ESTABLISHMENT OF ANALYTICAL METHOD AND MONITORING FOR THE DETERMINATION OF SOME HEAVY METALS IN HONEY SAMPLES

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ABSTRACT

An analytical method for determination of the heavy metals; lead, cadmium, copper and mercury in honey sample was introduced for validation into the scope of the Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food.

The limit of quantitation of the heavy metals; Pb, Cd, Cu and Hg was ranged between 0.002 and 0.5 µg/g. The mean recovery ranged from 94.0 to 111.0 % at low spike level, and from 100.0 to 112 % in high spike level. The tested concentrations were found to be linear from the limit of quantitation; 0.02, 0.002, 0.50 and 0.01 µg/g up to 0.10, 0.03, 4.0 and 0.10 µg/g for Pb, Cd, Cu and Hg, respectively. The method was found to be precise, which the RSDr for all the heavy metals studied were less than 9% and they having a reproducibility of less than 12.1%. The expanded uncertainty (at 95% confidence level) was found to be 28%, 24%, 14%, and 26% for Pb, Cd, Cu and Hg, respectively. The applicability of the accredited method to routine analysis was continuously assessed by analyzing eightteen honey samples, which were collected from three different Governorates; El Sharquia, El Fayum and Bani Suwayf during year 2006. All the collected honey samples were free from any traces of Hg. Copper was the most abundant element present in all the honey samples. The detected amounts of Pb and Cd in the all collected honey samples were lower than the Maximum Limits (MRL).

INTRODUCTION

Validity is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data (ISO 17025, 2005) validation of analytical method is recognized as a potentially week link in the quality chain of laboratories. The validation procedure needs to be considered the context of fitness for purpose and cost and cost benefit criteria.

Levels of heavy metals in the environment have recently increased as a consequence of human and industrial activity. Heavy metal pollution in environment is one of the important problems for human (Deming 2005; Mikkelsen *et al.,* 2005 and Yaman 2005). Thus, a study of environmental pollution in a specific zone, involves the evaluation of heavy metals in water, soil, vegetables and food stuff. Honey as food stuff used for healing purposes must be safe or free from any pollutants, such as heavy metals. In Poland, large amounts of heavy metals were found in honeys from hives located near

extra urban cross roads and steel works (Przybylowski and Wilczynska, 2001). Heavy metals, such as lead (Pb) has been widely studied due to its continuous assimilation in small quantities which causes toxic effects. Lead is released into the atmosphere from industrial sources (manufacturing operations, recycling efforts and automobile emissions from leaded fuels (Baird, 1998). Lead is still detected in dust and it has been incorporated in the trophic chain even through lead emissions from cars have recently decreased (Rodriguez *et al.*, 2004).

The determination of traces heavy metal ions in environmental samples is restricted by two main difficulties; the very low concentration of heavy metal ions, which may be lower than the detection limit of many analytical techniques and interfering effects of the matrix (Tuzen and Soylak, 2005). Various methods are recommended for the enrichment and separation of heavy metal ions from a sample matrix including; ion exchange, solvent extraction, cloud point extraction, electro-analytical techniques and membrane filtration (Ma *et al.*, 1994; Kabay *et al.*, 1998 and Saracoglu *et al.*, 2001). The evaluation of heavy metals contents in honey has a two fold significance; the former one lies in the toxicity of theses metals, with the consequent necessity to develop adequate analytical procedures for their monitoring. The latter one is suggested by the possibility of using bees and their products as bioindicator of possible environmental pollution (Munoz and Palmero, 2006). The present study aimed to study validity of analytical method for determination of Pb, Cd, Cu and Hg in honey samples and the detection of their levels in the samples collected from different apiaries located in three Egyptian governorates (El Sharquia, El Fayum and Bani Suwayf).

MATERIALS AND METHODS

1-Sampling:

Eighteen honey samples were collected from apiaries located in three Egyptian governorates (El Sharquia, El Fayum and Bani Suwayf) during 2006.

