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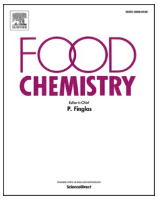

Food Safety and quality of some Italian honey: Study of long-persistent contaminants (heavy metals and PFAS) in different production areas in southern Italy

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ABSTRACT

In recent years, persistent environmental contaminants such as PFAS have emerged alongside traditional pollutants like heavy metals. Honey is a useful biomonitoring tool thanks to bees' foraging range, which reflects average pollution levels. The objective of this study was to determinate heavy metals and PFAS in honey from six areas of the Molise region (rural, industrial, and remote), to evaluate environmental impact on the matrix, verify if honey meets health standards, and assess potential consumer risks. 56 honey samples were collected over four months directly from honeycombs. Heavy metals were analyzed by ICP-AES; PFAS by SPE-LC-MS/MS. Aluminium, antimony, and selenium were the most detected metals; three PFAS (Bistriflimide, 6:2FTS, L-PFOS) were found at ultra-trace levels. Chemometric analysis identified arsenic, antimony, and selenium as area-specific markers. Risk assessment showed higher non-carcinogenic risk in areas A and E, and carcinogenic risk in C and D. Area B was the least impacted.

1. Introduction

Honey is made up of a super-saturated solution of carbohydrates and includes traces of minerals, protein, enzymes, polyphenols, and aromatic molecules. It has been utilized as a nutritious food and a medicinal product in the past and continues to be used now (Montenegro & Mejías, 2013; Rana et al., 2018). Honey has a long history of use, mostly as a medicine in addition to being used as food. This use is corroborated by several discoveries made in the Greek and Egyptian periods. As late as today, it is still very usual in our society to use or recommend honey as a remedy for coughs and sore throats, following Hippocrates' widespread recommendations; moreover, the Egyptians, the Christians, and the Muslims all considered honey to be a natural product (Arawwawala & Hewageegana, 2017; Saranraj et al., 2016). Nowadays, there is evidence of honey's protective effects on the cardiovascular, nervous, respiratory and gastrointestinal systems due to the antioxidant and/or antimicrobial effects which the substances it contains may exert. That is why it is often added to gels, dressings, ointments, creams, and syrups for the treatment and healing of wounds and skin infections. (Arawwawala & Hewageegana, 2017; El-Seedi et al., 2020; Hossain et al., 2021; Lee et al., 2011). It is also used for its antifungal, antiviral, antibacterial and anti-obesity properties (Al-Kafaween et al., 2023; Rana et al., 2018). In addition, the presence of some sugars that cannot be degraded by the human gut (oligosaccharides and polysaccharides that have β-glycosidic bonds) make honey a prebiotic product able to help the growth of probiotic microorganisms from other food and dietary sources (Mustar & Ibrahim, 2022). Due to its beneficial properties, it is important to take into account the possible presence of foreign substances in the composition of honey. In fact honey, as stated in Directive, 2001/110/EC, must be "as far as possible [...] free of organic and inorganic substances foreign to its

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composition" (Council Directive, 2001/110/EC of 20 December, 2001 Relating to Honey, 2001). The presence of foreign substances within honey is due to pesticide treatments, plant protection treatments on plants, and environmental pollution (Nowak & Nowak, 2023). Since bees "absorb" these compounds while gathering nectar and pollen, all of these substances can be found in honey. Furthermore, pollutants from the environment can also be adsorbed onto the body of the insect during flight. For these reasons, bees and honey are increasingly used as low-cost tools being increasingly used to conduct environmental biomonitoring in order to detect contaminants in the environment with a relatively large radius (1.5–3 km) around the hive (Di Fiore et al., 2023; Fuente-Ballesteros et al., 2025; Lambert, Piroux, et al., 2012; Lambert, Veyrand, et al., 2012; Negri et al., 2015).

Furthermore, the use of honey as a tool for environmental biomonitoring is now well known and validated especially for the inorganic fraction, which has chemical characteristics that are closely related to this food matrix, although bees are certainly the best tool for this purpose (Alnaggar et al., 2013; Di Fiore et al., 2023; Herrero-Latorre et al., 2017). About the alien substances that can be found in honey, many of them result from the scientific and industrial development that has taken place in the last century. Today these substances are recognized as a concern for human and environmental health (Glüge et al., 2020; Nowak & Nowak, 2023). Among the foreign substances in the composition of honey we can find heavy metals, which represent the inorganic fraction of chemical contaminants. Naturally present in the environment, they can also result from anthropogenic processes such as mining and smelting activities, emission from vehicular traffic, disinfectant and cosmetic products (Duruibe et al., 2007; Fu & Xi, 2020).

Heavy metals may readily attach to sulfur groups, affecting enzyme activity (Duruibe et al., 2007; Fu & Xi, 2020). Poly and perfluorinated compounds (PFAS), which are highly impactful for human and environmental health, belong to the group of emerging harmful substances within the Persistent Organic Pollutants (POPs) and are characterised by high chemical stability and environmental persistence due to the strong carbon–fluorine bond. (Podder et al., 2021). Since these chemicals have been identified as endocrine disruptors associated with polycystic ovarian syndrome and normal systems of prenatal and postnatal development, the scientific community is concerned about them (Bonato et al., 2020; Glüge et al., 2020; Iannone et al., 2024). Perfluorooctanoic acid (PFOA) is one of the chemicals that has been demonstrated to be hazardous as carcinogen and mutagenic agent (Bonato et al., 2020; Glüge et al., 2020). Furthermore, a recent study, through the assessment of various risk indices, demonstrated that the interaction between PFAS and heavy metals leads to a substantial increase in the incidence of cardiovascular diseases (Boafo et al., 2023). In this context, this interaction may affect biological processes associated with disease development (Lahera et al., 2007; Victor et al., 2009). This observation highlights the potential role of these environmental contaminants as contributing factors to the progression of the latter disease (Boafo et al., 2023). Cardiovascular disease (CVD) poses a significant public health concern, characterised by a worrisome escalation attributable to social, environmental, and economic factors (Deaton et al., 2011). Given that humans are constantly exposed to chemical mixtures of environmental contaminants (Hollander et al., 1999), it is particularly important to evaluate dietary exposure to pollutants such as toxic metals and PFAS (Boafo et al., 2023).

This study investigates the occurrence of heavy metals and fluorinated organic compounds (PFAS) in 56 honey samples collected from different locations in the Molise region (Southern Italy). Although these contaminants differ markedly in their chemical and physical properties, both are characterised by high environmental persistence, with metals accumulating in ecosystems and PFAS being widely recognized as "forever chemicals" (Teymourian et al., 2021; Wu et al., 2016). The aims of this study were: (i) to determine and quantify the concentrations of selected heavy metals and PFAS in honey samples; (ii) to compare the measured concentrations with regulatory limits established for human consumption and to perform a dietary risk assessment; (iii) to investigate the potential sources of heavy metal and PFAS contamination in the Molise region and to explore differences among sampling locations through the analysis of honey samples.

