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Elemental Composition and Biochemical Properties of Honey as a Reflection of Environmental Quality

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Abstract:

Honey represents the reflection of the surrounding environment and it is used as a biomarker for pollutants in the environment. It is noteworthy and necessary that the food be free of heavy metals and toxic compounds. In this research, a comprehensive survey of essential and non-essential elements along with heavy metals was carried out for honey samples collected from honey-rich areas in Iraqi Kurdistan region. The biochemical properties of honey and 20 elements were studied. The honey samples showed pH values ranged between (3.66 – 7.05), acidity (9 – 35 meq/kg), moisture percent (12-16.1%), conductance (0.203 – 1.063 mS/m), diastase enzyme activity (11.1 - 65 IU), HMF (1.46 – 48) and the average of sugar content (3.14, 27.2, 39.2, and 77.8) for sucrose, glucose, fructose and total sugar percentage, respectively. The mean values of the analyzed elements (Pb, Sb, As, Zn, Cu, Ni, Co, Fe, Mn, Cr, V, Ti, Mg, Al, Sr, Ba, Cd, Se, Mo and Be) were (0.050, 0.037, 0.005, 0.916, 1.096, 0.257, 0.034, 6.977, 1.232, 2.309, 1.355, 0.033, 0.0, 74.803, 61.565, 1.300, 0.178, 0.008, 0.366 and 0.039 mg/kg), respectively. A single sample from Erbil governorate showed a high quantity of aluminum, and the highest value for Pb was found in honey samples from Sulaymaniyah governorate. Additionally, the highest concentration of all the other important nutrients was detected in Sulaymaniyah honey samples. The final findings demonstrated that the honey from Kurdistan region is not contaminated with heavy metals (HM) and contains sufficient essential nutrients that are necessary to human health, supporting the high quality of the region's honey.

1.Introduction

Honey is a highly nutritious and sweet nutrient made up of several kinds of sugars, primarily glucose and fructose, as well as maltose raffinose, along with other elements, minerals, vitamins, proteins, amino acids, enzymes, polyphenols, antioxidant compounds and other solid matters (Bogdanov et al., 2015). In addition to the flower secretions or exudates of plant-parts, nectar plays a major role in the morphology of honey and its components (Marić et al., 2021). In the past, honey was utilized for both nutrition and many medical aspects (Brodschneider and Crailsheim, 2010; Sampath Kumar K. P., 2010) . Also, honey act as antioxidant and antibacterial agents, it suggested through many investigations as a potential treatment of many diseases and therapeutic agents (Lin et al., 2022; Mandal and Mandal, 2011).

Through biochemical parameters, the quality of honey and its properties can be evaluated according to Harmonised Methods of the International Honey Commission (IHC) such as moisture content, 6- hydroxy-methylfurfural (HMF), diastase activity, proline, acidity, pH, total sugar (glucose, fructose and sucrose) and conductance by measuring electrical conductivity (EC) (Bogdanov et al., 2002; Bogdanov S., 1997; Mohammad et al., 2023).

The elements with a high atomic weight are known as heavy metals (HM) including Pb, Sb, As, Zn, Cu, Ni, Co, Fe, Mn, Cr, V, Ti, Tl, Mg, Al, Sr, Ba, Cd, Se, Mo and Be (Solayman et al., 2016; Yadav et al., 2019).

A variety of factors can alter a metal's toxicity depending on its concentration in a target organ (Tchounwou et al., 2012). The high toxicity of the HM such as lead, arsenic, and mercury cause kidney dysfunction, the reproductive system abnormalities, and vascular disorders. The HM have shown carcinogenic effects, they are readily penetrating through internal organs and cell membranes, which resulting in the denaturation of blood or mucous membrane protein (Fu and Xi, 2020; Natasha et al., 2022).

Several biochemical reactions and processes require essential metals, such as copper, iron, cobalt, selenium, and zinc. A scant amount of these metals is not toxic, but after a certain concentration, these elements are becomes harmful (Fu and Xi, 2020) (Tchounwou et al., 2012).

The presence of pollutant metals in the environment including the biosphere, the atmosphere, the lithosphere, and the hydrosphere (organisms, air, land, and water) could lead to leak and transport them into food) Human activities, such farming (using herbicide, pesticide), industrial manufacturing process and storage of food, or vehicle emissions and radiation may cause contamination of food (Briffa et al., 2020).

