results for Copper which were reported by **Fakhimzadeh and Lodenius** (**2000**b); **Harmanescu *et al.*, 2007** and **Silva *et al.*** (**2012**) in Brazil. Also, **Al Naggar *et al.*** (**2013**) recorded the highest levels of copper (22.28 and 24.18 ppm) while, the lowest (0.95 and 1.90 ppm) in Kafr-El Zayat and Kafr-El Sheikh, respectively. **Szczesna** (**2007**) reported that copper content ranged from 5.60 to 23.90 ppm for the Poland samples, from 5.3 to 14.7 ppm for the Korea samples and from 3.20 to 10.0 ppm for the China samples with respective averages of 9.30, 10.10 and 7.50 ppm.

All the values obtained for copper contents in our study (Table, 3) were less than national accepted limits for this metal in similar products (200 mg/kg) (**Ordinance no. 975, 1998**), except the sample collected from Kafr El-Dawar (21.15 ppm) which exceeded the accepted limit. However, two samples collected from Al-Delengat and Abu-Hommos were greater than the Finland and Polish accepted level of copper for food (10.0 ppm) (**Fakhimzadeh and Lodenius 2000b**).

The pollen samples from Edko recorded the highest concentration of Manganese (44.87 ppm), while, the lowest value (13.02 ppm) was detected in samples from Kafr-El dawar (Table, 3).

Table 3. Means of Fe, Zn, Cu, Mn, Pb, Cd, As and Cr levels (mg/kg) in pollen samples of different locations from Alexandria and El-Behaira governorates:

Location	Minerals Levels (mg.kg^{-1})							
	Fe	Zn	Cu	Mn	Pb	Cd	As	Cr
Edko	118.67	42.367	5.120	**44.879**	0.966	0.145	N.D	N.D
Al-Delengat	88.959	27.109	11.236	15.456	0.245	0.245	N.D	N.D
Abu Hommoss	126.09	**124.932**	16.495	23.366	0.223	N.D	N.D	N.D
Etay El baroud	**167.362**	39.871	6.285	25.830	0.948	N.D	N.D	N.D
Al-Muntazah	61.064	35.069	3.125	17.638	0.231	N.D	N.D	N.D
Hosh eissa	120.171	38.725	9.240	20.563	0.490	0.196	N.D	N.D
Ezbet-Mohsen	81.304	40.962	5.033	25.646	1.183	0.477	N.D	N.D
Abis	145.829	57.749	6.564	17.398	2.221	0.616	N.D	N.D
El-Tarh	75.790	25.543	8.621	30.928	1.235	N.D	N.D	N.D
Khorshid	116.618	26.685	5.539	18.424	1.926	0.120	N.D	N.D
Al-Tabia	66.417	21.959	7.577	15.949	1.637	0.374	N.D	N.D
Kafr El-Dawar	68.201	27.003	**21.159**	13.024	**3.578**	**1.121**	N.D	N.D
L.S.D $_{0.05}$	9.366	2.581	0.749	1.694	0.262	0.189	N.D	N.D

N.D., not detected

Comparison between the present data and previous studies, indicated that, the concentration of Manganese in our samples were lower than those reported in pollen samples collected from South Korea and China with a mean value of 55.1 and 142.7 ppm, respectively (**Szczesna**, **2007**). Manganese concentration in pollen have been reported in the range of 21 to 73 ppm for industrial and Urban sites and from 68 to 110 ppm in rural, non-industrialized sites from Finland (**Fakhimzadeh and Lodenius, 2000 a &**b). **Dima *et al.*** (2012) reported that Manganese levels in samples collected from urban sites (43 – 134 ppm) were higher than those values from rural sites (21 – 52 ppm) of Dambovita region in Romania. **Zhelyazkova *et al*.** (**2010**) recorded low values of Mn (7.28 and 8.52 ppm) in pollen samples from two regions in Bulgaria. Also, the values obtained in our study were comparable

to those found in Poland (**Grembecka and Szefer**, **2013**) which had a mean of 1.9 mg/100g.

The maximum content of lead (Pb) was determined in beebread collected from Kafr El-Dawar with a mean value of 3.57 ppm, while the minimum content was from Abu-Hommoss (Table, 3). All these values exceeded the Polish permissible standard (0.4 – 0.5 ppm), the Finland accepted levels of lead in food (0.3 ppm) and the national accepted limits in similar products (1.0 ppm) in Romania. On the other hand, the lower concentrations of lead were detected in 3 samples collected from Abu-Hommoss, Al-Muntazah and Al-Delengat with means of 0.22, 0.23 and 0.25 ppm, respectively, their values were below the pre-mentioned permissible standards.

