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Dietary exposure to heavy metals through polyfloral honey from Campania region (Italy)

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ABSTRACT

Honey may have potential benefits due to its nutrient and bioactive molecules. On the other hand, it is a food that could be affected by environmental pollution; therefore, honey may contain contaminants such as heavy metals. The present study aimed to quantify eleven heavy metals and essential elements (Hg, Cd, V, Cr, Ni, Cu, As, Sb, Pb, Ba, Mn) in honey collected in the Campania region (Italy) and analyzed through Q-ICP-MS. Secondly, carcinogenic and non-carcinogenic risks due to ingestion of honey in toddlers, adolescents, and adults were estimated based on the Target Hazard Quotient (THQ) and Lifetime Cancer Risk (LTCR). No statistically significant difference emerged among the different areas. The risk assessment did not report concerns for non-carcinogenic risk. However, the three groups showed a potential carcinogenic risk for Ni, Cr, and As, even though toddlers reported higher exposure values. The finding of this study provides pieces of knowledge on levels of contaminants in honey in Campania. Furthermore, it can aid in understanding the resulting risk due to honey ingestion.

1. Introduction

Honey is a well-known sweet and viscous natural substance used as food since prehistoric times (Crane, 1983). Indeed, it is an excellent source of nutrients such as sugars (mainly glucose and fructose), proteins, vitamins, minerals, phenols, and others. According to Codex Alimentarius (2019) definition, honey is produced by honeybees from the nectar of flowers, from secretions of plants, as well as excretions of plant-sucking insects on the living parts of plants (in the case of honeydew honey), which the bees collect, transform through the addition of specific compounds, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. The type of plant characterizes the honey variety and its chemical-physical composition (Popek, 2002). In the last two decades, worldwide honey production has increased by about 67% (1260063 tonnes in 2000–1862598 tonnes in 2019) proving a most significant market interest for this food. Honey is not only appreciated for its organoleptic features but also for its health-promoting effect. The high number of bioactive compounds with antioxidant and anti-inflammatory properties significantly reduce cellular proliferation, glucose, fructosamine, and glycosylated hemoglobin serum concentration, oxidation of low-density lipoproteins, asthma, and bacterial infections (Cianciosi et al., 2018).

Furthermore, high microbiological stability due to acidity, low water availability, and microbial activity inhibitors was described (Omafuvbe and Akanb, 2009). For these reasons, honey consumption may be suggested following LARN (Levels of Absorption Reference of Nutrients and Energies for the Italian population) (SINU, 2014). However, honey may be a source of potentially toxic elements such as heavy metals and trace elements due to honeybee's bioaccumulation capability (Bibi et al., 2008; Pipoyan et al., 2020). Heavy metals are persistent pollutants, naturally occurring in the environment or widespread by anthropogenic activity. The primary emission sources of these chemicals are industrial processes, combustion of fossil fuel refining, vehicles emissions, disposal of municipal wastes, and application of pesticides and fertilizer (Li et al., 2019). Toxic elements can lead to acute or chronic intoxication, although some (e.g., Cu, Cr, Se, Mn, Zi) are essential for human metabolism and have detrimental effects only at high doses (Whitfield et al., 2010 Jun). They can alter the function of the immune and nervous

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system and organs such as lungs, liver, and kidneys (Fraga, 2005; Sankhla et al., 2017; Heo et al., 2017; Hashemi, 2018). Each element has a specific mode of action, but underlying toxicity mechanisms include ROS generation, glutathione depletion, and bonding to sulfhydryl groups damaging cells and activating carcinogenic processes (Engwa et al., 2019; Wu et al., 2016). As a result, the consumption of honey, which may be affected by the surrounding environmental conditions, could be a significant source of exposure to chemical contaminants, representing a potential public health issue. Previous research assessed the honey contamination in different regions, reporting high levels of heavy metals such as Pb, Cd, As, Cr, and Ni (Ullah et al., 2022; Pipoyan et al., 2020; Orisakwe et al., 2019). However, some studies described the peculiar ability of honeybees to "filter" the nectar, reporting no relevant heavy metals levels in honey (Dżugan et al., 2018; Borsuk et al., 2021). Therefore, this study set out to evaluate the levels of eleven heavy metals and essential elements (Hg, Cd, V, Cr, Ni, Cu, As, Sb, Pb, Ba, Mn) in honey collected from apiaries located in different Campania (Italy) municipalities, with the aim to fill the gap of data related to this area. To the best of our knowledge, although some research has been carried out on heavy metals in honey, few published studies have quantified the levels of these elements in samples collected in Southern Italy. Therefore, our study may provide a significant account of honey safety in the Campania region (Italy), ascertaining human exposure to these elements.

2. Materials and methods

2.1. Sampling

Thirtytwo honey samples in three suburban areas (eight sites) of the Campania region were collected: Caserta province (Cancello Arnone, Castel Volturno, and Mondragone), Vesuvius area (Torre del Greco, Trecase, Terzigno and Ottaviano), and Sorrento peninsula (Vico Equense). Four honey samples were collected for each site at the end of October 2020. 50 g of fresh honey belonging to polyfloral species were collected directly from beekeepers. The samples were placed individually in sterile test tubes, frozen at a temperature of − 80 °C, and then analyzed.

2.2. Chemical and instrumental analysis

0.50 g of sample were homogenized, and 5 mL of HNO_3 (65% w/w) and 2 mL of 30% H_2O_2 (30% w/w) were added. Then the samples underwent wet mineralization through a Milestone microwave for 30 min at 190 °C, and finally, the samples were cooled and transferred to a flask, and the final volume was adjusted to 25.0 mL by adding Millipore Mill-Q® water (18.2 MΩ cm resistivity).

