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Assessing Copper Risk in Honey Sold in City of Yerevan

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ABSTRACT

Mining, including copper (*Cu*) production, is one of the primary economic activities in Armenia. Besides having an adverse environmental impact, it can lead to *Cu* accumulation in food, including honey. Considering the wide production and consumption of honey, this study aims to assess the dietary exposure of *Cu* through honey consumption in Yerevan, Armenia. Seventeen honey samples were included in the study. Food Frequency Questionnaire was used to evaluate honey consumption. While *Cu* contents exceed the Maximum Residue Level, dietary exposure estimates do not exceed the oral reference dose. Hence, the consumption of honey sold in Yerevan does not have the potential to cause adverse health effects.

Introduction

Bee farming is one of the main agricultural branches. On the one hand, this industry is highly profitable, but on the other hand bees, as major pollinators of crops, contribute to the yielding capacity and seed development of fruit trees, herbaceous plants and vegetables (Nicholls, et al., 2013). Honey is essential for the human organism due to its natural nutritious, therapeutic and preventive power. The latter is determined by chemical composition, which even in the case of the same floral origin of honey can be different depending on climatic conditions (sunlight and moisture content), chemical contents of soil, presence of diverse minerals in the soil, and so on. As a natural product used for therapeutic purposes, honey must not contain undesirable substances such as heavy metals (Eteraf-Oskouei, et al., 2013).

Honey is being consumed worldwide not only as a source of food but also for medicinal purposes, thus, it must remain pure and nutritious (Bartha, et al., 2020). However, many studies have indicated its contamination with heavy metals, pesticides, bacteria and radioactive materials (Pipoyan, et al., 2020, Mititelu, et al., 2022). These chemicals are released into the environment from both anthropogenic and natural sources and may accumulate in the soil and plant for long periods (Magna, et al., 2018).

Copper (*Cu*) has an essential part in biological systems and thus, is regarded as a necessary element (Ajibola, et al., 2012). However, high concentrations of *Cu*, as well as prolonged consumption of honey containing *Cu* can pose a risk to consumer health and cause gastrointestinal disorders (Bartha, et al., 2020).

Since environmental pollution can negatively influence

the honey quality and cause probable health risks, it is essential to assess the presence of potential toxicants (Pisani, et al., 2008). Previously, the Center for Ecological Noosphere Studies of Armenia (CENS) regularly carried out investigations regarding concentrations of heavy metals in honey and health risk assessment through honey consumption across the regions of Armenia, including mining and non-mining areas. The results of these studies indicate that in several honey samples, the concentrations of *Cu* were above the maximum allowable level, meanwhile, non-carcinogenic risk values did not exceed the acceptable level (Pipoyan, et al., 2020, Belyaeva, et al., 2011, Saghatelyan, et al., 2013). Taking into consideration the absence of similar studies in the capital city of Armenia, Yerevan, this is the first-ever attempt to evaluate *Cu* concentrations and carry out a dietary exposure assessment of honey sold in the markets of Yerevan. The work was supported by the State Budgetary Fund of the Science Committee of RA.

Materials and methods

Sampling

The study involved 17 honey samples of different floral origins. Honey sampling was done in compliance with accepted international methods. The samples were obtained from all the major supermarkets of Yerevan as well as from small-scale honey producers. The decomposition of honey was carried out in the ISO-IEC 17025 accredited laboratory of the Ecology Center at the Research Center of the National Academy of Sciences of Armenia, using methods approved by EU legal acts. For determining the content of non-volatile chemicals, honey has been exposed to high thermal degradation according to AOAC 985.01 method (Fredes, et al. 2006).

Digestion of samples

Samples were digested with nitric (HNO_3), sulfuric (H_2SO_4), and perchloric ($HClO_4$) acids. The digestion program was carried out accordingly – 500 W/5 min up to 180 ^{0}C, 0 W/2 min, and then 500 W/10 min at 180 ^{0}C. Then, the samples were cooled and filtered. Eventually, the filtrates were moved to 50 ml Erlenmeyer flasks.

Analysis of Cu

The levels of *Cu* were determined with an atomic absorption spectrometer (AAS, Perkin Elmer AAnalyst 800, US). Solution preparation was made with double-distilled deionized water. The glassware was cleaned with 10 % HNO_3. The instrument was calibrated with a standard solution of the appropriate element. Blank standards from Perkin Elmer (an authorized distributor) were used. The solutions were diluted appropriately for calibration. The variation coefficients of replicate analysis were determined.