2-Method of analysis:

Two g of sample was weighed into the microwave digestion vessel, added to 5 ml of concentrated nitric acid (65%) and shake gently. After waiting for 30 min, 2 ml of hydrogen peroxide (30%) were added. One reagent blank and one spike sample must include in every set of samples. Each microwave digestion vessel was sealed carefully and placed in its holder in microwave oven. The thermocouple probe was introduced in reference vessel and closed the oven door. The microwave oven program was adjusted as the following.

Table (1): Microwave oven program

No. of Step	1	2	3	4	5	6	7	Venting
Power (watt)	2	0	300	0	400	0	600	0
Time (min.)	5	10	15	5	15	5	15	5

This program is adapted on the conditions that 10 samples are being digested.

At the end of heating cycle, the vessels were cooled inside microwave cavity about 5 min. The thermocouple probes were removed from the reference vessels, the vessels were cooled in a water bath for about 30 min. and then the vessels were opened carefully. The down lid and the walls were rinsed with de-ionized water inside the vessel. The residual solution was transferred in 50 ml volumetric flask with de-ionized water and completed to marked volume. The reagent blank was treated with the same way. The samples were kept in polypropylene tubes until analyzed by Atomic Absorption Spectrometer (AAS).

2.1. Atomic Absorption measurement

The instrument was started, calibrated by calculating of CM (Characteristic Mass) and programmed according to the instructions of the manufacture. Table (2) described the instrumental parameters of Unicam 929 AAS used in the determination of Pb, Cd, Cu, and Hg.

Table (2): Instrumental Parameters of Atomic Absorption Spectrometer (AAS).

Parameter	Pb	Cd	Cu	Hg
Technique.	Graphite	Graphite	Flame	Cold vapor
Wave length (nm)	217.0	228.8	324.8	235.7
Slit band pass (nm)	0.5	0.5	0.5	0.5
Lamp current %	75% - 100%	75%-100%	75% - 80%	75%- 100%
Signal type.	Transient	Transient	Continues	Continues
Back ground correction	On	On	On	On
Inert gas.	Argon	Argon	-	Argon
Heating source.	Electro thermal	Electro thermal	Air – acetylene with flow rate (1:1 l/min.)	No heating

Table (3): Furnace program for Pb and Cd

Phase	Temp. (^{0}C)		Time (sec.)		Ramp (^{0}C/Sec.)		Gas type		Gas flow (ml/min)	
	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd
Drying	130	130	40	35	10	30	3	3	2	2
Ashing	800	800	20	20	30	50	3	3	2	2
Atomization	1800	1800	3	3	0	0	3	3	0	0
Cleaning	2500	2500	3	3	0	0	3	3	2	2
Cooling	20	20	5	5	0	0	3	3	3	2

Table (4): Cold vapor system parameters for Hg

Flow rate	Baseline delay	Stabilize delay	Reductant
200-300 ml/min	20-30 sec	30-40 sec	Acidified borohydride solution 1 % (w/v)

2.2- Calibration

The calibration curves of Pb, Cd, Cu, and Hg must be constructed with every set of samples. The concentration levels of calibration curve were 5, 10, 20, and 30 µg/L for Pb, 0.5, 1, 2, 3 µg/L for Cd, 0.1, 1, 2, 4 mg/L for Cu and 5, 10, 20, 40 µg/L for Hg. The metal contents of each element in the sample were calculated from the following formula:

X = (a -b) vd / m
X = metal content in sample (mg/ kg)
a = metal content in measuring solution (mg/l)
b = metal content of reagent blank solution (mg/l)
v = volume of sample solution (ml)   d = dilution factor
m = weight of sample (g)

3- Validation procedure
3-1-Limit of quantitation (LOQ)

The limit of quantitation is the minimum concentration of analyte in the test sample that can be determined with acceptable recovery and precision under the stated conditions of the test. The lowest practical limit of quantitation was estimated by performing repeated spike samples at about the added lowest quantitation level on honey samples. Limit of quantitation was expressed as relative standard deviation (RSD %).