Metals analysis was performed using a Thermo Scientific™ ICAP™ RQ inductively coupled plasma mass spectrometer (Q-ICP-MS) with a Burgener Mira-Mist nebulizer, a Quartz cyclonic spray chamber, cooled to 2.7 °C, and skimmer cones. The instrument was operated using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. The operating conditions of the Q-ICP-MS equipment were optimized using a tuning solution (Ba, Bi, Ce, Co, In, Li, U 1.00 µg/L, Thermo Scientific) on masses ^{115}In, ^{7}Li, ^{59}Co, ^{238}U, ^{209}Bi, and ^{140}Ce was used for oxide and doubly charged interference checks. The analysis was performed in KED (Kinetic Energy Discrimination) mode, and the parameters were: collision gas: He, plasma gas flow (Ar): 14,8 mL/min; nebulizer gas flow: 0.98 L/min; auxiliary gas flow: 0.85 L/min; ICP RF Power: 1550 W; CeO/Ce = 0.0057. Cell gas flow was 4.8 mL/min for He.

The Q-ICP-MS was used for the determination of As, Ba, Cd, Cr, Cu, Mn, Hg, Ni, Pb, Sb, and V in honey. All samples were analyzed in duplicate, and each sample was measured in triplicate by Q-ICP-MS detection (Aliu et al., 2020; Bereksi-Reguig et al., 2020; Kılıç Altun et al., 2017).

The solutions were prepared using water (18.2 MΩ cm resistivity) purified with a Millipore Mill-Q® purification system, concentrated nitric acid (HNO_3 65% m/m, Suprapur®, Merck, Germany) and hydrogen peroxide (H_2O_2 30% m/m, Suprapur®, Merck). An HNO_3 1% v/v (Suprapur®, Merck, Ultrapure) solution was used to clean the Q-ICP-MS apparatus between quantifications.

The calibration standards were prepared with multi-element standard solution CertiPUR® (Merck, Darmstadt, Germany) 1000 mg L^{-1} at concentrations: 0.5, 1.0, 2.5, 5.0, 10.0 µg L^{-1}. An internal standard mix comprising 50 µg L^{-1} Ge, 5 µg L^{-1} Ir, 10 µg L^{-1} In and 25 µg L^{-1} Y was introduced online with an internal standard mixing kit. The internal standard elements were appropriately matched to analyte elements (de Oliveira et al., 2017; Wetwitayaklung et al., 2018).

2.3. Estimated daily intake

The exposure population assessment to the elements through the ingestion of honey was calculated by estimated daily intake (EDI) according to the following formula suggested by the United States Environmental Protection Agency (USEPA) (US EPA, 2021a):

$$EDI = (C \quad x \quad IR \quad x \quad EF \quad x \quad TE)/(BW \quad x \quad AT)$$

where C is the concentration of each PTE detected in the samples (mg/kg); IR is the Intake Rate of honey (kg/day) for toddlers, adolescents, and adult population; BW is Body Weight (11.3, 52.6, and 69.7 kg_{bw} for toddlers, adolescent, adult, respectively (Leclercq et al., 2009). EF is Exposure Frequency to the contaminant (350 day/year); TE is Total Exposure (70 years), and AT is the Average Lifetime time for non-carcinogenic risk (TE × 365 day/year). The data relating to IR were retrieved from Chronic Food Consumption per day (g/day) of EFSA (EFSA, 2021):

- o Toddlers = median 10.0 g/day, 95th percentile 25.3 g/day
- o Adolescents = median 13.3 g/day, 95th percentile 19.0 g/day
- o Adults = median 10.0 g/day, 95th percentile 35.8 g/day

2.4. Non-carcinogenic risk assessment

2.4.1. Target hazard quotient

The Target Hazard Quotient (THQ_m) for non-carcinogenic effects of each PTE through dietary exposure was calculated through the following formula suggested by USEPA:

$$THQ = \frac{EDI}{RfD_m}$$

RfD_m is Oral Reference Dose (mg/kg_{bw}/day) (Table 1) proposed by US EPA. Some studies used the same RfD for Hg (Kurniawati et al., 2021; Bat et al., 2019); Cr (Wei et al., 2019; Korkmaz et al., 2019; Real et al., 2017); Ni (Korkmaz et al., 2019; Obiora et al., 2019; Real et al., 2017); As (Qin et al., 2021; Real et al., 2017); Mn (Korkmaz et al., 2019). RfD_{Pb} is a particular case because, according to US EPA, setting a reliable threshold for Pb is challenging. We used RfD_{Pb} proposed by other studies (Obiora et al., 2019; Bat et al., 2019; Ullah et al., 2021; Real et al., 2017).

A THQ_m (dimensionless) > 1 entails a high non-carcinogenic risk.

2.4.2. Multiple exposure to toxic elements

In the case of multiple exposure to several contaminants, the cumulative risk arising from the dietary exposure to all elements was assessed through the Hazard Index (HI). This index stands for the sum of THQ_m for each element and is calculated as follows:

$$HI = \sum_m THQ_m$$

An HI > 1 entails a high risk as far as non-carcinogenic risk is concerned.

Table 1
Oral Reference Dose (mg/kg_{bw}/day) and Cancer Slope Factor (mg/kg_{bw}/day)$^{-1}$ for each element.

