

Honey as a bio indicator of the environmental pollution and its risk assessment in El-Minya province

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Abstract

Honey is a very nutritive food substance with highly therapeutic properties and wide range usage. The probability of heavy metals exposure *via* honey consumption is high particularly if honey collected from polluted areas either agricultural or industrial one. In the current investigation the concentration of Copper (Cu), Iron (Fe), Zinc (Zn), Lead (Pb), and Cadmium (Cd) were determined in honey samples which collected from different areas in El-Minya province by Atomic Absorption spectrophotometer (Agilent Technologies 200 series AA/240 FS AA), beside their risk analysis if they ingested with the contaminated honey. The results were revealed that the concentration of Cu, Fe, Zn, and Pb were lower than their proposed permissible limits by the Egyptian Organization Standard (EOS), whilst Cd element was not detected. Moreover, their risk assessment through the Target Hazard Quotients (THQ), the estimated weekly (EWI) and daily intakes (EDI) were reported to be less than the recommended levels by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In conclusion; honey samples from the investigated areas in El-Minya province were proved to be safe for human consumption because of the low heavy metal content (Cu, Fe, Zn, Pb, and Cd) and no potential public health risks were identified.

Keywords: Health risk; Copper; Iron; Zinc; Lead; Cadmium; Honey.

1. Introduction:

Biological monitoring is constantly concerned with surveying the state of environmental contamination as animal and plant species are able to give valuable data. In this regard, the use of bio environmental pollution sensors, such as honey bee products, over chemical or physical detectors, may seem to have the priority (Strzyż, 2004). Bees, especially foragers, readily occupy all environmental parts (soil, plants, air, and water) and are manageable insects that provide a significant amount of material for sampling and study (Conti and Borté, 2001).

Anthropogenic activities produce contaminants, which their quantity and toxicity exceed the homeostatic capacity of the environment to overcome it. Thus, the systematic analysis and monitoring of the environment is extremely urgent (Bargańska *et al.*, 2015).

Honey is a sweet natural substance and is derived from the secretion of living plant parts, the nectar of blossoms by honey bees (Sohaimy *et al.*, 2015). The classification, characterization and medicinal importance of the honey have been introduced by several researches (Gregorio *et al.*, 2019). Honey has possible medicinal properties for wound healing, leg ulcers, infections, and cancer and has antibacterial properties (Abeshu and Geleta, 2016).

Unorganized and rapid urban and industrial developments leading to increasing the levels of heavy metals in developing countries such as Egypt (Radwan and Salama, 2006), Iran (Maleki and Zarasvand, 2008), China (Wong *et al.*, 2003), and India (Sharma *et al.*, 2008a). The hairy bodies of pollen could be contaminated from the loaded heavy metal in air that could be returned to the hive, or they could be absorbed along with the flowers' nectar, or through the honeydew or the water (Porrini *et al.*, 2003).

Heavy metals are environmentally non-biodegradable persistent pollutants that can be accumulated on the surfaces then absorbed into the vegetable tissues. By absorbing heavy metals from deposits in areas of plants exposed to air from toxic conditions and contaminated soils, plants accumulate the heavy metals (Sharma *et al.*, 2008b).

Ingestion of repeated small quantities of heavy metals resulting in gradual and permanent accumulation of these elements in different body parts which triggering serious health problems such as cirrhosis of the liver, renal dysfunction, human hypertension, central, and peripheral nervous system neuropathy, diabetes mellitus, gastroenteritis, osteomalacia and anemia (Eife *et al.*, 1999).

For example; Pb poisoning primarily affects the gastrointestinal tract and the central nervous system (Markowitz, 2000), Cd exposure has been linked to "Itai-itai" sickness, a bone fracture epidemic in Japan (Nishijo, *et al.*, 2017). Cu toxicity can cause intravascular hemolysis, cardiac and renal dysfunction, hepatic necrosis, methemoglobinemia, rhabdomyolysis, encephalopathy, and mortality in its most severe forms (Harris, 2000). While hematemeses, peritonitis, coagulopathy, cardiomyopathy, hepatic dysfunction, renal failure (Baranwal and Singhi, 2003) and cancer could be caused by Fe toxicity (Bhasin *et al.*, 2002). On the other hand an acute respiratory distress syndrome (ARDS) (Murray *et al.*, 2018), metal fume fever, (Greenberg and Vearrier, 2015), and neurological disturbances were resulted from Zn toxicity (Sheqwara and Alkhatib, 2013).