$$RSD\% = \frac{S}{\bar{x}} \times 100$$

S = Standard deviation

$\bar{x}$ = Mean of found concentration in (*n*) samples

3-2-Recovery test

The honey samples were spiked at two levels of heavy metals and their recoveries were studied. All analyses were carried out in at least six replicates. Spike recoveries were determined by adding the heavy metals to a control honey sample at a final concentration of 0.02 or 0.10 µg/g for Pb, 0.002 or 0.03 µg/g for Cd, 0.5 or 4.0 µg/g for Cu and 0.1 or 0.10 µg/g for Hg and analyzing the spiked honey using the method of Anonymous (2003). The relative standard deviation (RSD %) was calculated according to the previously equation.

3-3- Linearity

Linearity was tested by performing recovery tests at concentrations of used heavy metals in honey samples. The correlation between the concentration and response of each heavy metal was studied.

3-4-Repeatability

The repeatability RSDr (intra-assay precision) was measured by comparing standard deviation of the recovery percentages spiked honey samples run at the same day. Honey samples were spiked at 100 ug/kg for Pb, 50 ug/kg for Cd, 2.5 mg/kg for Cu and 0.10 mg/kg for Hg. Replicated (*n*=8) samples were all run and the RSD value was calculated for each element.

3-5-Reproducibility

In this study intra-laboratory reproducibility was considered, spiking honey samples were analyzed in several days. The reproducibility (RSD$_R$) (as between – day precision) was determined by analyzing spiked honey samples for several alternative days. Replicated (*n*=15) samples were all run and the RSD$_R$ value was calculated for each element according to the following equation. The fortified samples, at levels 100 ug/kg for Pb, 30 ug/kg for Cd, 4 mg/kg for Cu and 0.1 mg/kg for Hg were analyzed 15 times by different operators at different times.

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RSD$_R$% = (SD / Mean recovery) x 100
SD = Standard deviation

3-6-Measurement uncertainty

Uncertainty is a parameter associated with the results of a measurement, which characterizes the dispersion of the values that could reasonably be attributed to the measured value. The parameter may be, for example, a standard deviation or the width of a confidence interval (Anonymous 2003**).** In estimating the overall uncertainty, it may be necessary to take each source of uncertainty and treat it separately to obtain the contribution of each source. Each of the separate contributions to uncertainty is referred to as an uncertainty component. When expressed as a standard deviation an uncertainty component is known as standard uncertainty. The total uncertainty, combined standard uncertainty**,** equals to the positive square root of the sum of the squares of the individual uncertainty components. For most purposes in analytical chemistry, an expanded uncertainty should be used. The expanded uncertainty provides an interval within which the value of the measured is believed to lay a higher level of confidence.

- Standard uncertainty

The following equation is used for standard uncertainty calculations:

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

S = Standard deviation $\qquad$ x_i = Found concentration in samples

$\bar{x}$ = Mean of found concentration in n samples $\qquad$ *n* = Number of samples

- Combined uncertainty (U$_C$)

The following equation is used for combined uncertainty calculations

$$U_C = \sqrt{(U_p)^2 + (U_{Rec})^2 + U_{Ref}}$$

- Expanded uncertainty

Expanded uncertainty was obtained by multiplying the combined uncertainty, by a coverage factor k, for confidence level of 95% k is 2.

RESULTS AND DISCUSSIONS

I- Validity of analytical method for determination of heavy metals in honey samples:

In recent studies, honey has been proposed as an environmental marker in order to evaluate heavy metals over a wide area where beehives are placed. Honeybees may forage in a variety of environments. Hence they effectively sample their surroundings for the constituents in or on forage plants, soil and atmosphere of a specific area. Honey could be used as indicator of soil, plants and air pollution.