Elements	RfD (mg/ kg_{bw}/day)	Reference	CSF (mg/ kg_{bw}/day)$^{-1}$	Reference
Hg	0.0003*	US EPA (2021b)	/	
Cd	0.0001	US EPA (2021b)	0.38	Gebeyehu and Bayissa (2020); Nduka et al. (2019) Real et al. (2017)
V	0.005**	US EPA (2021b)	/	
Cr	0.003***	US EPA (2021b)	0.50	(USDOE, 2011)
Ni	0.02▲	US EPA (2021b)	1.70°	(USDOE, 2011)
Cu	0.04	US EPA (2021b)	/	
As	0.0003▲▲	US EPA (2021b)	1.50	US EPA (2021b)
Ba	0.20	US EPA (2021b)	/	
Sb	0.0004▲▲▲	US EPA (2021b)	/	
Pb	0.0035	Obiora et al. (2019); Bat et al. (2019); Ullah et al. (2021) Real et al. (2017)	0.0085°°	(USDOE, 2011)
Mn	0.10	USEPA, 2021	/	

**Vanadium and Compounds
***Chromium VI
▲Nickel Soluble Salts
▲▲Inorganic Arsenic
▲▲▲Antimony (metallic) and Antimony Tetraxide
°Nickel subsulfide
°°Lead and Compounds
* Mercuric Chloride and other Mercury salts

2.5. Carcinogenic risk assessment

The carcinogenic effects related to the ingestion of food contaminated by Ni, Cr, Pb, As, and Cd were evaluated through the Lifetime Cancer Risk (CR) (US EPA, 2021a), that is:

$$LTCR = EDI \times CSF$$

CSF is the Cancer Slope Factor (mg/kg_{bw}/day)$^{-1}$ that estimates the probability of developing cancer through Ni, Cr, Pb, As, and Cd ingestion. The CSF_{Cd} proposed (Table 1) was previously used by Gebeyehu and Bayissa (2020) and Real et al. (2017). USEPA considers an LTCR (dimensionless) $> 1 \times 10^{-4}$ as an unacceptable risk of developing cancer over a human lifetime, whereas values between 1×10^{-6} and 1×10^{-4} are considered an acceptable range for carcinogenic risk (USEPA, 2001). Instead, Health Canada and AEP propose the value of 1×10^{-5} as the maximum safety threshold for the risk of developing cancer (Health Canada, 2010).

The cumulative cancer risk is the risk estimation due to exposure to multiple carcinogenic elements and was calculated as:

$$LTCR_{tot} = \sum_{k=1}^{n} LTCR_k$$

Where $LTCR_k$ is the Life Time Cancer Risk for the cancer element *k*.

2.6. Statistical analysis

Data analysis and graph processing were performed using R Software version 3.6.0 and ggplot2 package (RCore Team, 2019; Wickham, 2016).

3. Results and discussion

3.1. Elements concentration in honey

The levels of each element obtained by the analysis of honey samples are listed in Table 2. No statistically significant difference emerged among the areas and municipalities regarding the levels of heavy metals. The concentrations ranged between 0.70 μg/kg (Hg) and 1713 μg/kg (Mn). Higher mean values were observed for Cu (1049 ± 360 μg/kg), Mn (881.1 ± 405.9 μg/kg), Ba (260 ± 145 μg/kg), and Ni (167 ± 80.4 μg/kg). Other elements reported concentration equal to 5.48 ± 3.55 μg/kg for Cd, 12.0 ± 8.30 μg/kg for V, 72.3 ± 19.2 μg/kg for Cr, 14.9 ± 8.36 μg/kg for As, 3.34 ± 3.02 μg/kg for Sb, 30.1 ± 8.91 μg/kg for Pb, and 31.81 ± 26.03 μg/kg for Hg.

Previous studies assessed the levels of heavy metals in honey in several regions of Italy (Fig. 1.). However, these studies considered fewer elements. Perna et al., 2021 analyzed samples of honey collected from agricultural, forestry, urban, and industries areas of the Basilicata region. They reported similar values for Cd (3.31 ± 2.33 (range: 0.40–9.36) μg/kg) and Ba (187 ± 122 (range: 10.77–544 μg/kg), whereas higher levels for Mn (881 ± 406 (range: 523–1713) μg/kg). Squadrone et al., 2020 considered monofloral and polyfloral honey samples from the pre-Alpine area. They reported similar levels for Cr (68.0 ± 63.0 μg/kg), Cu (950 ± 940 μg/kg), Ni (220 ± 190 μg/kg), and As (16.0 ± 21.0 μg/kg), whereas higher levels for V (9 ± 49 μg/kg), Pb (58 ± 160 μg/kg), and Mn (2100 ± 1700 μg/kg). Bontempo et al. (2017) collected seven different botanical samples in Northern, Central, and Southern Italy, reporting similar data for Ni (200 ± 300 μg/kg) and Ba (200 ± 200 μg/kg) but lower levels of Cr (0 ± 0 μg/kg) and Cu (600 ± 1000 μg/kg) in polyfloral honey. Quinto et al., 2016 analyzed honey samples from Foggia, Naples, Caserta, Campobasso, Isernia, Matera, L′Aquila, and the province of Rome. As regards the area of the Campania region (Caserta and Naples), the levels of the elements that occurred in this area were lower than the levels reported in our study. Di Bella et al. (2015) reported mean values of Ni (220 ± 110 (range: 110–330) μg/kg) in line with our data, although levels of Cr (140 ± 70 (range: 70–190) μg/kg) and Pb (50 ± 30 (range: 30–70) μg/kg) were higher from an analysis of polyfloral honey in Sicily and Calabria. Meli et al., 2015 analyzed twenty-one honey samples collected from Marche, chiefly belonging to the polyfloral species. The analysis showed similar levels for Mn (870 ± 560 (range: 370–2930) μg/kg) but higher values for Cd (20 ± 18 (range: 1–53) μg/kg), Cr (270 ± 230 (range: 20–670) μg/kg), and Pb (180 ± 160 (range: 10–450) μg/kg). Naccari et al., 2014 reported higher levels of Cd (15.3 ± 4 (range: <LOQ - 24) μg/kg) and Pb (170.9 ± 68 (range: 78–390) μg/kg) in seven samples for each honey species (Carob, Chestnut and Eucalyptus). Pisani et al., 2008 considered several botanical origin (including polyfloral) honey samples of the Siena area. Their data-related to polyfloral honey showed similar values for Cd (4.25 ± 3.58 (range: 1.00–15.3) μg/kg) and Sb (3.40 ± 2.19 (range: 1.20–13.3) μg/kg), whereas levels of Ni (273 ± 413 (range: 77–2760) μg/kg), Ba (915 ± 424 (range: 408–2634) μg/kg), Pb (76.4 ± 55.9 (range: 28.2–304) μg/kg), and Mn (1680 ± 3610 (range: 130–1690) μg/kg) were higher than our data. According to the literature, as mentioned earlier, almost all elements reported a fair variability likely due to the area characteristics (urban, industrial, agricultural, volcanic), botanical origin, and collection period. On the other hand, Ni levels are often in agreement with those of other studies in different sites of Italy, namely Basilicata, Calabria, Sicily, Piedmont, Marche and Tuscany regions (Perna et al., 2021; Squadrone et al., 2020; Quinto et al., 2016; Di Bella et al., 2015; Meli et al., 2015; Pisani et al., 2008).