The concentrations of certain metals in honey (Cd, Barium (Ba), Cu, Aluminium (Al), calcium (Ca), manganese (Mn), Pb, Zn, and nickel (Ni)) have been shown to be increased in chemical waste and exhaust fumes generated by mines and steelworks, in industrial and urban areas, or in motorway vehicles close to the bees (Stankovska *et al.*, 2008). Thus, the aim of this study is identifying and measuring the concentration of Cu, Fe, Zn, Pb, and Cd as

contaminants in honey samples from El-Minya province with identifying the possible health hazards.

3. Material and methods:

3.1. Study area:

The bio monitoring study was performed in spring and summer seasons during the year 2019 and 2020 on eight different sites in El-Minya province (Fig. 1). Site one, El Mahras, is located in Mallawi center. The second is Twah is located in El Minya center. The third is Demshaw-hashem is located in Elminya center. The fourth is Kom-Elloufy is located in Samalut. The fifth is Shosha is located in west of Samalut center. The sixth is El-Qees is located in Beni mazar center. The seventh is Manshyet menbal is located in Matay center. The eighth is Etledem is located in AbuQurqas.



Figure (1):Centers of El-Minya province.

3.2. Sampling:

In order to avoid any kind of metal contamination, fresh honey samples harvested from bee hives during spring and summer, they are the routine honey production seasons in Egypt. A total number of 48 samples include different types of honey (clover, corn, sunflower, anise, marjoram, caraway and basil) were collected (2019-2020) from beekeepers and stationary apiaries that located in the previously mentioned 8 districts of El-Minya province (6 samples from each). Samples were collected in dark plastic cubs using plastic gloves and wooden sticks then stored at 4°C till analysis.

3.3. Determination of heavy metal concentration (Cu, Fe, Zn, Pb, and Cd):

3.3.1. Digestion of honey samples:

Samples were prepared according to 920,180 method of the AOAC (2000). Five mL of 75 (w/v) % HNO₃ were added to each 2 gm honey within a porcelain crucible. The acid was then evaporated on an electrically heated hot plate at 100-120 °C, and then it was gauged to 10 mL with distilled water (dilution 1:5).

3.3.2. Analytical procedures:

The detection and estimation of the metals were carried out by using Atomic Absorption spectrophotometer (Agilent Technologies 200 series AA/240 FS AA) in the Faculty of Postgraduate Studies for Advanced Science, Beni-Suef University, and Atomic Absorption Spectrometry (Perkin Elmer 2380) of Animal Health Research Institute, Dokki, Giza, Egypt, the instrumental conditions for each element were adjusted according to the instrumental manual. The readings were taken from the device and were converted to the actual concentration of metals in the samples using the following equation;

Heavy metal concentration (ppm) = Concentration x Rate of dilution / Weight of sample.

3.4. Risk Assessment:

Health risks assessment because of honey consumption containing heavy metals was determined through the following:-

3.4.1. The Target Hazard Quotient (THQ):

Considering the ratio between exposure and the reference exposure dose. The THQ for Cu, Fe, Zn, Pb and Cd was determined according to the methodology clarified by US-EPA 2000 and calculated for an adult average body weight of 60 kg and considering a food ingestion rate of 1g/ day (Naccari *et al.*, 2014), according to the following formula:

$$\text{THQ} = [(\text{Efr} \times \text{ED}_{\text{tot}} \times \text{IFR} \times \text{C}) / (\text{RfDo} \times \text{BWa} \times \text{ATn})] \times 10^{-3}$$

Where:

Efr: the exposure frequency (365 days/year);

ED_{tot}: is the exposure duration (70 years);

IFR: is the food ingestion rate (g/ day);

C: is the concentration (µg/ g);

RfDo: is the oral reference dose (µg/ g/ day);

BWa: is the adult body weight (60 kg);

ATn: is the average time for non-carcinogens (it is equal to EFr x EDtot).