Some authors in Poland evaluated the degree of environmental pollution with lead on the basis of its detection in bees and their products, including pollen (**Free *et al.*, 1983; Konopacka *et al.*, 1993; Jablonski *et al.*, 1995** and **Roman, 2009**). They proved that lead amount in pollen load exceeded permissible standard and the highest levels were found in pollen coming from bee colonies situated very close to the road. **Loper *et al.*** (**1980**) conducted their research near the highway and recorded lead content in pollen on a level of 6 to 15 ppm. In addition, **Conti and Botre** (**2001**) proved relatively low lead concentration in pollen ranging from 0.020 to 0.332 ppm. On the other hand, **Al- Naggar *et al.*** (**2013**) found that lead levels in bee pollen collected during summer from bee hives in El-mehala El-kobra (10.53 ppm), Kafr-El Sheikh (13.85 ppm) and Kafr-El Zyat (10.13 *ppm*) considerably exceeded permissible standard, while, it was below the standard limit in Al- Fayoum (2.85 ppm).

The data obtained from the present study (Table, 3) revealed that, maximum concentration of cadmium was observed in samples collected from Kafr-El Dawar with mean value of 1.12 ppm, while, the minimum content was found in Khorshid (0.120 ppm). On the other hand, only four samples collected from Abo-Hommoss, Etay- El baroud, Al-Muntazah and El-Tarh were below detection limit.The average concentrations of this metal (Table, 1) in beebread (0.51 ppm) significantly exceeded permissible standard. It was 10 times higher than the Polish and Trade Norms (0.05 ppm) and about 5 times greater than the Finland Norms and Kenya Bureau of Standard limits in food products (0.1 ppm).

Cadmium possesses the ability to accumulate, especially in soil. Due to the presence of cadmium in pesticides and mineral fertilizer, its concentration in rural regions may be significant (**Winiarski**, **1997**). For that reason, in beebread samples coming from Kafr-El Dawar, the concentration of that element was observed higher than other analyzed samples (1.12 ppm).

Some authors in Poland have pointed out that the level of cadmium in most apiaries exceeded many times the acceptable norms (**Lipinska and Zalewski, 1989**; **Migula and Kafel**, **1992; Szczesna *et al.* 1993; Jablonski *et al.*, 1995**and **Roman**, **2007**). They detected considerable cadmium concentration in pollen ranging from 0.03 to 0.50 ppm. The highest level (0.181 ppm) was recorded in pollen loads collected in the apiary situated near the road.

A comparable values of cadmium in pollen was detected by **Fakhimzadeh and Lodenius** (**2000a**), which reported that mean values of cadmium was in the range of 0.03 to 1.20 ppm, it was above the Finland and Polish norms in 66 % of tested samples. On the other hand, lower values of cadmium than obtained in our study were reported by **Harmanescu *et al.*** (**2007a**), it was 0.01 ppm. Also, **Conti and Botre** (**2001**) had recorded low values for the cadmium in bee pollen (0.015 to 0.09 ppm). However, **Al Naggar *et al.*** (**2013**) reported higher level for cadmium levels in pollen collected from Al-Fayoum (1.38 ppm) during summer, all the levels were higher than maximum admitted levels.

The high level of the detected metals in beebread samples from Kafr El-Dawwar, Abis Ezbet-Mohsen, and Khorshid may be attributed to existing of sources of pollution to the apiaries. These pollution sources were drainage of agriculture land (<10m), Kafr El-Dawwar Agriculture highway (20m), El-mansheia el-gadeda Sewage treatment company (300m) and Misr Co. for synthetic silk. (≥ 3km) near by Kafr El-Dawwar location and Abis bridge and highway (1km), Cairo-Alex. Agriculture Highway (≥1.5km), Abis drainage of agric. (100m) and Automatic slaughterhouse (300m) closed to Abis apiary location.The expected sources of pollution for the samples collected from apiaries in El Tabia, Al-Muntazah and El-Tarh is their location near Alex.-Fert for fertilizers Co, Abu-Qir-El tabia main street(<1km) and Abu-Qir for fertilizers Co. (<1km). Pollution of other districts could be due to their situation close to the Cairo-Alex Agriculture highway.