Table 2
Elements detected in honey in eight sites of the Campania.

	Concentration (μg/kg)										
	Cd	**V**	**Cr**	**Ni**	**Cu**	**As**	**Sb**	**Ba**	**Pb**	**Hg**	**Mn**
Mean	5.48	12.03	72.34	167.0	1049	15.0	3.34	259	30.09	31.81	881
SD	*3.55*	*8.29*	*19.18*	*80.35*	*360*	*6.36*	*3.02*	*145*	*8.91*	*26.0*	*406*
Median	5.08	8.53	67.4	137	1107	12.2	2.40	245	28.7	44.6	721
Min	1.15	5.35	48.1	64.3	510	5.35	1.50	103	16.5	0.70	522
Max	10.80	29.7	101	321	1465	26.1	10.60	502	42.8	55.9	1713

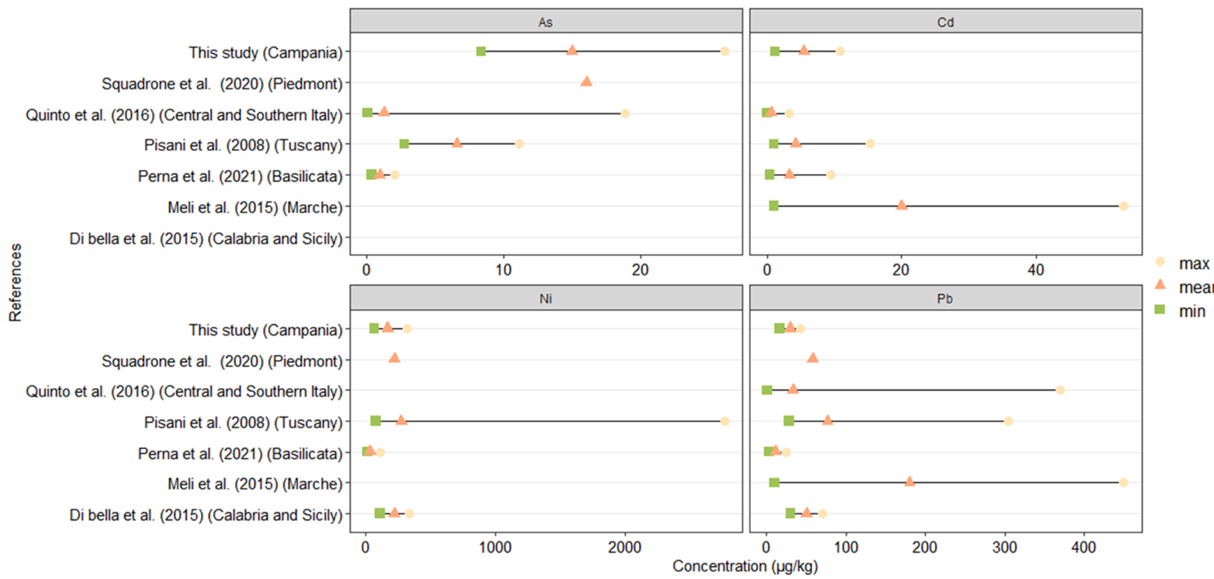

Fig. 1. As, Cd, Ni and Pb levels in honey collected in Italy according to different studies in Italian regions.

Where available, data on polyfloral honey were used for the comparison (Fig. 1).

Pb is the only element in honey that present legal limits in Europe. According to Commission Regulation (EU) 2015/1005 related to maximum levels of Pb in foodstuffs, the threshold value in honey is 100 μg/kg wet weight (EU, 2015). The higher level of Pb was 55.95 μg/kg: therefore, all samples showed concentrations essentially lower than the EU Regulation threshold limit.

3.2. Bioaccumulation comparison among honey, bee, and other beekeeping matrices

This study was part of a broader investigation that evaluated the heavy metals in honeybees and beekeeping matrices (wax and pollen) and the impact of the Covid-19 pandemic on environmental pollution (Scivicco et al., 2022). Unlike the other beekeeping matrices, honey showed the lowest concentration for most elements. This result corroborates the hypothesis that the activity of the worker bees during the honey elaboration process may have a bearing on the levels of metals in the finished product, and this evidence matches that observed in earlier studies (Formicki et al., 2013; Taha et al., 2017; Dżugan et al., 2018; Borsuk et al.,2021). It could conceivably be hypothesized that the levels of heavy metals in the honey is affected by enzymes and molecules (gluconic and ascorbic acid) responsible for chelation of elements and complex formation, leading to the absorption and accumulation of metals in specific body anatomic sections or excretion with feces, rather than their accumulation in honey (Borsuk et al., 2021). Furthermore, the different contamination levels may be due to the chemical-physical features of the matrices: lipophilic elements may preferentially accumulate in the lipids, whose concentrations are higher in honeybees, wax, and pollen than in honey. In addition, pollen and honeybee are more exposed to airborne particulate matter, accumulating elements on their outer surface.