2. Materials and methods

2.1. Study area and sampling

Honey samples were collected from hives situated in six different sites of Molise Region (Italy): Bojano (site A: coordinates 41°29′N 14°28′E; area: 49 km^2; elevation 480 m; population 8181), Campochiaro (site B: coordinates 41°24′N 14°33′E; area: 35.7 km^2; elevation 750 m; population 636), Petrella Tifernina (site C: coordinates 41°41′N 14°42′E; area: 26.36 km^2; elevation 553 m; population 1044), Riccia (site D: coordinates 41°29′N 14°50′E; area: 69.9 km^2; elevation 680 m; population 5152), Roccavivara (site E: coordinates 41°57′N 14°42′E; area: 21.05 km^2; elevation 652 m; population 805), Tufara (site F: coordinates 41°34′N 14°44′E; area: 35.52 km^2; elevation 420 m; population 887) (Fig. 1). In this area are located several apiaries belonging to professional, local beekeepers. While Bojano station is close to the Campochiaro/Bojano industrial region, Campochiaro station is situated within the WWF Guardiaregia-Campochiaro Oasis Nature Reserve. The area in question encompasses an expanse of over 3000 ha, incorporating woodlands, fluvial systems, and subterranean cavern networks. There is little agricultural impact from the Petrella Tifernina station, which is close to the Montagano landfill. The stations of Riccia, Tufara and Roccavivara are located in typical agricultural settings, but the latter is distinguished by the presence of an ecological island in the area. Hence, the presence of anthropogenic and industrial activities, as well as vehicular traffic, can compromise the state of environmental quality, requiring an accurate biomonitoring activity of the pollutants. During a four-month period from June to September 2021, honey samples were taken directly from a portion of the honeycomb inside the hives. On the same date of sampling, samples were taken with and without the smoker; the latter were labelled with an "s" at the end of their names. The number of honey samples collected varied among regions due to differences in the size of the apiaries involved. Sampling was designed to ensure representativeness of each area rather than enforcing an identical number of samples per site. A total of 56 samples were stored in sealed plastic containers in a dark and cool environment until analysis.

2.2. Reagents and chemicals

For the inorganic chemical analysis, $65\%_{w/w}$ nitric acid (Sigma-Aldrich, Milan, Italy) was used for the mineralization phase of the samples, together with ultrapure water, 18.2 MΩ cm, (Milli-Q system, Human Corporation, Seoul, Republic of Korea). A laboratory standard mixture of mineral elements (O2Si Smart Solution, North Charleston, SC, USA) in a water solution at 10% of HNO_3, containing Co, Fe, Mn, Ni, Cd, Pb, Zn, Al, Se, As, Sb and Hg, with a concentration of 1000 mg L^{-1} ± 0.5% was used for method development and quantification of heavy metals in honey samples (Table 1S of the Supplementary Material).

For the fluorine organic chemical analysis, Methanol (LC-MS grade) was purchased from Honeywell (Charlotte, NC, USA). Acetonitrile (LC-MS grade) and ammonium hydroxide solution 30% (NH_4OH) were supplied by Carlo Erba (Milan, Italy). Acetic acid was purchased from VWR International LLC (Radnor, PA, USA). *n*-nonane, ammonium acetate (99.2%) and ENVI-Carb™ SPE Bulk were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained using a Millipore Milli-Q Reference A+ purification system (Mohlseim, France). SPE Strata-X-AW (200 mg, 6 mL, code 8B-S038-FCH and Kinetex XB-C_{18} 100 A LC column (100 × 3 mm; 2.6 μm, code 00d-4496-Y0) were purchased from Phenomenex (Torrance, CA, USA). Oasis HLB (2.1 × 20 mm, 5 μm, code 186002034) and Oasis WAX (2.1 × 20 mm, 5 μm, code 1860022508) on-line columns were obtained from Waters Inc. (Milford,

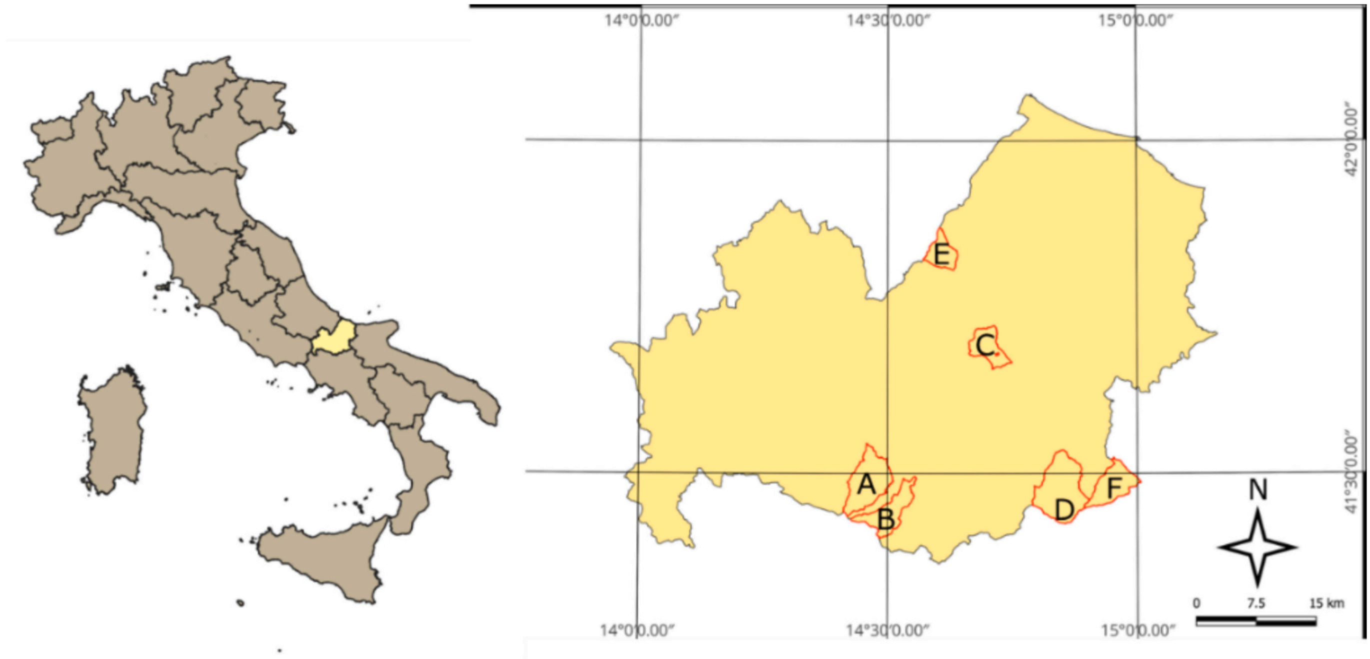


Fig. 1. Geographical position of the municipalities under study within the Molise region.

Table 1
Descriptive statistics[a] of metals found in all samples.

Heavy Metals	min (ng g^{-1})	max (ng g^{-1})	mean (ng g^{-1})	SD	RSD (%)
Al	104	233	143.9	26.8	18.6
As	<LOD	128.6	77.5	49.0	63.2
Cd	<LOD	19.5	8.3	6.9	82.4
Co	<LOD	4.7	0.5	1.1	235.3
Cu	8	75	30.5	13.4	44.0
Mn	21	143	52.8	28.9	54.8
Ni	<LOD	52.8	3.8	7.8	207.2
Pb	<LOD	24	10.1	7.2	71.4
Sb	33.8	137.2	96.4	34.5	35.8
Se	85.8	194.6	137.5	26.5	19.2

[a] Results below LOD were treated according to the EFSA bound scenario approach. For the calculation of the middle-bound estimate, values below the LOD were substituted with LOD/2, whereas the lower- and upper-bound estimates were obtained by assigning values of 0 and the LOD, respectively.