RfDo for Cu, Fe, Zn, and Pb were 0.040, 0.7, 0.3 and 0.0035 $\mu\text{g}/\text{g}/\text{day}$, respectively (USEPA IRIS, 2006).

If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects (Mohammadi *et al.*, 2019).

3.4.2. The Estimated Daily Intake (EDI):

The safety of honey consumption by adults and children was estimated on the base of the EDI which was calculated concerning the acceptable level (Joint FAO/WHO Expert Committee, 2011). The average daily honey consumption was set at (19 g) (Żak, 2017 and Winiarska-Mieczan *et al.*, 2021). The average weight for adults was (70 kg), and for children (15 kg). The EDI was calculated using the following formula (Winiarska-Mieczan and Kwiecień, 2016 and Winiarska-Mieczan *et al.*, 2021):

$$\text{EDI} = \text{Mean daily consumption of honey} \times C / \text{BW}$$

Where:

C: is the concentration ($\mu\text{g}/\text{g}$);

BW: is the body weight.

3.4.3. Provisional Tolerable Weekly Intake (PTWI):

Dietary intake of Cu, Fe, Zn, and Pb through honey consumption was calculated using the following equation:

$$\text{Estimated Weekly intake (EWI)} = (C \times \text{IR}) / \text{BW}$$

Where:

C: is the concentration ($\mu\text{g}/\text{g}$);

IR: is the ingestion rate;

BW: is the body weight.

The EWI were compared with the PTWI of the heavy metals (Cu: 3500 $\mu\text{g}/\text{kg}$ bw/week, Fe: 5600 $\mu\text{g}/\text{kg}$ bw/week, Zn 7000 $\mu\text{g}/\text{kg}$ bw/week, Pb: 25 $\mu\text{g}/\text{kg}$ bw/week (Council of Europe, (2001) and FAO/WHO, (2010).

3.5. Statistical analysis:

The results are stated as the mean values \pm Standard error (SE). Constant variables were analyzed with the one-way analysis of variance (ANOVA), then the Duncan post hoc test. A

P value of <.05 was considered significant statistically. All data analyses were performed through the IBM SPSS Statistics ver. 25.0 (IBM Co., Armonk, NY, USA).

4. Results

4.1. The concentration of the estimated heavy metals (Cu, Fe, Zn, Pb, and Cd):

The illustrated data in Table (1) revealed that the concentration of Cu was significantly lower than its permissible limit according to the EOS, while the minimum concentration was recorded in Manshet mnbal\Matay and Etledem\Abu qurqas and the maximum concentration was detected in El mahras\Mallawi and Kom ellofy\Samalut.

The concentration of Fe in honey samples of the investigated areas was significantly lower than the permissible limit; in contrast the minimum concentration was detected in Manshet mnbal\Matay whilst the maximum concentration was observed in Twa\Elminya.

In comparison to the permissible limit of the EOS, the concentration of Zn in honey samples was significantly low. On the other hand, its minimum concentration was reported in Etledem\Abu qurqas and the maximum concentration was detected in Kom ellofy\Samalut.

From the obtained data in Table (1) we observed that the Pb concentration in honey samples was significantly lower than its permissible limit according to EOS. Contrary, the minimum concentration was detected in honey samples of Twa and Demshaw\Elminya areas and the maximum concentration was detected in honey samples of Manshet mnbal\Matay area.

Regarding Cd element it could not be detected in the honey samples.