3.3. Risk assessment through honey consumption

3.3.1. Non-carcinogenic risk

To assess non-carcinogenic risk, THQ was calculated based on intake of honey for toddlers, adolescents, and adults (in a median and 95th percentile scenario) and RfD proposed by EFSA and US EPA (Table 1). The values ranged from 7.09E-05 (median exposure) in adults for Ba to 0.40 (95th percentile exposure) in toddlers for Hg (Fig. 2). THQ did not exceed the threshold value of 1 for each element for toddlers, adolescents, and adults in both scenarios indicating that non-carcinogenic health effects were not significant.

Likewise, HI reported values below the safety threshold (namely $<$ 1). The highest value was 0.69 and occurred in toddlers (95th percentile). Hence, the exposure to all elements showed a low probability to cause adverse non-carcinogenic health effects over a lifetime.

3.3.2. Carcinogenic risk

LTCR was used to assess carcinogenic risk based on CSF proposed by US EPA and US DOE (Table 1). Not all elements are carcinogenic; therefore, CSF is only available for Cd, Cr, Ni, As, and Pb (probably carcinogenic). According to USEPA, LTCR values above 1×10^{-4} are considered unacceptable regarding the risk of developing cancer (US EPA, 2001). For Ni exposure, LTCR $> 1 \times 10^{-4}$ was observed in toddlers, whereas the threshold was reached in adolescents and adults to the third quartile (75th percentile). Considering the Health Canada criteria (Health Canada, 2011), the levels of Cr for all age groups and As in toddlers exceeded the threshold of 1×10^{-5} (Fig. 3). Accordingly, a carcinogenic risk emerged from cumulative LTCR assessment: a higher

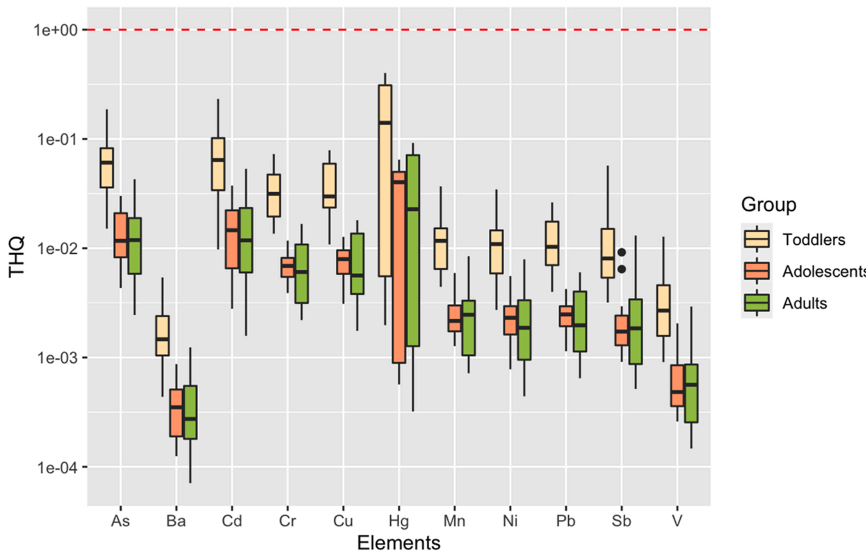

Fig. 2. Target Hazard Quotient (THQ) values for non-carcinogenic risk based on elements exposure in toddlers, adolescents, and adults.

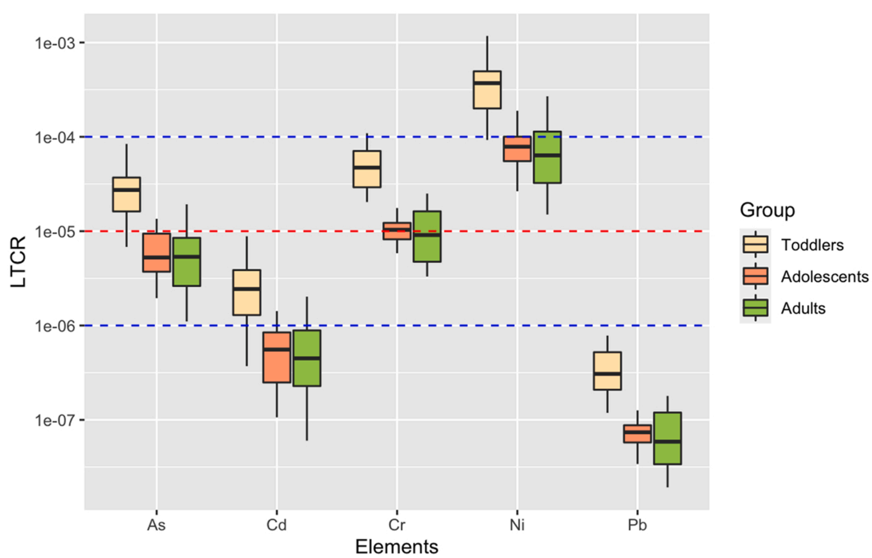

Fig. 3. Lifetime Cancer Risk (LTCR) values based on carcinogenic elements exposure in toddlers, adolescents, and adults.

risk was observed in toddlers (median: 2.44E-04; 95th percentile: 6.17E-04) than adolescents (median: 6.98E-05; 95th percentile: 9.95E-05) and adults (median: 3.95E-05; 95th percentile: 1.42E-04). Previous studies reported significant levels of these heavy metals. Ullah et al. (2022) declared an LTCR > 1E-04 and 1E-05 for Ni and Cd, respectively, based on an analysis of honey collected in Pakistan. Likewise, Pipoyan et al. (2020) reported a carcinogenic risk for Ni (ILCR > 1E-04) as well as As (ILCR in the range of 3.36E-06 and 1.94E-05) related to honey from Armenia. Instead, in Nigeria, Orisakwe et al. (2019) showed a higher LTCR for As, equal to 5.25E-02.