1- Limit of quantitation :

The limit of quantitation was calculated after analyzing eight or nein replicates. The lowest level of Pb, Cd, Cu and Hg were 0.02, 0.002, 0.5 and 0.01 µg/g and their recoveries were 108, 104.5, 94.0 and 111.0%, respectively. The relative standard deviations (RSD%) of Pb, Cd, Cu and Hg were 15.0, 11.2, 16.0 and 7.0 %, respectively. Elements with the highest coefficient of variability (RSD%) were Pb and Cu, while Hg and Cd exhibited the lowest RSD %. The limit of quantitation ranged between 0.002 and 0.5 µg/g. The limit of quantitation was set separately for each metal (Table 5).

Table (5): Limits of quantitation of Pb, Cd, Cu and Hg in honey samples.

Element	Spiked level (µg/g)	Number of replicates	Mean recovery (%)	RSD (%)
Lead	0.02	8	108.0	15.0
Cadmium	0.002	9	104.5	11.2
Copper	0.500	9	94.0	16.0
Mercury	0.010	9	111.0	7.0

2- Recovery test:

The low and high spiked concentrations of Pb, Cd, Cu and Hg revealed mostly the similar mean of recoveries. The mean recovery ranged between 94.0 to 111.0 % at low spike level, while it was from 100.0 to 112.0 % in high spike level. In general, recoveries were more variable as indicated by RSD% values at lower concentration of heavy metals. In contrary, the values of relative standard deviation (RSD%) at the high concentration of Pb, Cd, Cu and Hg were low and ranged from 2.0 to 9.0% (Table 6).

Table (6): Recovery percentage and relative standard deviation at two levels of concentrations of Pb, Cd, Cu and Hg.

Metals	Spiked level (µg/g)	No. of replicates	Mean recovery (%)	RSD (%)
Lead	0.02	8	108	15.0
	0.10	8	102	9.0
Cadmium	0.002	6	105	11.2
	0.030	6	105	6.8
Copper	0.50	9	94.0	16.0
	4.00	9	100	2.0
Mercury	0.01	6	111	7.0
	0.10	6	112	3.0

3- Linearity

Method linearity was conducted by managing recovery test of used heavy metals at different concentrations in honey samples. The tested concentrations were found to be linear from the limit of quantitation; 0.02, 0.002, 0.50 and 0.01 µg/g up to 0.10, 0.03, 4.0 and 0.10 µg/g for Pb, Cd, Cu and Hg, respectively. The tested levels were covered the routinely found concentrations and the maximum limits (ML's) for each element in honey sample.

4- Repeatability

Honey samples were spiked at levels 100 ug/kg for Pb, 50 ug/kg for Cd, 2.5 mg/kg for Cu and 0.10 mg/kg for Hg. Replicated (n=8) samples were all run and the RSD% value was calculated for each metal. The mean Recovery percentage of tested heavy metals varied between 95.9 and 104.5% as shown in Table (7). The method was found to be precise, which the RSDr % for all the heavy metals studied was less than 9%.

Table (7): Recovery (%) and repeatability (RSDr %) of the heavy metals; Pb, Cd, Cu and Hg at different spiking levels (n =8).

Replicate	Lead		Cadmium		Copper		Mercury	
	Amount (ug/kg)	Recovery (%)	Amount (ug/kg)	Recovery (%)	Amount (mg/kg)	Recovery (%)	Amount (mg/kg)	Recovery (%)
1	101	101	53	106	2.4	96	0.10	100
2	93	93	51	102	2.4	96	0.10	100
3	95	95	51	102	2.4	96	0.11	110
4	92	92	51	102	2.5	100	0.10	100
5	108	108	56	112	2.4	96	0.10	100
6	101	101	52	104	2.4	96	0.09	90
7	81	81	52	104	2.5	100	0.10	100
8	---	---	52	104	2.5	100	0.09	90
Mean	95.857	95.857	52.25	104.500	2.437	97.500	0.100	98.750
SD	±8.611	±8.611	±1.669	±3.338	±0.051	±2.07	±0.006	±6.409
RSDr(%)	8.983		3.194		2.123		6.490	

RSDr % = (SD / Mean recovery) x 100

5- Reproducibility:

The fortified samples, at levels 100 ug/kg for Pb, 30 ug/kg for Cd, 4 mg/kg for Cu and 0.1 mg/kg for Hg, were analyzed 15 times by different operators at different times. The mean recovery of Pb, Cd, Cu and Hg was 102.5%, 99.3%, 98.8% and 106.7%, respectively. The highest reproducibility (RSD_R) value of recoveries was observed with Cd (12.1%), while the lowest one was with Cu (6.6%). The precision can be considered optimal having a reproducibility of less than 12.1%.