Quality assurance and quality control

Appropriate quality assurance procedures were carried out for the reliability of the results. For error minimization, the determination of contaminant level was performed with three replications. For the accuracy of the results, repeated analysis of samples against Standard Reference Materials for all trace elements was made (SRM 1570a). The obtained results were within ± 2 % of the certified values, suggesting that the results were precise.

Data collection and statistical analysis

To obtain honey consumption data, a food frequency questionnaire (FFQ) was developed and used for conducting surveys. The FFQ study was conducted in 2018 and included all the districts of Yerevan to ensure representativeness. Seven hundred people (18-65 years old) residing in Yerevan city took part in the survey. The FFQ was paper-based and interviewer-administered. The average daily consumption of honey was calculated by multiplying daily consumption frequency (portions/day) with portion size and quantity. All the statistical analyses were conducted using SPSS software (SPSS Inc., Chicago, Ill., USA, version 28). To get a normal distribution of consumption values, the K-means cluster analysis method was applied. The optimal number of clusters was determined experimentally. Clustering was carried out for several times, by varying *K* from 2 to 10 clusters. For each case, the sum of squared deviations was identified using the ANOVA table. Afterwards, three homogeneous cluster groups were revealed.

Exposure assessment

By combining *Cu*'s concentration data with consumption data, estimated daily intake (EDI) was calculated (US EPA, 1997):

$$EDI = \frac{C \times IR}{BW},$$

where C is the mean concentration of contaminant (mg/kg), IR is the rate of ingestion of food (kg/day), BW is the body weight (kg) (mean body weights for males and females in studied regions were 70 and 60 kg, respectively).

Non-carcinogenic health risks associated with honey

consumption by residents were assessed using the Target Hazard Quotient (THQ). THQ was calculated as the ratio of the EDI to the oral reference dose (RfD) (US EPA, 1997).

$$THQ = \frac{EDI}{RfD}.$$

For the RfD of *Cu*, a dietary reference intake of 0.01 mg/kg/BW/day was used (ATSDR, 2004).

Results and discussions

Content of Cu in honey

The contents of *Cu* in honey samples of Yerevan are presented in Figure 1. The contents ranged from 0.26 to 1.57 mg/kg, with a mean of 0.99 mg/kg and standard deviation of 0.32 mg/kg. In Armenia there is no regulation limiting *Cu* content in honey, however, our results indicate that in several honey samples (H-1, H-2, H-5, H-10, H-11, H-14, H-15, H-17) *Cu* contents exceed the Maximum Residue Level (MRL) of 1 mg/kg set by EU (APEDA, 2008). The *Cu* concentrations assessed in the current study are comparable with the values estimated in other countries (Table). The findings are similar to those of neighboring countries of Iran and Turkey. It is noteworthy that no investigation was found regarding *Cu* content in honey in other neighboring countries of Georgia and Azerbaijan. In comparison to other countries (such as Romania, Ghana) where honey samples were collected mainly from polluted areas, the contents of *Cu* are remarkably higher than the ones obtained in the present study.

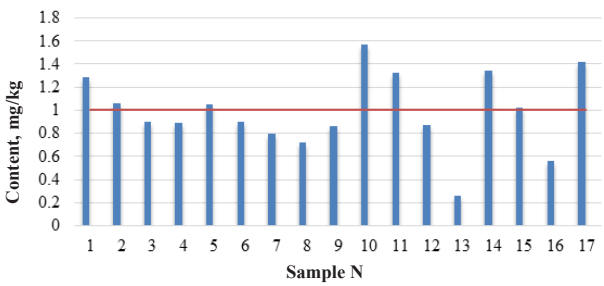

Figure 1. *Cu* contents (mg/kg) in honey samples sold in markets of Yerevan *(composed by the authors).*

Honey consumption

The average honey consumption for the studied population is 12.14 g/day; 60 % of consumers are female (10.16 g/day) and 40 % are male (14.80 g/day). The consumption amounts of honey for three different clusters are presented in Figure 2. For the first, second, and third clusters, the consumption amounts are equal to 6.39, 28.36, and 58.85 g/day, respectively. While the consumption amount increases from the first to the third cluster, the amount of people in each cluster decreases. Cluster 1 includes 584 people, cluster 2 includes 107 people, while cluster 3 includes only 39 people.