Table (1): The concentration of Cu, Fe, Zn, and Pb in honey samples:-

Metal \ Area	Cu	Fe	Zn	Pb
El mahras\Mallawi	0.62 ± 0.013 ^f	4.44 ± 0.15 ^c	0.64 ± 0.05 ^{c,d}	0.1 ± 0.02 ^{a,b}
Twa\Elminya	0.5 ± 0.009 ^e	5.61 ± 0.53 ^d	0.61 ± 0.04 ^{c,d}	0.07 ± 0.02 ^a
Demshaw\Elminya	0.5 ± 0.006 ^d	3.59 ± 0.46 ^{a,b,c}	0.56 ± 0.03 ^{b,c,d}	0.07 ± 0.01 ^a
Kom ellofy\Samalut	0.6 ± 0.006 ^f	3.38 ± 0.22 ^{a,b}	0.71 ± 0.06 ^d	0.1 ± 0.01 ^{a,b}
Shosha el gabal\Samalut	0.41 ± 0.009 ^c	3.69 ± 0.39 ^{a,b,c}	0.52 ± 0.14 ^{b,c}	0.09 ± 0.03 ^{a,b}
El qees\Beni mazar	0.34 ± 0.0007 ^b	3.86 ± 0.36 ^{b,c}	0.39 ± 0.02 ^{a,b}	0.19 ± 0.04 ^{b,c}
Manshet mnbal\Matay	0.29 ± 0.0004 ^a	2.74 ± 0.49 ^a	0.46 ± 0.03 ^{a,b,c}	0.3 ± 0.08 ^c
Etledem\Abu qurqas	0.28 ± 0.01 ^a	3.96 ± 0.67 ^{b,c}	0.29 ± 0.03 ^a	0.25 ± 0.05 ^c
Permissible limit* (ppm)	3.5 ^g	70 ^e	15 ^e	4.5 ^d

Data expressed as mean ± S.E. (n= 6)

*Permissible limit according to the Egyptian Organization standards (EOS) (1993).

4.2. Risk assessment:

4.2.1. THQ of the estimated heavy metals:

The obtained data in Table (2) revealed that the THQ of the estimated metals (Cu, Fe, Zn, and Pb) in honey samples from the investigated areas is less than 1.

Table (2): The THQ of Cu, Fe, Zn, and Pb in honey samples:-

Heavy metals The area	Cu	Fe	Zn	Pb
ELmahras/Mallawi	2.58×10^{-4}	1.06×10^{-4}	3.55×10^{-5}	4.75×10^{-5}
Twoa/ELminya	2.08×10^{-4}	1.34×10^{-5}	3.38×10^{-5}	3.33×10^{-4}
Demshaw hashem/ELminya	2.08×10^{-4}	8.55×10^{-5}	3.11×10^{-5}	3.33×10^{-4}
Kom ellofy/Samalat	2.5×10^{-4}	8.05×10^{-5}	3.94×10^{-5}	4.75×10^{-5}
Shosha el gabal/ Samalat	1.71×10^{-4}	8.79×10^{-5}	2.88×10^{-5}	4.286×10^{-4}
EL qees/Beni mazar	1.42×10^{-4}	9.19×10^{-5}	2.17×10^{-5}	9.05×10^{-4}
Manshet mnbal/Matay	1.21×10^{-4}	6.523×10^{-5}	2.55×10^{-5}	1.43×10^{-3}
Etledem/Abu qurqas	1.17×10^{-4}	9.43×10^{-5}	1.61×10^{-5}	1.19×10^{-4}
THQ	1*			

*If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects (Mohammadi *et al.*, 2019).

4.2.2. The EDI of the estimated heavy metals:

The consumption of 19 g honey from the investigated areas as an average daily consumption was reported to be safe for adults and children health in case of all the estimated metals in comparison to the recommended levels except the EDI of Cu was set to be unsafe for children in honey samples from ELMahras/Mallawi, Twoa/ELminya, Demshaw hashem/ELminya, Kom ellofy/Samalat, and Shosha El gabal/ Samalatset in which the values exceed the recommended levels (0.05-0.5 $\mu\text{g}/\text{kg}/\text{day}$).