Bio-monitoring can be defined as the use of bio-materials or living-organisms to get information on specific properties of the biosphere or environment. Picking the right organism as an indicator is the consequential factor of bio-monitoring. The use of honeybee products as bio-monitoring tools to detect environmental pollution helps to support this purpose (Conti and Botrè, 2001; Ruschioni et al., 2013).

This research aims at a comprehensive study of the 20 elements and HM in honey as bio-monitor to obtain integral information of the environmental purity or pollution found in predominant governorates of the Kurdistan region - Iraq through collection and testing of thirty two diverse honey samples distributed in these regions.

2.Materials and methods

Thirty two honey samples collected and prepared for analysis according to IHC methods and codex standards in the three main cities of Iraqi Kurdistan region (Erbil, Sulaymaniyah, and Duhok) to represent the entire area of north of Iraq (Bogdanov S., 1997; international food stander 1981).

All chemicals used were from Merck - Germany. To determine the metals concentrations, “inductively coupled plasma– optic emission” spectrophotometry (ICP-OES) Technique was used. These wavelengths were used to determine metals concentrations are demonstrated in Table (1).

Table 1 The wavelength (nm) that used for determination of metals.

Symbol	Wavelength (nm)	Symbol	Wavelength (nm)
Pb	220.353	Mn	257.61
Sb	206.833	Cr	283.563
As	189.042	V	309.311
Zn	213.856	Ti	334.941
Cu	324.754	Se	196.09
Ni	221.647	Mg	279.553
Co	228.616	Al	167.079
Fe	259.94	Sr	407.771

Elemental analysis

Aliquots of 1 g honey samples were digested using (9:1) mixture of HNO_3 (69%):H_2O_2 (30%). The samples were then heated at 200^{o}C for 20 minutes, and then held at this temperature for another 20 minutes. The digested mixture was filtered and the filtrate was then made up to 50 mL with deionized water prior to analysis. An iCAP 7600 Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) - A Thermo Fisher instrument was used to employ a detector (a high-performance solid-state CID86 chip). The analyzed elements include Cr, Co, Cu, Fe, Mn, Mo, Ni, Se, V, Zn, Sb, Ti, Sr, Ba, Al, As, Cd, Pb and Tl. The emission lines for the elements and their measuring modes were selected based on exhibiting highest signal to noise ratios and lowest interference. Table 2 lists method parameters and conditions for the analysis.

Table 2 Parameters and conditions for element analysis using ICP-OES method.

Method Parameter	Value
UV exposure time	15 ms
UV RF power	1150 W
UV nebuliser gas flow rate	0.5 L.min^{-1}
Vis exposure time	5 ms
Vis RF power	1150 W
Vis nebuliser gas flow	0.5 L.min^{-1}
Cool gas flow rate	12 L.min^{-1}
Auxiliary gas flow rate	0.5 L.min^{-1}

Biochemical test of honey: -

All honey samples were analyzed according to IHC; the following tests were used, the moisture content was estimated using the refractive index method. The acidity was measured by dissolving 10g of sample honey in 75 ml of water and titration against sodium hydroxide (0.1M), pH of honey measured using a pH meter, while the percentage of sugars (glucose, fructose and sucrose) was measured by HPLC coupled with a refractive index detector. The diastase enzyme was determined according to White's method and electrical conductivity assessment by electrical conductivity meter. HMF was estimated according to White's method by UV-visible spectrophotometer. All the details can be found in IHC methods for determining honey characterizations. (Bogdanov et al., 2002).

3.Results and discussion

The significance of this research consists of identifying and measuring the metal levels and HM present in honey as a bio-monitoring and vital environmental factor that reflects the extent of purity or contamination of foods and land in Iraqi Kurdistan region. Several biomarkers, including animals (terrestrial and aquatic), plants, and food have been used in recent years to monitor environmental toxicity and pollution (Al-Waili et al., 2012; Briffa et al., 2020). Thirty-one natural multifloral honey samples gathered from various locations in north of Iraq which distributed between Erbil, Sulaymaniyah and Duhok cities in order to accurately represent the reflection of environment's effect. The honey was first examined to assure its quality and naturalness according to standard methods by Harmonized methods of the European Honey Commission (HIC) (Bogdanov et al., 2002; Mohammad et al., 2023). The average diastase activity of the samples ranged between 24-41 IU/kg which indicate that the honey samples are natural and not artificial, also the electrical conductivity ranged between 0.203 - 1.068, reinforced this inference (Bogdanov S., 1997; Mohammad et al., 2023). Furthermore, it indicates that the honey is fresh and has not been heated or subjected to any additional treatments as included in HIC, if the quantity of HMF is less than 40 mg/kg, For these samples, the arithmetic mean of the results was determined to be 13.9mg/kg. Acidity and pH of the honey samples were within normal limits, and the percentage of sucrose was between 0-5%