MA, USA). PFASs native and labelled standards were supplied by Wellington Laboratories (Guelph, Ontario, Canada). The complete list of the reference materials and labelled standards is reported in Table S2 (Supplementary Material).

2.3. Inorganic compounds analysis

2.3.1. Sample treatment

Honey samples were mineralized using nitric acid and microwaves to remove the organic portion in the sample and prevent it from acting as an interferent. Specifically, 0.5 g of honey was weighed into a plastic centrifuge container and diluted with ultrapure water up to 5 mL. The sample was brought into solution by stirring for 3 min with a vortex (ZX3, VELP Scientific, Usmate, Italy) and two with ultrasound (Strasonic, 18–35, Liarres.r.l., Casalfiumanese, Italy). The formed solution was then transferred to the digester vessels and 10 mL of $65\%_{w/w}$ HNO_3 was added to it. Mineralization was performed using a MARS 5-CEM microwave digester, equipped with hermetically sealed Autovent HP-1500 containers, at a power of 600 W for 20 min. The resulting solution was filtered and diluted up to 25 mL with ultrapure water.

2.3.2. ICP-AES analysis

The metals were assessed employing Agilent Technologies 4210 MP-AES inductively coupled with plasma atomic emission spectroscopy (ICP-AES), in accordance with the EPA method 6010C (EPA, 2000). Quantitative analysis was performed using calibration curves with concentrations of 50, 100, 150, 200 and 250 ng mL^{-1} obtained from the dilution of the above-mentioned multi-element solution.

In such kind of studies, Quality Assurance and Quality Control (QA/QC) procedures are very important: they were implemented in accordance with EFSA recommendations to ensure the reliability and comparability of the analytical results. The determination of elemental contaminants in honey samples was carried out using external calibration with multi-element standard solutions. Procedural blanks were included in each analytical batch to monitor potential contamination, and blank levels were consistently negligible relative to sample concentrations. Instrument calibration was verified using calibration check standards, with acceptable deviations within ±10%. Analytical accuracy was evaluated by analyzing the certified reference material NIST SRM 1515 (Apple Leaves), which was subjected to the same digestion and analytical procedure as the honey samples. Measured concentrations were in good agreement with the certified values, with recoveries generally falling within the acceptable range (80–120%), in line with EFSA guidance. Analytical precision was assessed through replicate analyses, yielding relative standard deviations (RSDs) within acceptable limits. Limits of detection (LODs) were determined based on repeated measurements of procedural blanks.

All the analytical data of each element, such as the emission wavelength, calibration curve, R^2, LOD and limit of quantification (LOQ), are listed in Table S1 (Supplementary Material).

2.4. Organic fluorine compounds analysis

2.4.1. Sample preparation

Two grams of honey were spiked at 50 pg g^{-1} with the twenty-five isotopically labelled internal standards (10 μL of a solution at 10 ng mL^{-1}). The sample was solubilized in 20 mL of MeOH/ammonium acetate 100 mM (50:50, *v:v*) solution, vortexed, shaken for 10 min and then centrifuged (2235 ×*g*, 5 min, 20 °C). Strata X-AW SPE cartridges (200 mg, 6 mL) were firstly conditioned with 6 mL of MeOH, followed by

6 mL of water, rinsed three times with the eluting mixture (3 × 6 mL of 2% NH_4OH in MeOH) and conditioned again (6 mL MeOH +6 mL of water). After sample loading, the cartridges were washed with 12 mL of water, 8 mL of MeOH and dried under vacuum. Finally, the analytes were eluted using 6 mL of 2% NH_4OH in MeOH into a 15 mL polypropylene tube containing 80 mg of d-SPE Envicarb and 100 μL of acetic acid. After shaking (5 min) and centrifugation (3220 ×*g*, 10 min, 20 °C), the supernatant was transferred into a 15 mL polypropylene tube containing 50 μL of *n*-nonane. After near-dryness solvent evaporation (N_2, 40 °C), 0.2 mL of 80:20 MeOH/ammonium acetate 4 mM mixture were added. Matrix-matched standards were resuspended with the same mixture containing all the analytes. Finally, samples were carefully vortexed, sonicated (5 min) and centrifuged (3220 ×*g*, 5 min, 20 °C) prior to LC-MS injection.

2.4.2. LC/MS-MS analysis and MS conditions

Chromatography was performed on a Thermo Ultimate 3000 High Performance Liquid Chromatography system (Thermo Scientific, San Jose, CA, USA). Analytes were separated on a Kinetex XB C_{18} column (100 × 3.0 mm; 2.6 μm) equipped with a C_{18} guard column. Between pump and injector two mini-LC columns (Oasis WAX 20 × 2.1 mm and Oasis HLB 20 × 2.1 mm) were installed. Mobile phases were water (A) and methanol (B) both containing 5 mM of ammonium acetate. The gradient started with 100% of eluent A at 0.05 mL min^{-1}. In 1 min flow and eluent B were increased to 0.3 mL min^{-1} and 30%, respectively. The gradient continued reaching 60% B in 10 min, 70% after 15 min and 100% B after 21 min. This condition was maintained for 5 min. The system returned to initial conditions (0.05 mL min^{-1} 100% A) in 2 min. The re-equilibration step was maintained for 2 min for a total run time of 30 min. The column temperature was 40 °C and the sample temperature was kept at 16 °C. The injection volume was 20 μL. To avoid background contamination from mobile phases and instrument components, two mini-LC columns (2.1 × 20 mm) packed with weak anion exchange (WAX) and hydrophilic-lipophilic balance (HLB) stationary phase, respectively, were installed between the pumping system and the injector.

The mass analyser Q-Orbitrap (Q-Exactive, Thermo Scientific, San Jose, CA, USA) was equipped with heated electrospray ionization (HESI-II) source. The optimized HESI-II temperature was set at 320 °C, the capillary temperature at 300 °C, the electrospray voltage at 3.5 kV (negative mode) and S-lens value was adjusted at 50 V. Sheath and auxiliary gas were 40 and 15 arbitrary units, respectively. The MS experiments performed to detect the analytes, are reported in a previous work (Barola et al., 2020), whereas retention times (RTs), monitored ion species, LOD and LOQ are reported in Table S3 (Supplementary Material). The mass extraction window was 5 ppm.

2.5. Statistical analysis

Statistical analyses were performed to investigate relationships among inorganic elements and PFAS in honey samples and to assess differences among sampling locations within the Molise region. Descriptive statistics, including minimum, maximum, mean, standard deviation (SD), and relative standard deviation (RSD), were calculated using Microsoft Excel.

Values below the limit of detection (<LOD) were handled differently depending on the type of analysis. For descriptive statistics and exposure assessment calculations, <LOD values were treated as zero in order to provide a conservative estimate of exposure. For multivariate statistical analyses (PCA, ANOSIM, and SIMPER), <LOD values were replaced with the corresponding LOD to avoid computational issues associated with zero values and to allow proper matrix calculation.

Differences in heavy metal levels among sampling locations within the Molise region were statistically evaluated using multivariate approaches, rather than element-specific univariate tests, in order to account for the multivariate structure of the dataset and the presence of correlated variables.

Principal component analysis (PCA) and Pearson correlation analysis were performed using the R statistical environment to explore relationships among elements and identify common patterns. Correlations were considered statistically significant at $p < 0.05$.