6- Measurement uncertainty

6-1-Standard uncertainty

Validation studies were used to quantify different uncertainty components. The random effects were estimated as the relative standard deviation of replicates of real contaminated samples. Standard uncertainty due to repeatability experiments (U_r), expressed as relative standard deviation was found to be less than 10 % (9%, 3%, 2%, and 6% for Pb, Cd, Cu and Hg, respectively). The standard uncertainty due to the reproducibility experiments (U_R), was found to be 10.4%, 12.0%, 6.6%, and 9.8% for Pb, Cd, Cu and Hg respectively.

6-2-Combined uncertainty (U_C)

Combined uncertainty, is the positive square root of the sum of the squares of different uncertainty components. It was found to be 14%, 12 %, 7 % and 13 % for Pb, Cd, Cu and Hg, respectively.

6-3-Expanded uncertainty

Expanded uncertainty is obtained by multiplying the combined uncertainty, by a coverage factor k, for confidence level of 95% k is 2. The expanded uncertainty (at 95% confidence level) was found to be 28%, 24%, 14%, and 26% for Pb, Cd, Cu and Hg, respectively (Table 9).

Table (8): Recovery (%) and reproducibility (RSD$_R$%) of the heavy metals; Pb, Cd, Cu and Hg at different spiking levels (n =15).

Replicat.	Lead		Cadmium		Copper		Mercury	
	Amount (ug/kg)	Recovery (%)	Amount (ug/kg)	Recovery (%)	Amount (mg/kg)	Recovery (%)	Amount (mg/kg)	Recovery (%)
1	79	79	33	110	3.8	95.0	0.11	110
2	99	99	33	110	3.8	95.0	0.10	100
3	95	95	35	116.7	3.9	97.5	0.10	100
4	113	113	35	116.7	4.0	100.0	0.12	120
5	109	109	28	93.3	3.9	97.5	0.11	110
6	120	120	29	96.7	3.8	95.0	0.11	110
7	108	108	28	93.3	3.6	90.0	0.11	110
8	94	94	32	106.7	3.6	90.0	0.10	100
9	115	115	30	100.0	4.0	100.0	0.11	110
10	103	103	29	96.7	4.2	105.0	0.08	80
11	98	98	31	103.3	4.2	105.0	0.11	110
12	93	93	31	103.3	3.8	95.0	0.11	110
13	109	109	24	80.0	3.9	97.5	0.12	120
14	108	108	24	80,0	4.2	105.0	0.11	110
15	94	94	25	83.3	4.6	115.0	0.10	100
Mean	102.5	102.5	29.8	99.3	3.95	98.83	0.107	106.7
SD	±10.669	±10.669	±3.589	±11.977	±0.261	±6.537	±0.009	±9.759
RSD$_R$(%)	10.412		12.057		6.614		9.759	

RSD$_R$% = (SD / Mean recovery) x 100

Table (9): Summary of uncertainty estimation of Pb, Cd, Cu and Hg

Metal	Standard uncertainty						(U_C)*	($2 \times U_C$)**
	Repeatability			Reproducibility				
	No.	Mean Conc. (ug/kg)	U_r	No. replicates	Mean Conc. (ug/kg)	U_R		
Pb	7	95.9	9	15	102.5	11	14	28
Cd	8	52.3	3	15	29.8	12	12	24
Cu	8	2.4	2	15	4.0	7	7	14
Hg	8	0.10	6	15	0.11	10	13	26

* (U_C) = Combined uncertainty   ** ($2 \times U_C$) = Expanded uncertainty

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be validated before their introduction into routine use. This methodology showed to be very simple, rapid and requiring small amounts of honey sample (2g). also depending on that the honey samples were digested in closed vessels in microwave oven using mixture of conc. nitric acid and hydrogen peroxide. The digested solution was diluted with water and the metal contents were determined by atomic absorption spectrometry.