Based on the above, honey collected in some municipalities of the Campania region can accumulate high levels of Ni, Cr, and As, similarly to other areas of the world, pointing out a potential risk for its consumption, mainly among toddlers.

4. Conclusions

Eleven heavy metals and essential elements were analyzed in polyfloral honey collected in different municipalities of the Campania region. No statistically significant difference was observed among sampling sites. The analysis revealed that no sample exceeded the threshold limit of Pb (100 μg/kg), set by Regulation (EU) 2015/1005. Risk assessment in toddlers, adolescents, and adults based on median and 95th percentile honey intake showed no concern for non-carcinogenic risk; In contrast, closer attention deserves to be paid to the exposure to Ni, Cr, and As for carcinogenic risk in the three groups, even though higher values emerged among toddlers. In conclusion, these findings suggest that environmental pollution has a lower impact on the occurrence of heavy metals in honey, which could probably benefit from the capability of honeybees in the detoxification of this product. However, because of the possible risks from honey consumption, setting regulation threshold limits for the Ni, Cr, and As should be considered.

CRediT authorship contribution statement

Marcello Scivicco: Methodology, Investigation, Writing – original draft. **Jonathan Squillante:** Writing – original draft, Writing – review & editing, Data curation. **Salvatore Velotto:** Writing – review & editing. **Francesco Esposito:** Validation, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Teresa Cirillo:** Writing – review & editing, Supervision, Resources. **Lorella Severino:** Conceptualization, Writing – review & editing, Resources, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

References

Aliu, H., Makolli, S., Dizman, S., Kadiri, S., Hodolli, G., 2020. Impact of environmental conditions on heavy metal concentration in honey samples. J. Environ. Prot. Ecol. 21, 351–358.

Bat, L., Şahin, F., Öztekin, A., Arici, E., Yardim, Ö., 2019. Assessment of Cd, Hg, Pb, Cu and Zn amounts in muscles of Cyprinus carpio from Karasu Stream, Sinop. Curr. Agric. Res. J. 7 (2), 171.

Bereksi-Reguig, D., Allali, H., Bouchentouf, S., Adamczuk, A., Kowalska, G., Kowalski, R., 2020. Analysis of trace-elements and toxic heavy metals in honeys from Tlemcen Province, north-western. Agric. Conspec. Sci. 85, 367–374.

Bibi, S., Husain, S.Z., Malik, R.N., 2008. Pollen analysis and heavy metals detection in honey samples from seven selected countries. Pak. J. Bot. 40 (2), 507–516.

Bontempo, L., Camin, F., Ziller, L., Perini, M., Nicolini, G., Larcher, R., 2017 1. Isotopic and elemental composition of selected types of Italian honey. Measurement 98, 283–289. https://doi.org/10.1016/j.measurement.2015.11.022.

Borsuk, G., Sulborska, A., Stawiarz, E., Olszewski, K., Wiącek, D., Ramzi, N., Nawrocka, A., Jędryczka, M., 2021. Capacity of honeybees to remove heavy metals from nectar and excrete the contaminants from their bodies. Apidologie 52 (6), 1098–1111. https://doi.org/10.1007/s13592-021-00890-6.

Cianciosi, D., Forbes-Hernández, T.Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P.P., Zhang, J., Bravo Lamas, L., Martínez Flórez, S., Agudo Toyos, P., Quiles, J.L., Giampieri, F., Battino, M., 2018. Phenolic compounds in honey and their associated health benefits: a review. Molecules 23 (9), 2322. https://doi.org/10.3390/molecules23092322.

Codex Alimentarius , 2019. Standard for honey. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B12–1981%252FCXS_012e.pdf.

Commission Regulation (EU) 2015/1005 of 25 June 2015, amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs.

USDOE, 2011. The Risk Assessment Information System (RAIS). US Department of Energy's Oak Ridge Operations Office (ORO. https://rais.ornl.gov/cgi-bin/tools/TOX_search?select=chemtox.

Crane E.: 1983. "The Archaeology of Beekeeping." London: Gerald Duckworth & Co., 1983.

Di Bella, G., Turco, V.L., Potortì, A.G., Bua, G.D., Fede, M.R., Dugo, G., 2015. Geographical discrimination of Italian honey by multi-element analysis with a chemometric approach. J. Food Compos. Anal. 44, 25–35.

Dżugan, M., Wesołowska, M., Zaguła, G., Kaczmarski, M., Czernicka, M., Puchalski, C., 2018. Honeybees (Apis mellifera) as a biological barrier for contamination of honey by environmental toxic metals. Environ. Monit. Assess. 190 (2), 1–9.

EFSA, 2021. EFSA. https://www.efsa.europa.eu/en/data-report/food-consumption-data.

Engwa, G.A., Ferdinand, P.U., Nwalo, F.N., Unachukwu, M.N., 2019. Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the modern world-new tricks for an old dog. Poisoning in the Modern World - New Tricks for an Old Dog? Intechopen, p. 10.