Differences among sampling areas were then assessed using one-way analysis of similarities (ANOSIM), and similarity percentage analysis (SIMPER) was applied to identify the variables contributing most to the observed dissimilarities. Multivariate statistical analyses were performed using PAST software (version 4.11).

3. Results and discussion

3.1. Inorganic compounds

The general results of the inorganic compounds, given the large number of samples as well as the large number of chemical elements analyzed, are shown in Table 1, which reports the descriptive statistics concerning the totality of samples for each analyzed metal, whereas the complete dataset is available in Table S4 (Supplementary Material). Comparisons between samples collected under different sampling conditions (with and without a smoker) showed both increases and decreases in metal concentrations, without a univocal trend. Looking at Table 1, the most frequently occurring elements in the samples are: aluminium, with an average of 143.9 ng g^{-1}; selenium, with an average of 137.5 ng g^{-1}; antimony, with an average of 96.4 ng g^{-1}; copper, with an average of 30.5 ng g^{-1}; and manganese, with an average of 52.8 ng g^{-1}. The elements with the greatest variability within the sample are cobalt, nickel and cadmium, which have a percentage standard deviation of 253.3%, 207.2% and 82.4%, respectively. From the perspective of the presence of individual elements, cadmium has values that range between LOD and 19.5 ng g^{-1}, with an average of 8.3 ng g^{-1}; lead has values between LOD and 24 ng g^{-1} with an average of 10.1 ng g^{-1}; and arsenic has values between LOD and 128.6 ng g^{-1}, with an average of 77.5 ng g^{-1}. Among these elements, particular attention is given to cadmium, lead and arsenic, as they are included in Commission Regulation (EU) 2023/915. Although only lead has a maximum limit established for honey, these metals are discussed due to their regulatory relevance (*Commission Regulation (EU)* 2023/915, 2023).

To evaluate spatial patterns in metal occurrence, data from each surveyed area are reported and discussed below. The data for the above-mentioned variables are shown in Figs. 2 and 3, respectively, and their spatial distribution is discussed below by individual sampling area. Average concentrations were calculated treating values below the limit of detection (< LOD) as zero, as described in the Materials and Methods, section 2.5.

Area A is characterised by the presence of Aluminium (144.3 ng g^{-1}), Selenium (144.2 ng g^{-1}), Antimony (106.30 ng g^{-1}) and Arsenic (86.4 ng g^{-1}). Within area A, Aluminium, Selenium, Manganese and Copper showed a decrease during the specified period of investigation. Conversely, arsenic and antimony exhibited a consistent increase. Additionally, while there was a marginal increase in cadmium, all other metals under scrutiny remained constant. Taking into account the sources in common for aluminium (Alasfar & Isaifan, 2021; Zhang et al., 2016) and copper (Di Fiore et al., 2023; Rehman et al., 2019), the observed decrease in the given period could be attributed to reduced environmental pollution as a consequence of the prohibition on the initiation of fires and the combustion of brushwood during the designated sampling period. Furthermore, the decrease in Al and Cu may also be due to the gradual reduction in the use of fertilisers on growing fields in the spring and summer period, as is the case for Se. Aluminium is used as a fertiliser to increase the length of the roots and shoots, the antioxidant activity, the leaf area and therefore the photosynthetic activity, which favours the growth of the plant and the fruit (Alghofaili et al., 2025). Copper can be used as a fungicide with the aim of crop yield enhancement. This includes tomato crops, with the fungicide

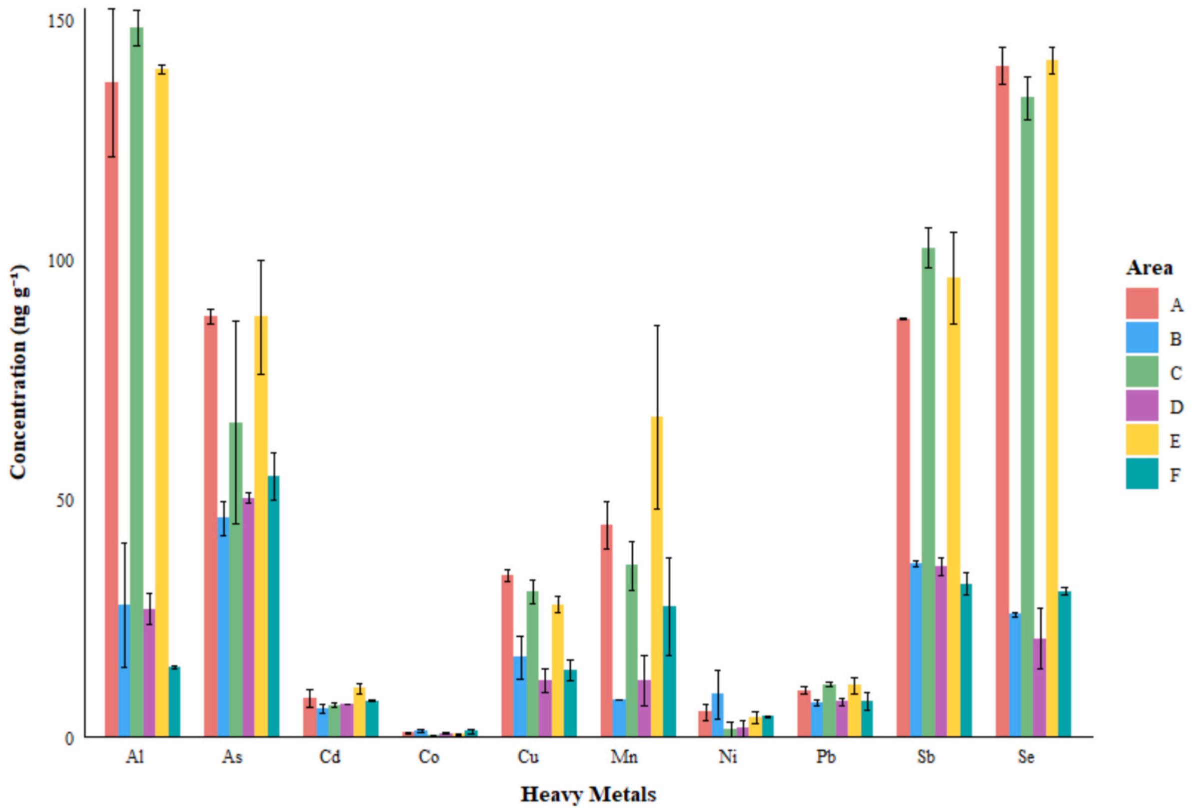


Fig. 2. Average dispersion of heavy metals analyzed within each zone during the sampling period, with relative standard deviations.

administered via both soil and leaf treatments (Zhu et al., 2012). Selenium is also employed to enhance the quality of tomato fruit (total soluble solids, soluble sugars, titratable acid, sugar-acid ratio, vitamin C and lycopene) by fertilising the soil and spraying the leaves (Xu et al., 2022) thus entering the food chain of pollinators and flower visitors (Quinn et al., 2011). The reduction of manganese, on the other hand, may be due to a constant decrease of this substance in the environment due to the non-use of heating systems during the considered period (Barrio-Parra et al., 2018; De Miguel et al., 1999). Arsenic and antimony have been observed to exhibit a consistent increase over time. The former contamination is attributable to a number of factors, including the combustion of fossil fuels (Ungureanu et al., 2015), the latter is used in car brake pads and discs, which release antimony trioxide (a potential carcinogen) during braking (Ungureanu et al., 2015). Consequently, we may hypothesise that a decline in vehicular traffic in the region, attributable to reduced work-related commuting, will be associated with a greater persistence, and therefore an increase in air pollutants, due to the lack of rainfall in summer (Duhanyan & Roustan, 2011; Elperin et al., 2011).