II- Monitoring of heavy metals in honey samples:

The applicability of the validated method to routine analysis was carried out by analyzing eighteen honey samples, which collected from three

different Egyptian governorates (i.e. El Sharquia, El Fayoum and Beni Suief) during 2006. All samples were subjected to the four metals analysis. Maximum Limits (ML's) of Codex Committee of Food Additives and Contaminants (CCFAC, 1993) were used as a point of reference for Pb, Cd, Cu, and Hg.

Table (10) demonstrated that most of samples analyzed contained Pb, Cd, and Cu. However, Hg was not detected in all analyzed samples. Data showed that copper was the highest frequently detected metal, where it was detected in all samples analyzed (i.e the contamination percentage was 100%), followed by Pb and Cd which were detected in 83.3% and 33.3% of all samples analyzed, respectively. No exceeding of the levels of Cd and Pb above their maximum Limits (ML's). However, the maximum limit of Cu (1000 µg/kg) was exceeded in only one sample which collected from Beni Seuif (i.e. the violation percentage was 5.5% out of 18 samples analyzed).generally the mean concentration of Cu ranged from 377 µg/kg to 738 µg/kg. The highest mean level was recorded in the samples taken from Beni Seuif. The lowest Cu was found in the samples collected from El Fayoum.

Table (10): Minimum, maximum, mean in ug/kg of the detected elements, number and percentages of contaminated sample, and violated element in analyzed honey samples collected from three Egyptian governorates during 2006.

Governorate	Total no. of samples analyzed	Analyzed element	Contaminated samples with each element		ML's (µg/kg)	Minimum conc. in (µg/kg)	Maximum Conc. In (µg/kg)	Mean Conc. In (µg/kg)	Violated element	
			No.	(%)					No.	%
El Sharquia	3	Pb	3.0	100.0	80	9.81	28.1	18.2	0.0	0.0
		Cd	1.0	33.3	8.0	0.88	0.88	0.88	0.0	0.0
		Cu	3.0	100.0	1000	350	540	433	0.0	0.0
		Hg	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0
El Fayum	7	Pb	5.0	71.4	80.0	0.16	77.0	26.67	0.0	0.0
		Cd	3.0	42.9	8.0	0.25	0.54	0.40	0.0	0.0
		Cu	7.0	100.0	1000	104.0	750.0	377.0	0.0	0.0
		Hg	0.0	0.0	10	0.0	0.0	0.0	0.0	0.0
Bani Suwayf	8	Pb	7.0	87.5	80.0	0.55	68.0	28.5	0.0	0.0
		Cd	2.0	25.0	8.0	1.45	1.95	1.7	0.0	0.0
		Cu	8.0	100.0	1000	100.0	1130.0	738.0	1	13
		Hg	0.0	0.0	10	0.0	0.0	0.0	0.0	0.0
Total	**18**	**Pb**	**15**	**83.3**					**-**	
		Cd	**6**	**33.3**					**-**	
		Cu	**18**	**100**					**1**	**5.6**
		Hg	**0**	**0**					**-**	**-**

The highest contamination percentage of Pb detected in the samples collected from El Sharquia governorate. However, the lowest contamination percentage recorded in the samples from Beni Seuif. The mean concentration of Pb ranged from 18.2 µg/kg to 28.5 µg/kg. the highest mean concentration value recorded in samples collected from Beni Seuif, while the lowest mean detected in the samples from El Sharquia. Also no exceeding of the levels of Pb in the samples above its Maximum Limit (80 µg/kg)

The contamination percentage of Cd ranged from 25% to 42.9%. The highest contamination percentage was observed in the samples collected from El Fayoum, while the lowest percentage detected in the samples from Beni Seuif. The mean. No increasing of Cd levels in the samples above its maximum Limit (8 µg/kg)