which indicates that the bees have not been fed with sugars. Additionally, the water content was within the normal range (14-16.1%) of natural honey. Overall, the obtained results for honey samples were agreed with those of the international standards and (IHC).

Table 3 Descriptive of honey samples properties.

	pH	Acidty	Water content	Total sugar	Conductance	Diastase activity	Sucrose	Glucose	Fructose	HMF
		meq/kg	% (g)	% (g)	mS/m	IU/kg	% (g)	% (g)	% (g)	mg/kg
Minimum	3.66	9	12	70	0.203	11.1	0.6	23.1	36.4	1.46
Maximum	7.05	35	16.1	81	1.063	65	5.2	32.8	51.8	48.6
Range	3.39	26	4.05	11	0.86	53.9	4.6	9.7	15.4	47.1
Mean	4.35	19	14.2	77.8	0.385	29	3.14	27.2	39.2	13.9
Std. Deviation	0.567	6.82	0.977	2.65	0.166	12.4	1.31	2.2	2.71	12.3
Std. Error of Mean	0.102	1.23	0.176	0.476	29.8	2.38	0.235	0.423	0.522	2.22

The construction of a standard curve for each of the minerals and HM was employed; examples are illustrated in Figure 1, and the R^2 value of 0.9999, the data from the standard solution curve confirms the accuracy of the analytical technique.

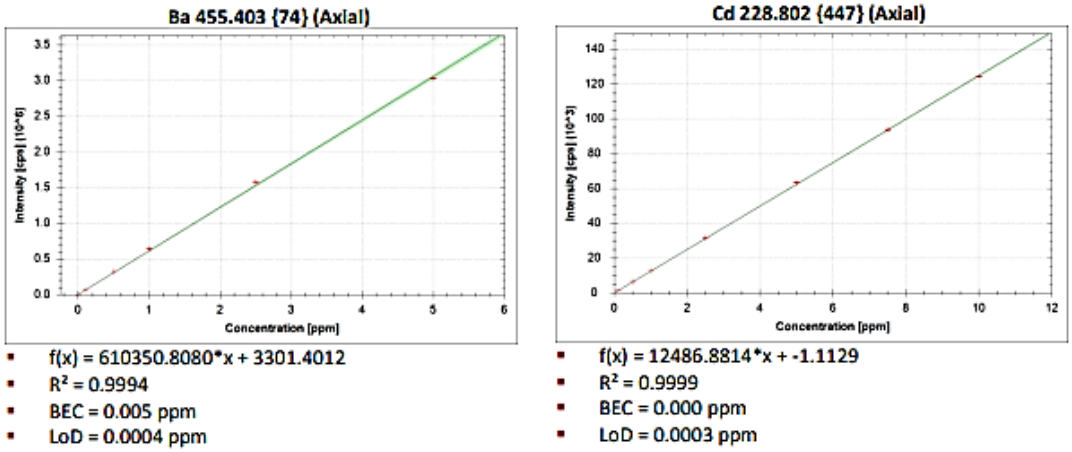

Figure 1 Calibration curve of Ba and Cd

Honey's minerals and HM content are mainly derived from soil and water. Bees come into contact with air, water, soil, and plants branches, and leaves during their transition from one flower to the next(Ruschioni et al., 2013).

The honey samples were split into two distinct groups: the first group which contains toxic HM (Pb, As, Cd, and Al), the second group which have nutritional values (Cu, Zn, Mn, Mg, Ni, Co, Cr, Fe and Mo) which are essential and beneficial to human's body (Flora et al., 2012; Mehri, 2020). According to the scientific researches, the most popular: Pb, Hg, Ni, Cd, Cr, Mn, Cu, Zn, and Ag the most popular HM are: Pb, Hg, Ni, Cd, Cr, Mn, Cu, Zn, and Ag metals. Some of heavy metals are toxic such as Pb or Hg (Zugravu and others 2009). Heavy metals can become toxic and hazardous due to their capacity to accumulate in living organisms, even in tiny amounts and the toxicity of these HM are influenced by various factors such as the dosage, the exposure technique, the chemical composition, as well as the exposed person's gender, age, heredity, and nutrition (Tchounwou

et al., 2012).