Table (3): The EDI of Cu, Fe, Zn, and Pb in honey samples:-

Heavy metals The area	Cu		Fe		Zn		Pb	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
ELmahras/Mallawi	0.168	0.785	1.21	5.62	0.174	0.811	0.027	0.127
Twoa/ELminya	0.136	0.633	1.52	7.11	0.166	0.773	0.019	0.089
Demshaw hashem/ELminya	0.136	0.633	0.974	2.15	0.152	0.709	0.019	0.089
Kom ellofy/Samalat	0.163	0.76	0.917	4.28	0.193	0.899	0.027	0.127
Shosha El gabal/ Samalat	0.111	0.519	1.001	4.67	0.141	0.659	0.024	0.114
EL qees/Beni mazar	0.092	0.431	1.045	4.89	0.106	0.494	0.052	0.241
Manshet mnbal/Matay	0.079	0.367	0.744	3.47	0.125	0.583	0.081	0.38
Etledem/Abu qurqas	0.076	0.355	1.075	5.02	0.079	0.367	0.068	0.317
Permissible limit of EDI ($\mu\text{g}/\text{kg}/\text{day}$)	0.05-0.5**		0-500*** 8000****		0.3-1**		15.8*	

* Permissible limit according to FAO/UNEP/WHO, 1991.

** Permissible limit according to JECFA, 1982.

***Is the Acceptable daily intake according to JECFA, 1980.

**** The provisional maximum tolerable daily intake (PMTDI) for all sources except for iron oxide colouring agents, supplemental iron for pregnancy and lactation, and supplemental iron for specific clinical requirements according to JECFA, 1983.

4.2.3. The EWI of the estimated heavy metals:

In comparison the values of the EWI of the estimated heavy metals in the honey samples to the recommended PTWI we noticed that the values are greatly low in case of adults and children as indicated in Table (4).

Table (4): The EWI of Cu, Fe, Zn, and Pb in honey samples:-

Heavy metals The area	Cu		Fe		Zn		Pb	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
ELmahras/Mallawi	0.009	0.04	0.06	0.3	0.009	0.04	0.0016	0.007
Twoa/ELminya	0.008	0.035	0.08	0.4	0.009	0.04	0.001	0.005
Demshaw hashem/ELminya	0.007	0.03	0.05	0.24	0.008	0.037	0.001	0.005
Kom ellofy/Samalut	0.009	0.04	0.048	0.23	0.01	0.05	0.0014	0.007
Shosha el gabal/Samalut	0.006	0.027	0.053	0.25	0.007	0.035	0.0013	0.006
EL qees/Beni mazar	0.005	0.023	0.06	0.26	0.006	0.026	0.003	0.013
Manshet mnbal/Matay	0.004	0.019	0.039	0.18	0.007	0.033	0.0043	0.02
Etledeem/Abu qurqas	0.004	0.02	0.057	0.26	0.004	0.019	0.004	0.017
PTWI($\mu\text{g}/\text{kg}$ bw/week)	3500*		5600*		7000*		25*	

* PTWI according to Council of Europe, (2001) and FAO/WHO, (2010).

5. Discussion

Honey has many nutritional and medicinal advantages as a food and therapy (Ajibola *et al.*, 2012). Since honey is a low-quantity source of nutrients, it would be recommended to take it in a greater amount (70 to 95 g/day) for adults in order to achieve the maximum desired nutritional benefits (Munstedt *et al.*, 2009).

The mineral compound concentration of honey varies from 0.1% to 1.0%; the major element is K, then Ca, Mg, Na, S, and P (Sampath Kumar *et al.*, 2010). Elements are inorganic substances essential for activities, so it must remain in the human body. For normal metabolism, elements such as, Cu, Mn, selenium (Se), nickel (Ni), Zn, and Fe are necessary (Altundag *et al.*, 2015), however above the tolerance limits they are harmful for human health as it will be considered as an environmental contaminants, moreover trace elements such as Cd, Al, and Pb are known to be toxic and are predicted to harm human metabolism (Altundag *et al.*, 2015). Due to their carcinogenic and cytotoxic effects, the levels of Pb, Cd, Ni, and chromium (Cr) are inadmissible (Kova'cik *et al.*, 2016). Trace elements are beneficial when found at optimal concentrations, but lead to toxicity in humans at higher concentrations. The

toxicity exists because the body is unable to metabolize the heavy metal, causing accumulation in human or animal soft tissues without becoming inactivated or killed completely (Ajibola *et al.*, 2012). Honey's minerals and toxic metal content have been used as a quality measure (Citak *et al.*, 2012). The amount of toxic metals in honey depends on its geographical and biological origin (Madejczyk and Baralkiewicz, 2008).