Formicki, G., Greń, A., Stawarz, R., Zyśk, B., Gał, A., 2013. Metal content in honey, propolis, wax, and bee pollen and implications for metal pollution monitoring. Pol. J. Environ. Stud. 22, 1.

Fraga, C.G., 2005. Relevance, essentiality and toxicity of trace elements in human health. Mol. Asp. Med. 26 (4–5), 235–244. https://doi.org/10.1016/j.mam.2005.07.013.

Gebeyehu, H.R., Bayissa, L.D., 2020. Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia. PloS One 15 (1), e0227883. https://doi.org/10.1371/journal.pone.0227883.

Hashemi, M., 2018. Heavy metal concentrations in bovine tissues (muscle, liver and kidney) and their relationship with heavy metal contents in consumed feed. Ecotoxicol. Environ. Saf. 154, 263–267. https://doi.org/10.1016/j.ecoenv.2018.02.058.

Health Canada Federal Contaminated Site Risk Assessment in Canada, 2010. Part V: Guidance on Human Health Detailed Quantitative Risk Assessment for Chemicals (DQRAchem) Contaminated Sites Division Safe Environments Directorate, Ottawa, Ont (2010).

Heo, J., Park, H.S., Hong, Y., Park, J., Hong, S.H., Bang, C.Y., Lim, M.N., Kim, W.J., 2017. Serum heavy metals and lung function in a chronic obstructive pulmonary disease cohort. Toxicol. Environ. Health Sci. 9 (1), 30–35. https://doi.org/10.1007/s13530-017-0300-x.

Kılıç Altun, S., Dinç, H., Paksoy, N., Temamoğulları, F.K., Savrunlu, M., 2017. Analyses of mineral content and heavy metal of honey samples from south and east region of Turkey by using ICP-MS. Int. J. Anal. Chem. https://doi.org/10.1155/2017/6391454.

Korkmaz, C., Ay, Ö., Ersoysal, Y., Köroğlu, M.A., Erdem, C., 2019. Heavy metal levels in muscle tissues of some fish species caught from north-east Mediterranean: Evaluation of their effects on human health. J. Food Compos. Anal. 81, 1–9. https://doi.org/10.1016/j.jfca.2019.04.005.

Kurniawati, S., Damastuti, E., Adventini, N., Syahfitri, W.Y., Kusmartini, I., Lestiani, D. D., Santoso, M., 2021 24. Determination of several heavy metals in staple foods from traditional markets in Jakarta using neutron activation analysis. InAIP Conference Proceedings, Vol. 2349. AIP Publishing LLC. https://doi.org/10.1063/5.0053376.

Leclercq, C., Arcella, D., Piccinelli, R., Sette, S., Le Donne, C., 2009. The Italian National Food Consumption Survey INRAN-SCAI 2005-06: main results in terms of food consumption. Public Health Nutr. 12 (12), 2504–2532. https://doi.org/10.1017/S1368980009005035.

Li, C., Zhou, K., Qin, W., Tian, C., Qi, M., Yan, X., Han, W., 2019. A review on heavy metals contamination in soil: effects, sources, and remediation techniques. Soil Sediment Contam.: Int. J. 28 (4), 380–394. https://doi.org/10.1080/15320383.2019.1592108.

Meli, M.A., Desideri, D., Roselli, C., Benedetti, C., Feduzi, L., 2015. Essential and toxic elements in honeys from a region of central Italy. J. Toxicol. Environ. Health, Part A 78 (10), 617–627. https://doi.org/10.1080/15287394.2014.1004006.

Nduka, J.K., Kelle, H.I., Amuka, J.O., 2019. Health risk assessment of cadmium, chromium and nickel from car paint dust from used automobiles at auto-panel workshops in Nigeria. Toxicol. Rep. 6, 449–456. https://doi.org/10.1016/j.toxrep.2019.05.007.

Obiora, S.C., Chukwu, A., Chibuike, G., Nwegbu, A.N., 2019. Potentially harmful elements and their health implications in cultivable soils and food crops around lead-zinc mines in Ishiagu, Southeastern Nigeria. J. Geochem. Explor. 204, 289–296. https://doi.org/10.1016/j.gexplo.2019.06.011.

de Oliveira, F.A., de Abreu, A.T., de Oliveira Nascimento, N., Froes-Silva, R.E.S., Antonini, Y., Nalini, de Lena, J.C., 2017. Evaluation of matrix effect on the determination of rare earth elements and As, Bi, Cd, Pb, Se and in in honey and pollen of native Brazilian bees (Tetragonisca angustula–Jataí) by Q-ICP-MS. Talanta 162, 488–494. https://doi.org/10.1016/j.talanta.2016.10.058.

Omafuvbe, B.O., Akanbi, O.O., 2009. Microbiological and physico-chemical properties of some commercial Nigerian honey. Afr. J. Microbiol. Res. 3 (12), 891–896. https://doi.org/10.5897/AJMR.9000494.

Orisakwe, O.E., Ozoani, H.A., Nwaogazie, I.L., Ezejiofor, A.N., 2019. Probabilistic health risk assessment of heavy metals in honey, Manihot esculenta, and Vernonia amygdalina consumed in Enugu State, Nigeria. Environ. Monit. Assess. 191 (7), 1–4. https://doi.org/10.1007/s10661-019-7549-2.

Perna, A.M., Grassi, G., Gambacorta, E., Simonetti, A., 2021. Minerals content in Basilicata region (southern Italy) honeys from areas with different anthropic impact. Int. J. Food Sci. Technol. 56 (9), 4465–4472. https://doi.org/10.1111/ijfs.15112.