Bani Suief samples revealed more concentrations by the detected metals as compared with the other two governorates. This could be due to the presence of apiaries nearly from polluted and industrial areas. However, honey may not be the most sensitive tool for evaluating the environmental contamination with heavy metals due to the low concentration present and the great variability caused by several factors, e.g. botanical origin, floral density, season of the year and rainfall Fredes and Montenegro, (2006). Tuzen et. al., (2007), studied the metal contaminations in honey in Turkey. They found that the contamination levels in honey samples collected from the West Anadola was higher than those collected from the East Anadolia of Turkey. Because of the industry has been well in West Anadola and possibly apiaries were located at a distance not far from the polluted habitat and the East Anadolia doest have industrially polluted apiaries. On other hand, Braziewicz et. al., (2002), determined trace element concentrations in three places of Poland; in the centre of Warsaw (a highly polluted region) about 100 km east of Warsaw and 70 km southwest of Warsaw (as regions free from industry). They reported that the concentrations of trace elements were similar in the honey samples collected from these places and the honey was not useful as a bioindicator of air or ground pollution. On comparison our results with another studies, we found that the minimum and the maximum levels of both of lead and cadmium detected in samples collected from three governorates were lower than those reported by Tuzen and Duran (2002) and Tuzen *et. al.*, (2006).

Conclusion:

The established method was found to be precise, which the RSDr for all the heavy metals studied were less than 9% and they having a reproducibility of less than 12.1%. The expanded uncertainty (at 95% confidence level) was found to be 28%, 24%, 14%, and 26% for Pb, Cd, Cu and Hg, respectively. The applicability of the accredited method to routine analysis was assessed by analyzing eighteen honey samples, which were collected from three different Governorates; El Sharquia, El Fayum and Bani Suwayf during year 2006. The results showed that heavy metals concentration levels in the honey samples collected from different regions were generally conformable. The copper was the most frequently detected element, followed by lead and cadmium. However, no contamination with Hg has been recorded. No exceeding of the limits of both of lead and cadmium in all analyzed honey samples collected from three governorates. While the copper limit was exceeded in only one sample, where the violation percentage was 5.6% of the total number of samples analyzed.

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تطوير طريقة تحليل وتقصي مستو بعض العناصر الثقيلة في عينات عسل النحل

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تم استخدام طريقة تقدير العناصر الثقيلة في عسل النحل بعد أجراء خطوات أثبات كفاءة الطريقة (validation) ضمن متطلبات المعمل المركزي لتحليل متبقيات المبيدات والعناصر الثقيلة في الأغذية وكانت حدود التقدير الكمي للعناصر الثقيلة (الرصاص – الكادميوم – النحاس –الذئبق) يتراوح بين ٠,٠٠٢ و٠,٥مج/كجم ومتوسط كفأه الاسترجاع يتراوح بين ٩٤-١١١% للتركيزات القليلـة و يتراوح بـين ١٠٠-١١٢% للتركيزات العاليـة وأثبتت الطريقـة خطيـة عند التركيزات المختلفة من حدود التقدير الكمي ٠,٠٠٢-٠,٠٢ - ٠,٥ و ٠,٠١ مج/كجم للرصاص والكادميوم والنحاس والذئبق على الترتيب أعطه الطريقة انحراف معياري أفل من ٩% وبعد أن تم أجراء أثبات كفاءة الطريقة (validation) تم اعتماد هذه الطريقة من هيئة الاعتماد الفنلندية واستخدمت هذه الطريقة في تقصي مستو العناصر الثقيلة في ١٨ عينـة جمعـة من ثـلاث محفظـات هـى الفيوم والشرقية وبني سويف خلال عام ٢٠٠٦

كل العينات كانت خاليه من عنصر الذئبق HG بينما النحاس كان موجود في كل العينات وعناصر الرصاص والكادميوم كانت موجودة فى كل العينات بقيم أقل من MRL.