CNS disorders, respiratory problems, metabolic disturbances, nausea, and vomiting are examples of health issues caused by heavy metals (Monisha *et al.*, 2014) Pb, for example, can harm the brain, kidney, nervous system, and red blood cells (Garca-Fern 'andez *et al.*, 1996). The most harmful heavy metals are As, Pb, and Cd, according to the Agency for Toxic Substances and Disease Registry. As and Cd toxicity with honey are relatively less common due to its lower use, but Pb contamination is often documented (Bogdanov, 2006). Cr, Zn, Mercury (Hg), Mn, and silver (Ag) are other heavy metals in honey, all are harmful to human health, and serve as bio-indicators of environmental contamination ('Vit *et al.*, 2010).

Cu is trace element that found naturally in rocks, soils, sediments, and some natural waterways since it is a naturally occurring element in the earth's crust (USEPA, 2003). It may be accumulated in the soil and be absorbed by plants as a result of man's anthropogenic and industrial activities, so it may be found in a variety of foods, including almonds, wheat germ, avocados, and bran, and so on (ATSDR, 2004). The concentration of Cu in honey samples that collected from different areas in El-Minya province in the current investigation was lower than it's the permissible limit according to the EOS (3.5 ppm) and the standards of Codex Alimentarius Commission (5 ppm) (Codex Alimentarius Commission, 1981). Contrary, in comparison to the Italian legislation (0.216 mg/kg) (Caroli *et al.*, 1999) all the investigated honey samples were contaminated. This contamination of Cu may be come from pesticides or from water that kept in containers made of copper metal (Ahmida *et al.*, 2013).

Fe is the 2nd most prevalent metal in the earth's crust (U.S.EPA, 1993), and it is a critical component for practically all living creatures (Valko *et al.*, 2005). It's found in algae, enzymes like cytochromes and catalase, and oxygen-transporting proteins like hemoglobin and myoglobin (Vuori, 1995). The anthropogenic mining activities are the source of Fe in surface water (Valko *et al.*, 2005). The most important sources of Fe pollution are metal corrosion, drilling, and digging (Markert, 1993). In the current investigation the Fe concentration was significantly lower than the standards of the EOS (70 ppm) and Codex Alimentarius Commission (15 ppm) (Codex Alimentarius Commission, 1981).

Zn is a transitional heavy metal, which present in earth crust (Alloway, 2013). It is a vital element in the human body (Murgia *et al.*, 2006). It is necessary for normal function of different organ systems, as well as for growth, development, and tissue repair (Zalewski, 2006). Zn can be found in soil, air, and water, as well as other anthropogenic sources such as fertilizer use, sewage sludge, and the increased pesticide input to the environment. Soil Zn is also influenced by erosion of older rocks (Alloway, 2008). The Zn concentration of honey samples is significantly lower than its permissible limit according to the EOS (15 ppm) and the standards of Codex Alimentarius Commission (5 ppm).

Pb is a metal that is bluish-gray in nature which found in trace levels in the earth's crust. Despite the fact that lead occurs naturally in the environment, human activities such as the burning of fossil fuels, mining, and industrialization result in the release of high amounts, but it is used in a variety of industrial, agricultural, and household uses, nowadays it's utilized to make metal items such as solder and pipes, lead-acid batteries, munitions, and X-ray shielding devices (Gabby, 2006). In the current investigation, the Pb concentration in honey samples was significantly lower than its permissible limit according to EOS (4.5 ppm), while in comparison to the standards of Codex Alimentarius Commission (0.3 ppm) the honey samples of Manshet mnbal\ Matay reached to this level, and in comparison to the regulations of the European Commission (2015) (the maximum level of Pb is 0.10 mg/kg) samples of El qees\ Beni mazar, Manshet mnbal\ Matay and Etledem\ Abu qurqas areas are considered polluted by Pb which may be due to the contamination from car exhausts as the apiary of El-Qees\ Beni mazar is about 100 m away from high way, this high way is characterized by intense motor vehicle circulation and about 1 km away from drainage canal and also the apiary of Etledem /Abu Qurqas is about 5 km away from high way.