The maximum content of Pb ranged from 0.103 - 0.181 mg/kg were detected mostly in Sulaymaniyah city honey samples along with a sample (E16, 0.115 mg/kg) from Erbil which also showed high arsenic content. The level of Pb in Sulaymaniyah honey samples was higher than its content in Erbil and Duhok honey samples with ratios of 2.6 and 3.3, respectively. Generally, cadmium content was ranged from 0 - 0.024 mg/kg, where the honey sample E11 from Erbil showed the highest level of cadmium (0.024 mg/kg) among all the honey samples. Compared to the expected value, the highest level of aluminum (363 mg/kg) was found in a sample taken in Erbil, as illustrated in table (4). The most untreated foods contain small amounts of Al (<5 mg/kg), except for certain herbs. The amount of Al obtained by typical adults from natural sources is ranged from 1 to 10 mg (Greger, 1992).

Table 4 The Range, mean and standard deviation of minerals and heavy metals contents of all samples

Elements	Minimum mg/kg	Maximum mg/kg	Range	Mean	Std. Deviation	Std. Error of Mean
Pb	0	0.18	0.18	0.050	0.049	0.009
As	0	0.043	0.043	0.005	0.001	0.004
Cd	0	0.024	0.024	0.008	0.006	0.001
Al	22	364	342	62	57	10
Sb	0	0.1	0.1	0.037	0.038	0.008
Zn	0.07	4.6	4.6	0.92	0.77	0.13
Cu	0	3.1	3.1	1.1	1.1	0.18
Ni	0.017	1.8	1.8	0.26	0.3	0.052
Co	0	0.07	0.07	0.034	0.028	0.005
Fe	3.5	12	8.6	7	2.8	0.49
Mn	0.4	2.3	1.9	1.2	0.46	0.081
Cr	1.8	2.8	0.99	2.3	0.22	0.038
V	0	1.9	1.9	1.4	0.43	0.074
Ti	0	0.14	0.14	0.033	0.035	0.006
Mg	46	121	75	75	17	3
Sr	0.43	2.8	2.4	1.3	0.48	0.084
Ba	0	0.82	0.82	0.18	0.22	0.038
Se	0.24	0.51	0.27	0.37	0.073	0.013
Mo	0.02	0.055	0.035	0.039	0.009	0.002
Be	0	0	not detected			
Bi	0	0	not detected			

Some evidence shows of a beneficial role for arsenic, nickel, and vanadium in certain physiological processes, but inorganic arsenic has been proven to cause human poisoning (Belitz et al., 2008). Thus, Arsenic doses exceeding 10 mg/kg/day could cause encephalopathy and gastrointestinal symptoms (Fincher and Koerker, 1987). The maximum concentration of arsenic in honey samples was 0.043 mg/kg (Figure 2), which is indicated that the allowed does of toxic HM (Pb, Cd, Al and As) in all honey samples is compliant with global standards of satisfaction as shown in table (5).