Area B, on the other hand, is characterised by the presence of selenium (144.20 ng g^{-1}), aluminium (140.29 ng g^{-1}) and antimony (86.23 ng g^{-1}). In this area, the concentrations of arsenic and antimony exhibit an increase over time, while the concentrations of all other elements remain relatively constant. However, it is observed that the concentration of copper tends to decrease, while those of selenium and lead fluctuate. As previously stated, the observed increase in the concentrations of arsenic and antimony over time may be attributable to an enhanced environmental persistence of pollutants emanating from vehicular traffic (Ungureanu et al., 2015) due to a lower frequency of rainfall (Duhanyan & Roustan, 2011; Elperin et al., 2011). As in zone A, the use of copper is decreasing due to the ban on lighting fires.

Area C has selenium (138.80 ng g^{-1}), among other elements such as aluminium (117.80 ng g^{-1}), arsenic (102.40 ng g^{-1}) and antimony (87.12 ng g^{-1}). In a similar trend to areas A and B, arsenic and antimony increase significantly over time in area C. The remaining metals, although fluctuating, remain almost constant over time, with the exception of aluminium and copper, which tend to increase in September. The slight increase in these two elements could be due to the use of fertilisers (Alghofaili et al., 2025; Zhu et al., 2012) or to small fires and the burning of brushwood (Di Fiore et al., 2023; Rehman et al., 2019), which are not permitted by local laws, could account for the slight increase in these two elements.

Area D is characterised by the presence of aluminium (151.7 ng g^{-1}), selenium (138.5 ng g^{-1}), antimony (98.0 ng g^{-1}), arsenic and manganese (89.4 and 86.0 ng g^{-1} respectively). In this area there is a clear increase in arsenic and antimony between the end of August and September, as in the previous areas. Manganese rose at the end of August and then fell in September, but remained higher than in June, as did aluminium, although to a lesser extent. For the latter two, the trend could stem from their use in industrial activities such as producing metal alloys and painting metals (Baukal & Jr., 2000; Howe et al., 2004; Ipeaiyeda et al., 2012; Li et al., 2014) and lack of rainfall during this period, which helps pollutants persist in the environment (Duhanyan & Roustan, 2011; Elperin et al., 2011). Indeed, the area boasts a number of manufacturing companies specialising in metal construction, doors and windows. While the trend for the remaining metals can be defined as constant, selenium exhibited a significant negative fluctuation in the latter half of July.

The analysis of Area E reveals the presence of Aluminium (138.6 ng g^{-1}), Selenium (136.4 ng g^{-1}), Antimony (105.5 ng g^{-1}) and Arsenic (86.8 ng g^{-1}). It is noteworthy that the levels of antimony and arsenic in this area also increased in August and stabilised in September most likely for the same reasons as areas A, B, C and D. Selenium exhibited a decreasing tendency (as for area A) and aluminium an upward tendency during the sampling period together with a slightly increase of copper. It is hypothesized that the trend of the latter is probably due to an increase of Al pollution due to vehicle traffic (Zhang et al., 2016) as for Sb and As for others areas or due to fertiliser use (Alghofaili et al., 2025). The

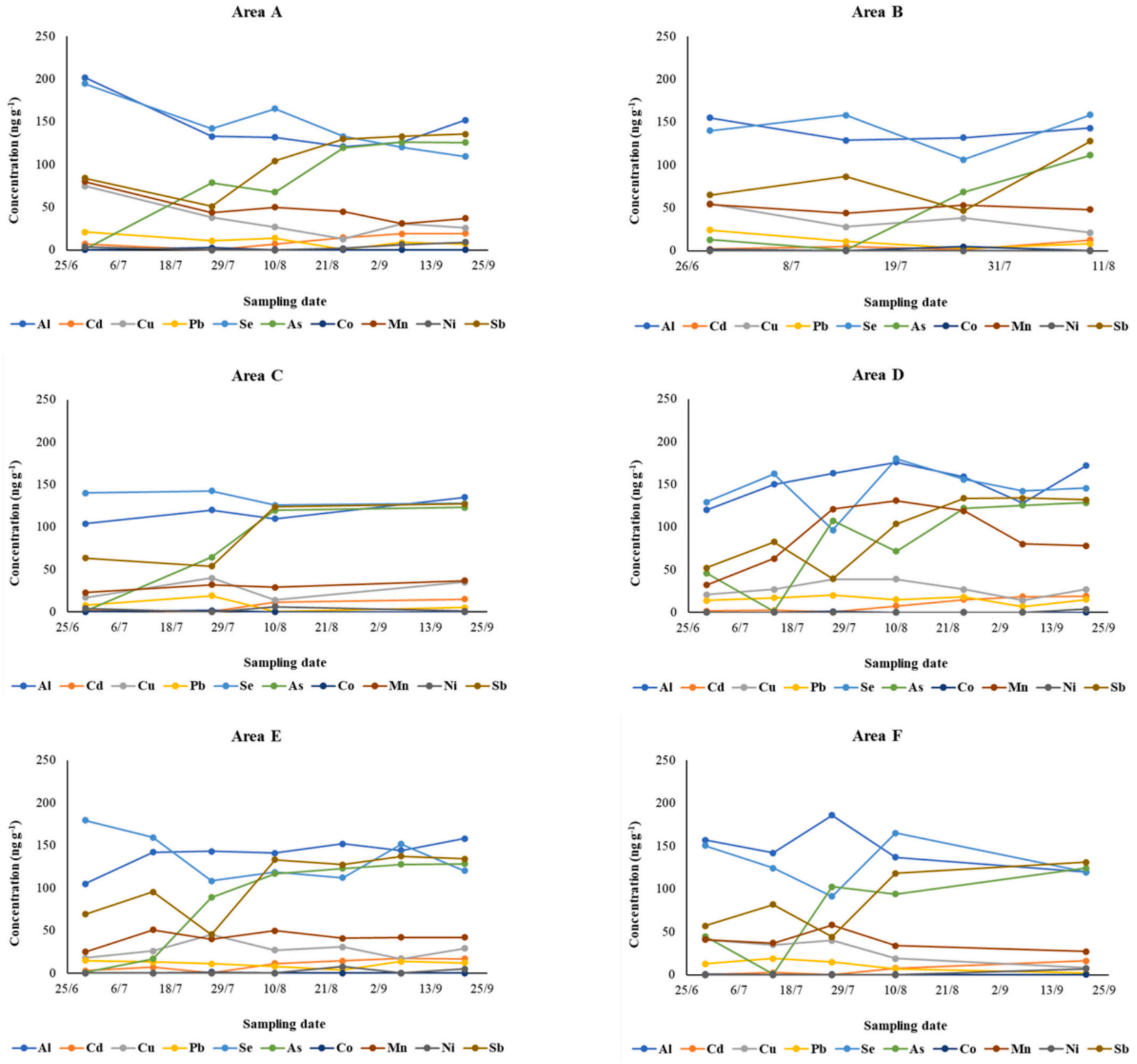


Fig. 3. Heavy metal fluctuations during the span of the sampling period in the areas under investigation.

remaining metals are almost constant over time.