Cd is a non-essential element with no role in the human body, but if present even at low doses will cause serious effects (Pretto *et al.*, 2014). It naturally occurs in the environment and could be released during smelting and mining, as well as from companies that produce alloys like plastics and batteries (Rashid *et al.*, 2013). In the present investigation all El-Minya honey samples were free from Cd contamination, it could not be detected which revealed that they are free from pollution. Because of its high rate of soil-to-plant uptake, Cd is primarily detected in fruits and vegetables (Satarug *et al.*, 2011) which may be the cause of non-detection in our samples. Both Pb and Cd elements haven't any vital function in human body, however their presence causing health problems, in which their

toxicity leading to severe damage to organs including the liver, kidneys, heart, and male gonads.

In fact all honey samples which collected from El-Minya provines are free from the investigated heavy metals (Cu, Fe, Zn, Pb, and Cd) according to the EOS (1993) thus means there is no pollution in these samples and honey is safe for consumption.

Hazard and risk analysis may be identify various distresses related to human and animal health, the Codex Alimentarius Commission (CAC) focuses on the domestic risks with identification to those correlated to the chemical, physical, and biological agents of life-threatening public health concern (CAC 2002). The risk assessment has been evaluated by the THQ (Naccari *et al.*, 2014), identifying the possible risks to human health through the THQ that associated with the intake of heavy metals *via* honey consumption is commitment for Egyptian population safety. It's a useful and valid method for risk estimation (Akoto *et al.*, 2014). THQ is calculated using the RfDo, an estimation of the daily exposure to which the human population is probably to be without any substantial risk of detrimental effects during a lifetime (Naccari *et al.*, 2014). In the current investigation, the calculated THQ of Cu, Fe, Zn, and Pb according to their RfDo indicates that there is no carcinogenic risk, as THQs values below 1 reveals a level of exposure smaller than the RfD (Naccari *et al.*, 2014).

International scientific committees like the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), the JECFA, and the regional scientific committees as national regulatory agencies and the scientific committees of the European Union are usually based on the safety factor approach for verifying the acceptable or tolerable intakes of those elements that may display thresholds of toxicity (Herrman and Younes, 1999).

The ADI is generally used to clarify the "safe" levels of intake; other means that are utilized are the RfD, and tolerable intakes that are expressed on either a daily tolerable daily intake (TDI) or weekly basis (TWI). TWI is commonly headed by "provisional" to refer to the insufficient data exists. JECFA uses the term PTWI for contaminants that may accumulate in the body. The weekly description is used to focus on the magnitude of limiting intake over a period of time for these elements (Herrman and Younes, 1999).

Calculating the EDI of the estimated heavy metals in the present investigation was proved to be less than the recommended levels by (JECFA, 1980; JECFA, 1982, and FAO/UNEP/WHO, 1991). PTWI "An endpoint used for food contaminants such as heavy

metals with cumulative properties". Its value characterizes the permissible human weekly exposure to those pollutants unavoidably connected with the consumption of otherwise healthful and nutritious foods (Wu, 2014). In the present investigation the EWI of the estimated heavy metals in honey samples were lower than the PTWI that recommended by the Council of Europe, (2001) and FAO/WHO, (2010).

6. Conclusion

The concentration of Cu, Fe, Zn, and Pb in honey samples from different areas in El-Minya province is lower than the established limits by the EOSs, whilst the Cd element isn't detected.

From the obtained results in our investigation we could concluded that the daily metals intake of honey from El-Minya province is not expected to cause any deleterious consequences during the lifetime in humans, Furthermore the analysis of carcinogenic risk comparative to the estimated metals indicated a minimum health risk for consumers (THQ values < 1).

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