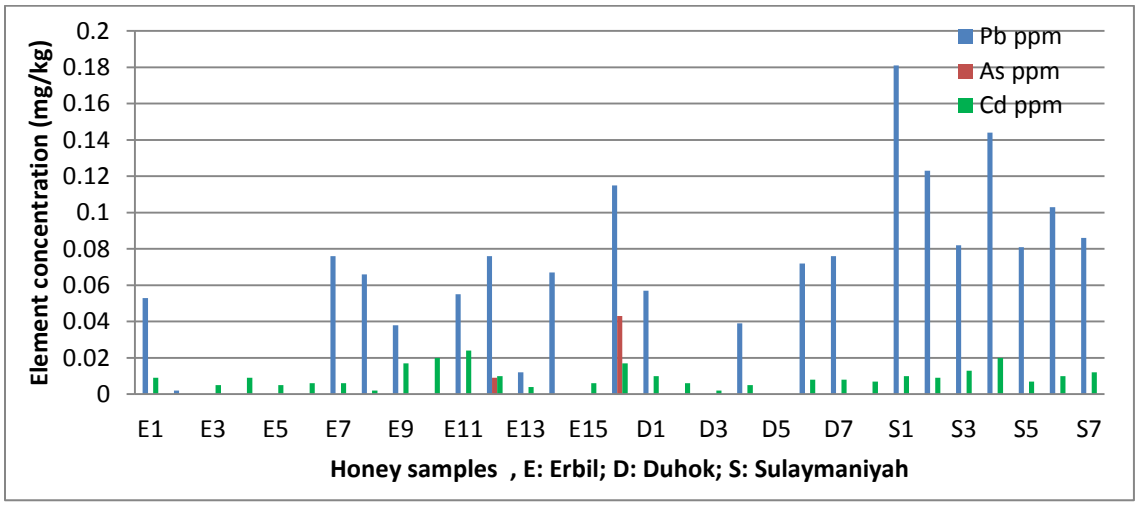

Figure2 The concentration level of Pb, Cd and As in honey samples.

It is difficult to compare between the observed results with other countries due to the variations in HM levels within the same country. Also there is a disparity and equivalence of the observed level of these elements and honey samples from neighboring Asian and European countries, for example, the lead content of Kurdistan honey is below the allowable limits which is parallel to Turkey, Iran, Greece, and Manuka honey from New Zealand and Saudi Arabia (Karabagias et al., 2017; Kılıç Altun et al., 2017; Saghaei et al., 2012; Solayman et al., 2016; Vanhanen et al., 2011). However, it is possible to detect a glaring contrast between the obtained results for Pb (0.05 mg/kg) and those of Mono-floral honey (4.95 mg/kg) and Honeydew honey (8.3 mg/kg) from Poland, as shown in Table (5).

Also, the concentration level of other toxic heavy metals such as As and Cd, are close to their concentrations obtained from countries mentioned above, except for Greece, which has a higher concentration of As (0.69 mg/kg). While aluminum content in Iraqi Kurdistan honey samples showed similar level to Turkish honey, but more than other countries of Greece, New Zealand and Saudi Arabia (Table 5).

The concentration of the rest elements and HM observed in Kurdistan honey are close to the other countries, except that the concentration level of zinc and iron in Turkish honey are abnormal, which are 49.9 and 268 mg/kg, respectively. The second type of HM such as Zn, Cu, Ni, Co, Fe, Mn, Mg, Cr, Se and Mo are essential nutrient elements and needed for different biochemical and bio-physiological processes. If the amount of micronutrients is not enough, it can lead to various diseases and syndromes (Tchounwou et al., 2012).

Table 5 Comparison of the mean value of HM (mg/kg) contents in honey samples between Iraqi Kurdistan honey and some other countries.

Elements	Iraqi Kurdistan mg/kg	Turkey mg/kg	Iran mg/kg	Greek mg/kg	Poland mg/kg	New Zealand / Manuka mg/kg	Saudi Arabia mg/kg
Pb	0.05	<1 ppb	0.07	0.18	4.95* 8.3**	0.04	0.055
As	0.005		0.0006	0.69		0.08	
Cd	0.008	<1 ppb			0.021	0.01	0.037
Al	62	69.7		1.6	10.5	1.22	
Sb	0.037			0.49			

Zn	0.92	49.9	24.3	1.24	3.22	0.37	0.746
Cu	1.1	68.5		56.9	0.021	0.35	0.341
Ni	0.26	<1 ppb	0.003	0.41	0.429	0.2	
Co	0.034		0.001				
Fe	7	268	0.7	2.94		1.86	3.195
Mn	1.2	45.6	0.08	2.76	4.96	2.15	0.373
Cr	2.3	<1 ppb	8.4			ND	
V	1.4						
Ti	0.033						
Mg	75			62.88	18.5	27.9	23.12
Sr	1.3						
Ba	0.18			0.22	0.121		
Se	0.37	54.1		0.42			
Mo	0.039			0.03		ND	

* Monofloral honey ** Honeydew honey (Sitarz-Palczak et al., 2015)

The biological roles of non-essential metals including Ni, Sb, V, Ti, Sr, Ba, Be and Bi are not recognized (Chang et al., 1996). The maximum values for these non-essential minerals are listed in Table (4) (Chang et al., 1996).