The presence of aluminium (155.7 ng g^{-1}), selenium (128.1 ng g^{-1}) and antimony (87.5 ng g^{-1}) is evident in Area F. It is notable that antimony and arsenic levels exhibit an increase during the months of August and September, a phenomenon that is similar to the other areas. It is evident that the aluminium and manganese and copper and selenium pairs demonstrate a slight downward tendency. The remaining metals appear to be constant over time. Given the proximity of area F to area D, it is plausible that the isolated increase in aluminium and manganese is due to airborne migration of this pollution (Ghio & Samet, 1999; Li et al., 2014; Sahu & Basti, 2021). Copper and selenium levels increase slightly during the period of decrease, suggesting a migration of pollution from other areas, as in the case of aluminium and manganese, or due to the application of fertilisers during the growth phase of the plants (Xu et al., 2022; Zhu et al., 2012).

3.1.1. *Comparison with other studies on heavy metals*

In this paragraph, the concentrations of the metals under investigation were compared with those reported in several regions, including Abruzzo (IT) (Flamminii et al., 2024), Campania (IT) (Scivicco et al., 2022), Serbia (Jovetić et al., 2018), Poland (Ligor et al., 2022), Algeria (Bella et al., 2022), and Australia (Hungerford et al., 2020). The corresponding literature data are summarised in Table 2.

For Aluminium (Al), our data were significantly lower, approximately 100 and 10 times less than those recorded in Abruzzo and Australia respectively. Arsenic (As) concentrations were comparable to those found in Abruzzo, both being about ten times higher than those from non-Italian samples. In contrast, the Campania region exhibited lower levels compared to both our study and Abruzzo, but higher than the non-Italian samples. Regarding cadmium (Cd), our results were similar to those from Campania, Serbia, and Australia, but lower than those from Abruzzo and Algeria. Cobalt (Co) levels in our study aligned with those found in Poland, yet were significantly lower than those from

Table 2
Mean concentrations and standard deviations of under investigation metals reported in the literature for various geographical locations (data expressed in ng g^{-1}). The symbol '-' indicates that the substances were not investigated in the referenced study.

Chemical	Abruzzo – IT	Campania – IT	Serbia	Polland	Algeria	Australia
Al	12,555.56 ± 385.00	–	–	–	–	1200.00 ± 1500.00
As	52.22 ± 15.20	15.00 ± 6.36	<1.00–9.00	0.06	8.42 ± 0.88	2.60 ± 1.00
Cd	35.56 ± 14.20	5.48 ± 3.55	<2.00–9.00	<LOD	23.98 ± 1.13	3.10 ± 4.00
Co	55.56 ± 10.40	–	nd	0.93	335.71 ± 61.20	16.70 ± 44.00
Cu	12,966.67 ± 176.90	1049.00 ± 360.00	<15.00–1781.00	136	1900.00 ± 954.00	200.00 ± 400.00
Mn	37,333.33 ± 650.00	881.00 ± 406.00	79.0–2428.00	458	2554.29 ± 262.10	3800.00 ± 4100.00
Ni	1258.89 ± 27.50	167.00 ± 80.35	<10.00–538.00	13.34	531.43 ± 53.10	33.10 ± 107.00
Pb	2084.44 ± 85.10	30.09 ± 8.91	<3.00–107.00	<LOD	711.43 ± 52.70	28.60 ± 74.00
Sb	–	3.34 ± 3.02	–	0.06	101.43 ± 4.80	5.10 ± 1.00
Se	–	–	–	–	430.00 ± 11.04	5.20 ± 2.00
Reference	(Flamminii et al., 2024)	(Scivicco et al., 2022)	(Jovetić et al., 2018)	(Ligor et al., 2022)	(Bella et al., 2022)	(Hungerford et al., 2020)

Australia, Abruzzo, and Algeria, in that order. Copper (Cu) concentrations were similar but lower than those reported in Poland, and markedly lower than those found in other regions. Manganese (Mn) levels in our study were considerably lower than those reported in all other studies, with Abruzzo displaying the highest concentrations. Nickel (Ni) levels were lower but comparable to those observed in Poland and Australia, and considerably lower than the levels in other regions, with Abruzzo again presenting the highest concentrations. Lead (Pb) concentrations in our study were lower than those from all other regions, with Abruzzo and Algeria exhibiting the highest values. Antimony (Sb) levels in our study were comparable to those found in Algeria and higher than in other regions. Selenium (Se) was only analyzed in Algeria and Australia, with our data falling between the values observed in these two regions. Overall, the comparison with literature data indicates that the mean concentrations of metals measured in honey samples in the present study are generally lower than those reported for several other Italian and non-European regions, particularly for aluminium, copper, manganese, and lead. For some elements, such as arsenic, antimony, and selenium, mean concentrations are within the same order of magnitude as those reported in other studies, although a high variability among the different geographical areas considered is observed.

3.2. *Fluorine organic compounds*

About PFAS under investigation, most of the honey samples analyzed showed the near absence of such compounds. Precisely, only 3 substances out of the 39 were determined. In sample E3s, Bistriflimide was found in the amount of 0.008 ng g^{-1}, for sample E4 the compound 6:2FTS had a concentration of 0.005 ng g^{-1}, in sample E6 was found L-PFOS (0.010 ng g^{-1}), while in sample F2s the 6:2FTS was determined (0.007 ng g^{-1}). From these results, it can be observed that PFAS were found in 3 samples from area E and one from area F. In addition, 6:2FTS was found in 2 out of 4 contaminated samples. Fig. 4 shows the L-PFOS chromatogram for the E6 sample, the reinforced blank, and the blank. Therefore, the PFAS found in the current study demonstrate very little contamination. Bistriflimide is an ionic fluid whose anion is involved in lithium battery production and, among others, in lubrication technologies, in fact it has been recently detected in recycling plants. Moreover, it has been associated with electronic waste (Barola et al., 2023). Being particularly mobile in the environment, its presence in Site E could be due to contamination from a nearby ecological island. PFOS is now a ubiquitous pollutant known since the 1950s in Teflon production. It is a surfactant with hydrophobic and oleophobic properties used, for