Pipoyan, D., Stepanyan, S., Beglaryan, M., Stepanyan, S., Asmaryan, S., Hovsepyan, A., Merendino, N., 2020. Carcinogenic and non-carcinogenic risk assessment of trace elements and POPs in honey from Shirak and Syunik regions of Armenia. Chemosphere 239, 124809. https://doi.org/10.1016/j.chemosphere.2019.124809.

Pisani, A., Protano, G., Riccobono, F., 2008. Minor and trace elements in different honey types produced in Siena County (Italy). Food Chem. 107 (4), 1553-60.

Popek, S., 2002. A procedure to identify a honey type. Food Chem. 79 (3), 401–406. https://doi.org/10.1016/S0308-8146(02)00391-6.

Qin, J., Niu, A., Liu, Y., Lin, C., 2021. Arsenic in leafy vegetable plants grown on mine water-contaminated soils: Uptake, human health risk and remedial effects of biochar. J. Hazard. Mater. 402, 123488 https://doi.org/10.1016/j.jhazmat.2020.123488.

Quinto, M., Miedico, O., Spadaccino, G., Paglia, G., Mangiacotti, M., Li, D., Centonze, D., Chiaravalle, A.E., 2016. Characterization, chemometric evaluation, and human health-related aspects of essential and toxic elements in Italian honey samples by inductively coupled plasma mass spectrometry. Environ. Sci. Pollut. Res. 23 (24), 25374–25384. https://doi.org/10.1007/s11356-016-7662-5.

R. Core Team. 2019, R: A language and environment for statistical computing.

Real, M.I.H., Azam, H.M., Majed, N., 2017. Consumption of heavy metal contaminated foods and associated risks in Bangladesh. Environ. Monit. Assess. 189 (12), 1–4. https://doi.org/10.1007/s10661-017-6362-z.

Sankhla, M.S., Sharma, K., Kumar, R., 2017. Heavy metal causing neurotoxicity in human health. Int. J. Innov. Res. Sci. Eng. Technol. 6, 5.

Scivicco, M., Nolasco, A., Esposito, L., Ariano, A., Squillante, J., Esposito, F., Cirillo, T., Severino, L., 2022. Effects of Covid-19 pandemic lockdown and environmental pollution assessment in Campania region (Italy) through the analysis of heavy metals in honeybees. Environ. Pollut. 307, 119504 https://doi.org/10.1016/j.envpol.2022.119504.

Società Italiana di Nutrizione Umana (SINU), 2014. LARN: livelli di assunzione di riferimento di nutrienti ed energia per la popolazione italiana. SICS.

Squadrone, S., Brizio, P., Stella, C., Pederiva, S., Brusa, F., Mogliotti, P., Garrone, A., Abete, M.C., 2020. Trace and rare earth elements in monofloral and multifloral honeys from Northwestern Italy; A first attempt of characterization by a multi-elemental profile. J. Trace Elem. Med. Biol. 61, 126556 https://doi.org/10.1016/j.jtemb.2020.126556.

Taha, E.K., Al-Jabr, A.M., Al-Kahtani, S.N., 2017. Honey Bees, Bee-collected pollen and honey as monitors of environmental pollution at an industrial cement area in Saudi Arabia, 1-0 J. Kans. Èntomol. Soc. 90 (1). https://doi.org/10.2317/151230.1.

Ullah, A.K., Afrin, S., Hosen, M.M., Musarrat, M., Ferdoushy, T., Nahar, Q., Quraishi, S. B., 2021. Concentration, source identification, and potential human health risk assessment of heavy metals in chicken meat and egg in Bangladesh. Environ. Sci. Pollut. Res. 1–2. https://doi.org/10.1007/s11356-021-17342-4.

Ullah, R., Jan, F.A., Gulab, H., Saleem, S., Ullah, N., 2022. Metals contents in honey, beeswax and bees and human health risk assessment due to consumption of honey: a case study from selected Districts in Khyber Pakhtunkhwa, Pakistan. Arch. Environ. Contam. Toxicol. 1–4. https://doi.org/10.1007/s00244-021-00910-7.

US EPA, 2021a https://www.epa.gov/iris Accessed December 1, 2021.

US EPA, 2021b. https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide https://epa-prgs.ornl.gov/cgi-bin/chemicals/csl_search Accessed December 1, 2021.

USEPA (United States Environmental Protection Agency), USEPA Risk Assessment Guidance for Superfund:Volume III - Part A, Process for Conducting Probabilistic Risk Assessment (2001).

Wei, J., Gao, J., Cen, K., 2019. Levels of eight heavy metals and health risk assessment considering food consumption by China's residents based on the 5th China total diet study. Sci. Total Environ. 689, 1141–1148. https://doi.org/10.1016/j.scitotenv.2019.06.502.

Wetwitayaklung, P., Wangwattana, B., Narakornwit, W., 2018. Determination of traceelements and toxic heavy minerals in Thai longan, litchi and Siam weed honeys using ICP-MS. Int. Food Res. J. 25, 1464–1473.

Whitfield, J.B., Dy, V., Mcquilty, R., Zhu, G., Heath, A.C., Montgomery, G.W., Martin, N. G., 2010. Genetic effects on toxic and essential elements in humans: arsenic, cadmium, copper, lead, mercury, selenium, and zinc in erythrocytes. Environ. Health Perspect. 118 (6), 776–782. https://doi.org/10.1289/ehp.0901541.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, NY, USA. ISBN 978-3-319-24277-4.

Wu, X., Cobbina, S.J., Mao, G., Xu, H., Zhang, Z., Yang, L., 2016. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ. Sci. Pollut. Res. 23 (9), 8244–8259. https://doi.org/10.1007/s11356-016-6333-x.