A variety of biochemical and biological functions rely on essential nutrients. The maximum values of essential elements (Zn, Cu, Ni, Co, Fe, Mn, Mg, Se, Mo and Cr) found were (4.6, 3.1, 1.8, 0.07, 12.0, 2.3, 121, 0.51, 0.005 and 2.8 mg/kg), respectively, as shown in table 4. The highest value of Zn, Mg, Se and Mo was belonged to S2, S1, S1 and S3 in Sulaymaniyah city, respectively, while the maximum values of Ni and Fe were detected in Erbil city samples (E11 and E12). In addition, the highest value of the other three elements Cu, Co, Mn and Cr were obtained in Duhok city samples (D2, D3, D5 and D8), respectively, as shown in figure (3).

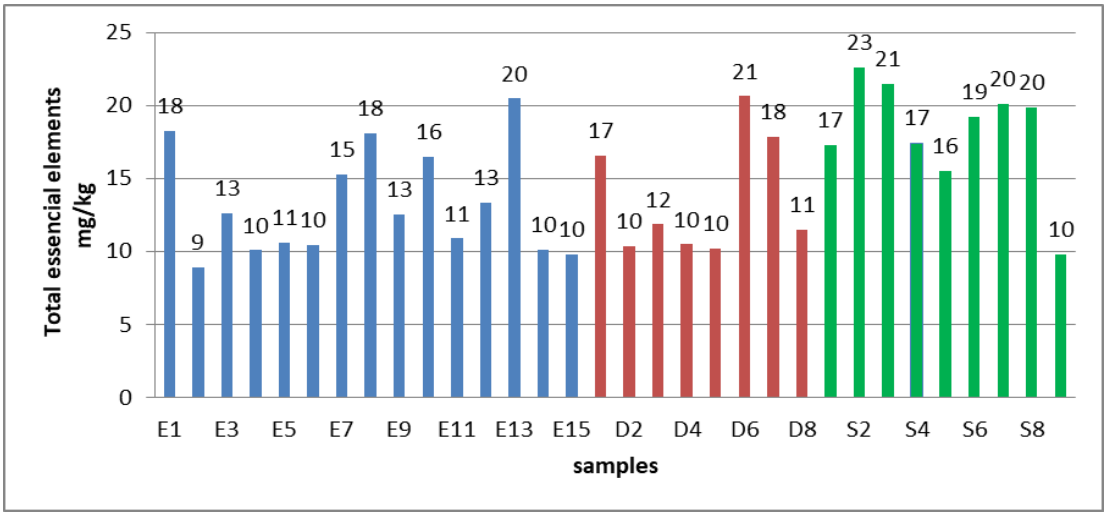

Figure 3 the total essentials nutrient elements in honey samples (where E: Erbil; D: Duhok; S: Sulaymaniyah samples).

The high value of the total essential nutrient elements (more than 100 mg/kg) was found in the samples E3, E12, D6, S1, S2, S3, S6 and S8, whereas the minimum value was observed in

the samples E2 and D5, as shown in Figure (3). Nevertheless, excessive levels of these metals can cause harm to cells and tissues, resulting in a range of negative effects and human disorders.
The human requirement for trace elements such as Zn, Cu, Mn, Mg, Mo, Co, Cr, Fe, and Se is less than 50 mg/day, whereas ultra-trace elements like Cd, Al, Sb, Ba, and Ti usually require less than 50 ng/g, which is a common amount of this substance in the diet's dry matter (Belitz et al., 2008; Solayman et al., 2016). But there is no sufficient knowledge available to meet the requirements for quantity of some metals yet, such as Ni, V and As.
The results of preceding findings demonstrate that the honey samples collected in Kurdistan region were clean and uncontaminated when compared to the Recommended Dietary Allowance (RDA), table (6) shows the quantities of Upper Intake Level (UL) and Recommended Dietary Allowance (RDA) that are allowable for the daily intake of the elements in food. (Flora et al., 2012; Pennington and Jones, 1987) (Institute of Medicine and Nutrition, 1994). The contaminated source is more prevalent in those beehives which are close to towns or big cities or placed near human activities. The environmental factors and tools used in honey processing are the two main sources of honey contamination. The main source of this contamination is due to factory residues or vehicle emissions or other human activities that pollute water, air and soil. These heavy metals pollutants are transmitted by bees to honey.

Table 6 The daily intake of elements (mg/day) allowed in food according to Recommended Dietary Allowance (RDA) and the Tolerable Upper Intake Level (UL).