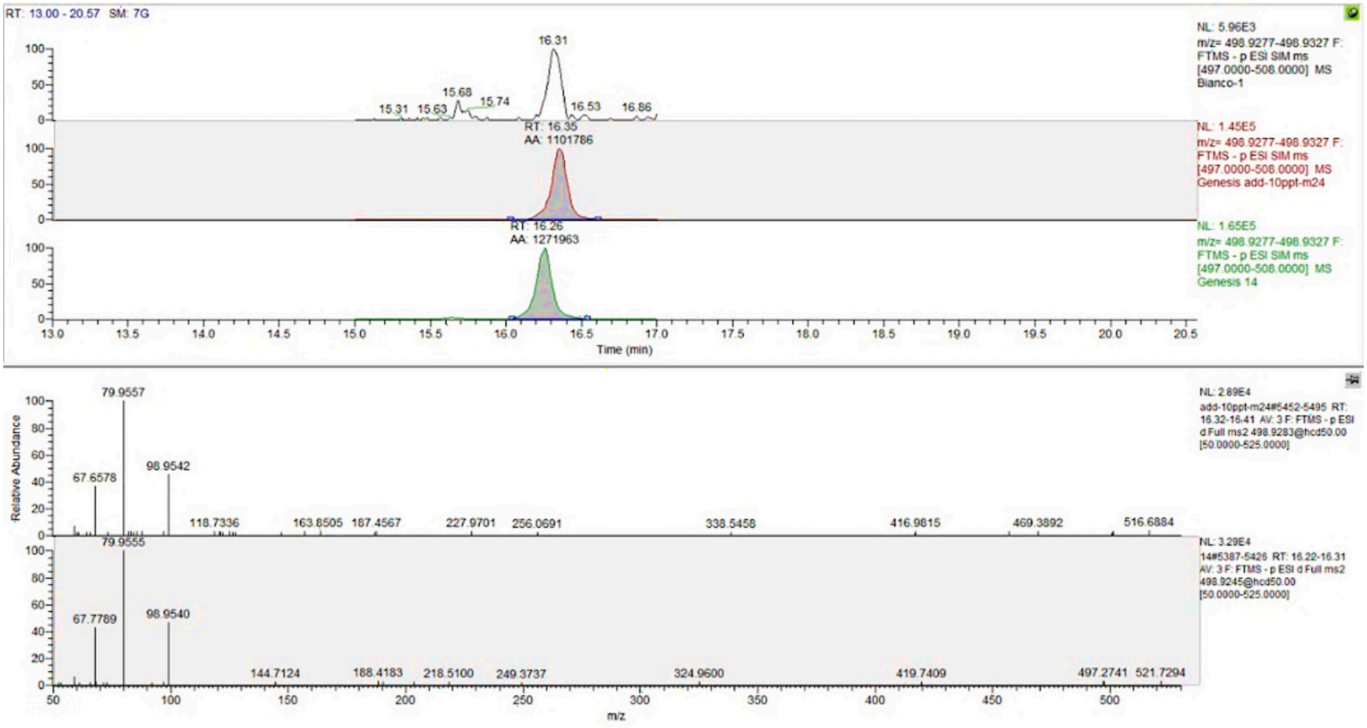


Fig. 4. Chromatogram concerning L-PFOS: blank sample; fortified blank, E6 sample; MS/MS spectrum fortified sample; MS/MS spectrum E6 sample.

example, in coatings. Besides being persistent, it is bioaccumulative, toxic and motile in the environment especially in aquatic environments because of its low volatility in air. Its sources are difficult to identify because of its multiple fields of application (paper treatment, chromium plating, fire-fighting foams, and semiconductors) although the primary source remains perfluorooctanosulfonylfluoride (POSF) degradation (Abunada et al., 2020; Lim et al., 2011). 6:2 FTS belongs to the class of fluorotelomers used in surface coatings, paints, as additive for adhesives and waxes, and in printer inks until 2014. Recently they were used to replace long-chain PFASs in anti-fog products and chrome plating. The main source of emissions is leachate from landfills (Hamid et al., 2020); both Zone E and Zone F are near waterways that could therefore act as a source of pollution.

3.2.1. Comparison with other studies on PFAS

With regard to the presence of perfluoroalkyl substances (PFAS) in honey, the extant literature is very limited. Among these, a 2016 survey investigating the presence of PFAS in honey from different European countries (Surma et al., 2016) and a preprint article of March 2025 investigating the presence of PFAS in Italian honey from some municipalities near Ferrara (Fe) (Pes et al., 2025) were considered. However, both of these studies analyse only a subset of the PFASs studied in this work (with the exception of PFBA, which is not analyzed in this study but is present in the last Italian study), which is the first to investigate simultaneously the presence of 39 PFASs in honey. The results of the results of these studies are summarised in Table 3.

The most prevalent molecules in the aforementioned studies are as follows: PFHpA, which varies (average value for the countries) from 0.191 to 0.350 ng g^{-1} concerning the work of Surma et al.; PFBA, which has an average of 0.342 ng g^{-1} in samples from the Ferrara area; PFOA, which varies between 0.037 and 0.224 ng g^{-1} considering both works previously mentioned. As outlined in section 3.2, the molecules detected in the samples under investigation in this study are 6:2FTS, Bistriflimide and L-PFOS. However, the substances in question were identified in only 4 out of 56 samples. It is noteworthy that the concentrations identified in the samples of the present study are, in the majority of cases/general, significantly lower than those reported in the aforementioned works.

3.3. Multivariate and statistical analyses

Multivariate and statistical analyses were applied to explore relationships among the investigated contaminants and to assess similarities and differences among sampling areas. All statistical analyses were conducted considering both inorganic contaminants and PFAS; however, due to the very limited number of PFAS detections and their low concentrations, these compounds did not contribute significantly to the multivariate outputs, which were therefore mainly driven by heavy metals.

3.3.1. Principal component analysis and Pearson correlation

Principal component analysis (PCA) was carried out to simplify the number of components under study. In fact, PCA makes it possible to greatly reduce the variables involved without reducing the information in the dataset. The choice of the number of principal components (PCs) to be considered usually entrusted to the scree graph that shows the eigenvalues of the individual new components generated by the software. In this case, the scree plot (Fig. S1) shows that a number of PCs equal to 4 should be considered because as that many PCs have eigenvalues greater than 1 (Carbone & Quici, 2020; Lucero et al., 2019; Passarella et al., 2022). In this case, however, only 3 PCs are sufficient since they alone account for 59.78% of the dataset examined. Fig. 5a shows the dispersion of the samples taking into account only the first two PCs (PCs1: Cd, Sb and As; PCs2: Al, Mn and Cu); Fig. 5b, on the other hand, shows the dispersion of the samples taking into account also the third PCs (PCs3: Se, Co and Pb). Please consult Table S5 in the supplemental material for the loading tables specific to each PCs. Table S6 (Supplementary Materials) displays the individual PCs scores for each sample.

In light of the data obtained from the principal component analysis (PCA), whereby the principal components (PCs) are represented exclusively by metals, the univariate linear r (Pearson) correlation between the latter chemicals was investigated. In this way, it is possible to understand which substances increase together, probably indicative of the same source of apportionment of the entire area under investigation. As illustrated in Fig. 6, there are notable (value greater than 0.7) and significant (p value <0.05) positive correlations between Cu and Al and between Sb and Cd. In contrast, Pearson's correlation revealed no statistically significant relationship between PFAS and heavy metals. However, a clear relationship was evident among the three PFAS molecules identified in the four samples, albeit with a p-value greater than 0.05. Hence, these values are not shown in Fig. 6.

The correlation between antimony and cadmium can be attributed to the anthropogenic sources of these two metals. In addition to general metallurgical processes and the leaching of mining waste, antimony can also be found in toys, paints, batteries, ammunition, and firefighters' equipment that acts as a flame retardant (Bolan et al., 2022). Food packaging plastic can also be a source of antimony as well as plastic bottles (Periferakis et al., 2022). At the same time, cadmium is especially utilized in the mining sector as well as in the manufacturing of plastics and batteries (Wu et al., 2016). It is thus hypothesized that the primary sources of these two metals may stem from the leaching of mining waste or the improper disposal of electronic and plastic waste. Here one may note that two of the six sampling areas, namely areas A and E, have recycling centres within their territories. Both the hypotheses outlined in the identification of pollution sources in section 3.2 and those presented in the current section corroborate the hypothesis that the mismanagement of electronic and plastic waste constitutes a potential source of environmental pollution in the areas under investigation, thereby impacting the studied food matrix.