Table. Comparative results on honey contamination with *Cu* in different countries*

Country	Source of Honey Samples	*Cu* Concentration (mg/kg)	Literature Source
Iran, North West province, Ardabil	Multifloral honey collected from individual beekeepers	0.027-2.872	Aghamirlou, et al., 2015
Iran	Collected from different honey brands sold in markets	<0.5	Akhbari, et al., 2012
Turkey, Western Region (Budur, Antalya, Isparta)	Collected from individual beekeepers	0.24-0.54	Tutun, et al., 2019
Turkey, South and East Region	Monofloral and multifloral honey collected from local beekeepers	<0.001-0.93	Kılıç Altun, et al., 2017
Romania, Copșa Mică	Collected from private apiaries located in a polluted area	2.0-33.0	Bartha, et al., 2020
Romania	Collected from accredited beekeepers from polluted areas, multifloral honey	0.030	Mititelu, et al., 2022
Poland, Southwest region	Freshly ripened honey samples collected from a market	0.01-1.42	Stecka, et al., 2014
Ghana, Tamale Metropolis	Collected from honey harvesters from within 5 km of the industrial city	6.5-39.0	Magna, et al., 2018
Pakistan, Khyber Pakhtunkhwa	Honey samples collected from different districts	1.902	Ullah, et al., 2022

*Composed by the authors.

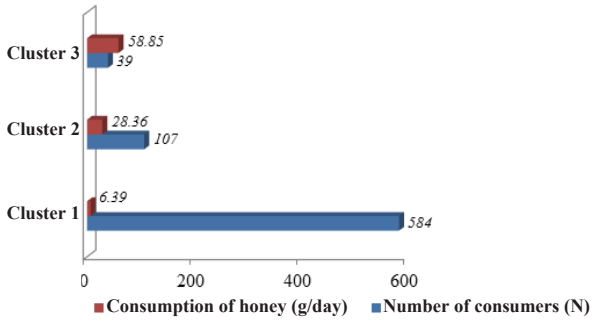

Figure 2. Honey consumption (g/day) for three clusters *(composed by the authors).*

EDI and THQ of Cu

The EDI values for trace elements are firmly based not only on contamination level but also on the consumption of honey (Pipoyan, et al., 2018a).

The estimated EDI values are presented in Figure 3. The amount for cluster 1 is equal to 0.0001 mg/kg BW/day, for cluster 2 – 0.0004 mg/kg BW/day, and for cluster 3 – 0.0008 mg/kg BW/day. The average EDI is equal to 0.0002 mg/kg BW/day. The EDI for both male and female consumers is equal to 0.0002 mg/kg BW/day, which does not exceed the oral reference dose of 0.0100 mg/kg BW/day (ATSDR, 2004).

The THQ values for males and females were 0.017 and 0.02, respectively. The THQ values for Cluster 1, Cluster 2 and Cluster 3 were 0.01, 0.4 and 0.08 respectively. In all cases, THQ values are less than the threshold of 1. THQ values for Cu are lower than 1 in several other countries as well, showing no health risk for consumers due to honey intake (Mititelu, et al., 2022, Ullah, et al., 2022). The intake of Cu from honey was below the provisional tolerable daily intake in Iran (Akbari, et al., 2012).

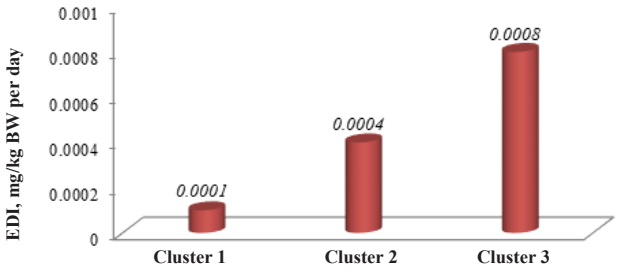

Figure 3. Estimated daily intake of *Cu* (mg/kg BW per day) *(composed by the authors).*

Conclusion

The obtained results indicate, that although in the investigated honey samples contents of *Cu* were higher than MRL, the consumption of honey sold in Yerevan, presented above, does not have the potential to cause adverse health effects. Due to fact that honey can contain other heavy metals, constant monitoring and risk assessment are crucial for maintaining consumer health protection (He, et al., 2013, Ru, et al., 2013). Hence, in our next investigations, we will assess the risk of other heavy metals for understanding the overall risk of honey consumed in Yerevan. Also, we plan to assess the risk of *Cu* in various honey samples produced in other cities and villages of Armenia to get a holistic view of *Cu* risk through honey consumption.

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