	Metals	RDA mg/Day	UL mg/day
oxic Elements	Pb	0.18	ND
	Cd	0.063	0.062
	As	30	0.012-0.025
Essential Trace Elements	Cr	0.035	20
	Zn	40	15
	Cu	10	1.2 -1.6
	Co	ND	ND
	Fe	45	16-18
	Mn	11	2.3
	Mg	400	ND
	Se	0.07	0.4
	Mo	2.0	0.045
Non-essential Elements	Ni	1.0	0.074-0.1
	Al	70	ND
	Sb	0.006	ND
	V	8.37	1.8
	Ti	0.01	
	Sr	0.13	
	Ba	0.21	ND

There is a source claims that the machinery, tools and artificial wax used by beekeepers and honey processing could emit metal pollutants (lead, cadmium, zinc, chromium, aluminum, copper, nickel and iron) into honey., Also, the devices used to extract honey from beehives and packing metals or container which used to store honey could release the HM into honey (Figure 4) (Stankovska et al., 2008; Üren et al., 1998).

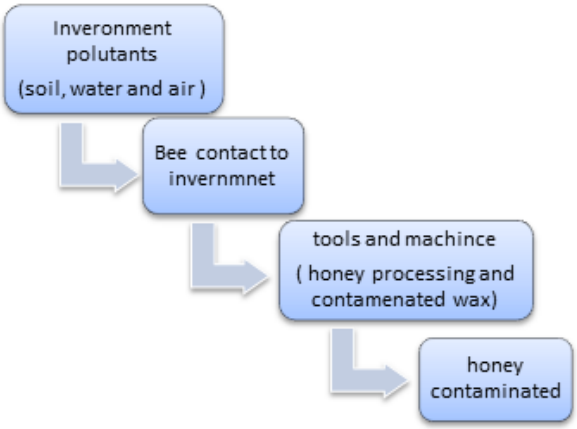

Figure 4 The source of honey pollutants.

It is worth noting that, through this research, there were relationships between the content of some elements with each other in honey samples. The most prevalent was the correlation of lead with the elements (Cu, Fe, Mg and Sr) and its negative correlation with Co. at the same time, Cu had a significant correlation with each of Fe, Mg and Sr respectively, table (7).

Table 7 Statistical correlation between HM in honey samples

	Pb mg/kg	Sb mg/kg	As mg/kg	Zn mg/kg	Cu mg/kg
Pb mg/kg	1				
Sb mg/kg	-0.032	1.000			
As mg/kg	0.255	-0.081	1.000		
Zn mg/kg	0.391	-0.155	-0.036	1.000	
Cu mg/kg	0.685	0.003	0.249	0.366	1.000
Ni mg/kg	0.125	-0.141	0.138	-0.114	0.019
Co mg/kg	-0.614	-0.096	-0.229	-0.175	-0.389
Fe mg/kg	0.785	0.087	0.304	0.343	0.878
Mn mg/kg	0.394	0.256	0.248	0.361	0.566
Cr mg/kg	-0.180	0.311	-0.036	-0.117	-0.072
V mg/kg	-0.140	-0.129	-0.190	0.038	-0.226
Ti mg/kg	0.194	-0.045	0.375	0.002	0.301
Mg mg/kg	0.604	0.200	0.233	0.172	0.528
Al mg/kg	0.244	0.352	0.374	-0.035	0.302
Sr mg/kg	0.698	0.294	0.180	0.254	0.625
Ba mg/kg	0.377	0.099	0.332	-0.027	0.319
Cd mg/kg	0.411	-0.018	0.266	-0.031	0.244
Se mg/kg	-0.260	-0.299	0.206	-0.441	-0.309
Mo mg/kg	0.254	0.023	-0.187	-0.044	0.189

4.Conclusion

In conclusion, the results of this study indicate that Kurdistan honey is quite pure, despite a few samples from the Sulaymaniyah city contained a few trace levels of Pb in average 0.18 mg/kg which were identical or less than what is present in many international honey Furthermore, it is regarded as being uncontaminated and within allowable limits. Likewise, honey from this region contains essential nutrient elements like copper, zinc, iron, cobalt, chromium, and others that are crucial to human health and contribute to the great quality of the honey produced in this area.

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Conflict of interest

Regarding the publication of this manuscript, the

authors declare that there are no conflicts of interest

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