Table (4) demonstrates the daily intake value (DIM) of (Fe), zinc, cu, Mn and Pb which were calculated by taking the average value of metals in all beebread samples and considering that each person (assuming 70 kg of body weight) consumes approximately 36 g (about 4 teaspoons) of pollen per day. It is noted that all DIM values of the studied metals are below the recommended standers by WHO (1994) and USAPA, 2010 except for manganese which lies in the range of recommendation. Therefore, it could be concluded that there is no health hazard associated with consumption of bee pollen in the studied districts.

Table 4. Estimated Daily Intake of Metals (DIM) through consumption of 36 gram (4 teaspoons) from bee pollen by human (Komosinska-Vassev *et al.,* 2015):

Trace elements	Average Conc. Of pollen (µg/g)	Intake of metals by human (mg/g)	RfD (mg/day)	References
Fe	101.65	3.66	10 – 60	WHO 1994
Zn	42.36	1.52	15	WHO 1994
Cu	8.83	0.318	2 – 3	WHO 1994
Mn	22.18	0.798	0.5 – 5	WHO 1994
Pb	1.24	0.045	0.245	WHO 1993
Cd	0.51	0.018	0.070	US EPA 2010

RfD is the oral dose for metal (mg/kg body weight/day)

Table (5), presented the correlation matrix between measured minerals pairs over the detected districts on bee pollen. Significant positive correlations were detected between cadmium and lead (r = 0.387), cadmium and copper (r = 0.442), lead and copper (r = 0.354) and zinc and iron (r = 0.041). Negative insignificant correlations were detected between cadmium and manganese (r = – 0.219), cadmium and Iron (r = – 0.150), lead and each of zink, manganese and iron (r = – 0.301, – 0.253 and – 0.150), respectively. Also, negative insignificant correlations were detected between manganese and copper (r = – 0.316) and between iron and copper (r = – 0.114).

Table 5. Correlation matrix between mineral apairs over the detected location for mineral content of pollen grains:

	Cd	Pb	Zn	Mn	Fe	Cu
Cd		0.387*	0.163$^{n.s.}$	-0.219 $^{n.s.}$	-0.150 $^{n.s.}$	0.442**
Pb			-0.301	-0.253	-0.179	0.354*
Zn				0.128	0.041**	0.310
Mn					0.220	-0.316
Fe						-0.114
Cu						

n.s – Non Significant coefficient. * - Significant coefficient of correlation at P0.05 level.
** - Highly Significant coefficient of correlation at P0.01 level.

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تقديرمحتوى بعض المعادن في خبز النحل من مناطق مختلفة من محافظتى الاسكندرية و البحيرة

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تم فى هذا البحث تقدير مستوى بعض المعادن في عينات من خبز النحل تم جمعها في الفترة من شهر مارس إلى سبتمبر 2013 من 12 منحل في مناطق مختلفة بمحافظتى الأسكندرية والبحيرة. وقد جهزت هذه العينات لتقدير أيونات المعادن الثقيلة لكلا من الحديد، الزنك، النحاس، المنجنيز، الرصاص، الكادميوم، الكروم والزرنيخ بإستخدام جهاز مطياف الإمتصاص الذرى للعناصر. وتم تقييم ومقارنة النتائج في عينات خبز النحل بالمعايير القياسية البولندية (1997) والفنلندية (2000) الموضوعة لحبوب اللقاح، وكذلك الحدود القصوي العالمية المصرح بها من العناصر الثقيلة في منتجات مشابهه في رومانيا (1998) وكذلك تم مقارنتها بالحدود اليومية المسموح للإنسان بتناولها من هذه العناصر والموضوعه من قبل منظمة الصحة العالمية(1994) .

أوضحت النتائج أن عنصرى الكروم والزرنيخ في عينات خبز النحل المختبرة كانت تحت مستوى التقدير بالطريقة المتبعة للتحليل. و قد شكل عنصر الحديد أعلى قيم في كل العينات المختبره. حيث كانت نسبتة 57.5%، يليه الزنك (23.96%) ثم المنجنيز (12.5%)، وأخيرا" عنصر النحاس (5%). وبالتالي فإنه أمكن ترتيبهم تبعا لمحتواهم في خبز النحل كما يلي: حديد > الزنك > منجنيز > النحاس > الرصاص > الكادميوم.

و قد سجلت القيم الاعلى و الادنى للعناصر محل الدراسة فى المناطق المختلفة كالتالى:

- كان أعلى مستوى من الحديد في عينة خبز النحل من منطقة إتاى البارود بالبحيرة بمتوسط قيمة 167.36 مجم/كجم. بينما كانت أقل قيمة تم تقديرها فى منطقة المنتزة بالأسكندرية بمتوسط 61.06 مجم/كجم. وبحساب الجرعة اليومية المسموح للإنسان بتناولها من الحديد وجد أنها (3.66 مجم/وجبة خبز نحل) وكانت أقل من الجرعه المسموح بها من قبل منظمة الصحة العالمية 1994 (10–60 مجم/يوم).
- كان أعلى مستوي من الزنك في عينات خبز النحل من مناطق أبوحمص (124.93 مجم/كجم). بينما كان أقل مستوى من هذا العنصر من عينات منطقة الطابية بمتوسط 21.95 مجم/كجم. تم أيضا حساب الجرعة اليومية المسموح للإنسان تناولها ووجد أنها (1.52 مجم/وجبة) وكانت أقل من الحد المسموح به من قبل منظمة الصحة العالمية 1994 (15 مجم/كجم/يوم).
- كانت أعلى قيمة للنحاس من منطقة كفر الدوار بمتوسط تقدر بـ 21.15 مجم/كجم. بينما كان أقل مستوى في عينات منطقة المنتزة (3.12 مجم/كجم). وأيضا قدرت الجرعة اليومية للإنسان (0.318 مجم/وجبة حبوب لقاح) وتحت الحد المسموح للانسان بتناوله من هذا العنصر من قبل منظمة الصحة العالمية 1994 (2–3 مجم/كجم/يوم).
- كان اعلى مستوى من المنجنيز (44.87 مجم/كجم) من منطقة إدكو. بينما أقل مستوى (13.02 مجم/كجم) في عينات منطقة كفر الدوار. وجد أن الجرعه اليومية المحسوبة للأنسان في هذه الدراسة تقدر بـ 0.79 مجم عند تناوله وجبة خبز نحل وهي أقل من الحدود المسموحه من قبل منظمة الصحة العالمية 1994 (0.5–5 مجم/يوم) .
- كان أعلى مستوى من عنصر الرصاص في عينات خبز النحل المجمعة من كفر الدوار و أقل مستوى كان من إيتاى البارود (0.95 مجم/كجم). و قد تعدت نسب الرصاص القياسية للمواصفات البولندية المسموح بها لحبوب اللقاح كمنتج (0.4–0.5 مجم/كجم)، وكذلك المواصفات القياسية الفنلندية لحبوب اللقاح (0.3 مجم/كجم). بينما كانت مقبولة أو في حدود النسب لمنتج شبيه والموضوعه من قبل دولة رومانيا (1.0مجم/كجم) . في المقابل تم تسجيل أقل مستويات من الرصاص عن المسموح بها دوليا فى ثلاث عينات بمتوسطات 0.22، 0.23 و0.25 مجم/كجم وذلك من مناطق أبو حمص، المنتزة والدلنجات، على التوالي.
- كان أعلى مستوى من عنصر الكادميوم من عينات خبز النحل لمنطقة كفر الدوار (1.12 مجم/كجم)، بينما أقل مستوى من عينات منطقة خورشيد (0.12 مجم/كجم). تم تقدير متوسط تركيز هذا العنصر في عينات خبز النحل (0.51 مجم/كجم)، حيث كان أعلى بمقدار 10 مرات عن الحدود المسموحه له في المواصفات القياسية البولندية لحبوب اللقاح (0.05 مجم/كجم)، وأعلى بمقدار 5 مرات عن نسبته في المواصفات الفنلندية وعن الحدود القياسية الكينية لهذا العنصر في الغذاء (0.1 مجم/كجم).

من نتائج التحليل الأحصائى وجد أنه توجد علاقة ارتباط إيجابية بين تواجد كل من الكادميوم والرصاص و بين الكادميوم والنحاس و كذلك الرصاص والنحاس وأيضا بين الزنك والحديد. بينما كانت علاقة الارتباط سلبية أو عكسية بين الكادميوم والمنجنيز و بين الكادميوم والحديد وأيضا بين الرصاص وكلا من الزنك، المنجنيز والحديد.