Table 3

Average data and relative standard deviations of PFAS reported for each examined area. Concentrations are expressed in ng g^{-1}, except for the Ferrara (Italy) samples, which are reported in ng mL^{-1}. The symbol "–" indicates that the substances were not investigated in the referenced study.

Chemical	Scotland	England	Poland	Slovakia	France	Spain	Italy	Ferrara (IT)
PFOA	0.18 ± 0.01	0.21 ± 0.01	n.d.	n.d.	0.22 ± 0.10	0.19 ± 0.00	0.12 ± 0.00	0.04 ± 0.45
PFHpA	0.32 ± 0.02	0.35 ± 0.01	0.31 ± 0.01	0.19 ± 0.00	n.d.	0.22 ± 0.01	0.25 ± 0.00	–
PFNA	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.00	0.18 ± 0.01	0.15 ± 0.01	–
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28 ± 0.02	–
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	0.15 ± 0.01	0.08 ± 0.00	–
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	–
PFBA	–	–	–	–	–	–	–	0.34 ± 2.86
PFPeA	–	–	–	–	–	–	–	4.74 ± 2.93
PFHxA	–	–	–	–	–	–	–	0.21 ± 1.78
L-PFBS	–	–	–	–	–	–	–	0.13 ± 2.00
Reference	(Surma et al., 2016)	(Surma et al., 2016)	(Surma et al., 2016)	(Surma et al., 2016)	(Surma et al., 2016)	(Surma et al., 2016)	(Surma et al., 2016)	(Pes et al., 2025)

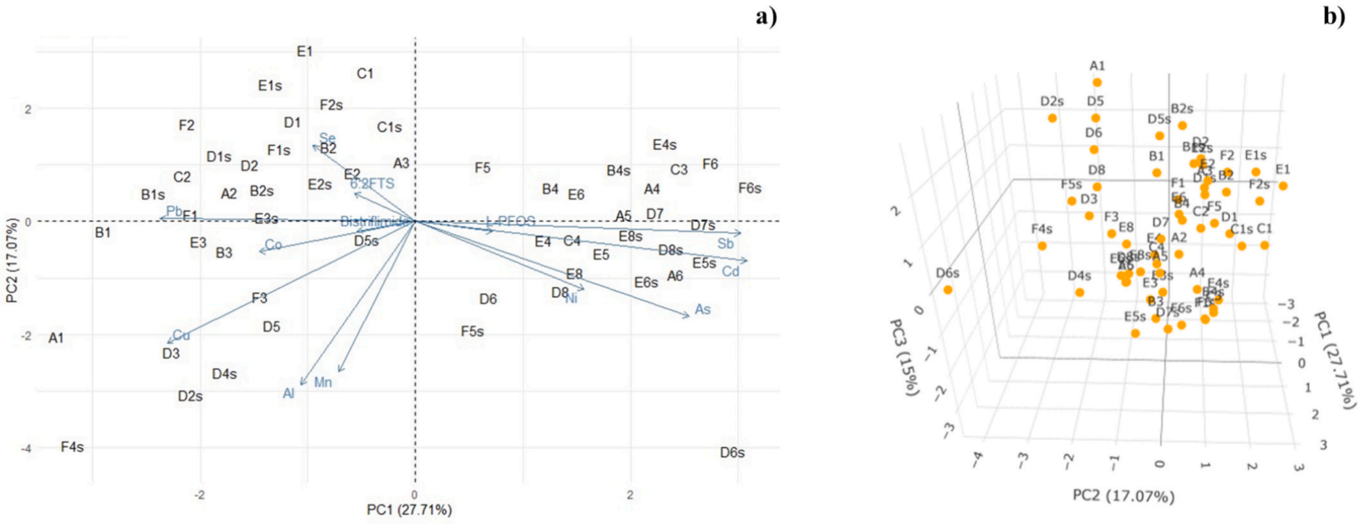
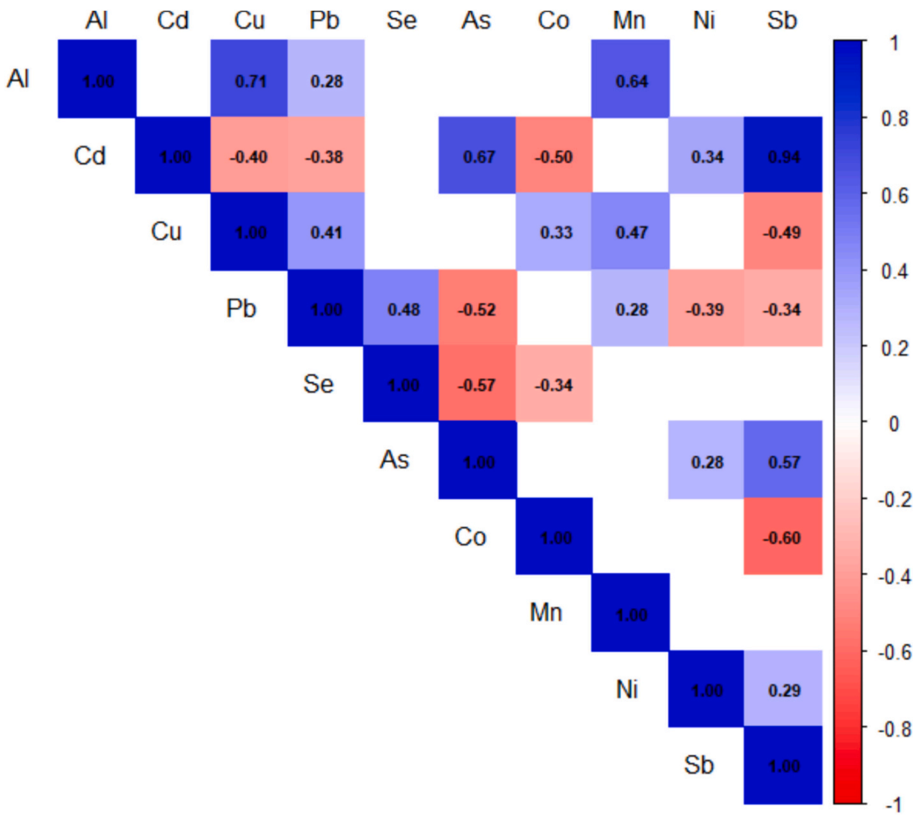


Fig. 5. a) Scatter plot of 2 PCs (PCs1: Cd, Sb and As; PCs2: Al, Mn and Cu); **b)** 3D Graph of 3 PCs (PCs1: Cd, Sb and As; PCs2: Al, Mn and Cu; PCs3: Se, Co and Pb).



	Al	Cd	Cu	Pb	Se	As	Co	Mn	Ni	Sb
Al	1.00		0.71	0.28				0.64		
Cd		1.00	-0.40	-0.38		0.67	-0.50		0.34	0.94
Cu			1.00	0.41			0.33	0.47		-0.49
Pb				1.00	0.48	-0.52		0.28	-0.39	-0.34
Se					1.00	-0.57	-0.34			
As						1.00			0.28	0.57
Co							1.00			-0.60
Mn								1.00		
Ni									1.00	0.29
Sb										1.00

Fig. 6. Univariate linear r (Pearson) correlation between heavy metals under investigation.

Besides, the anthropogenic sources of copper and aluminium contamination are analogous. Accordingly, these metals are ubiquitous in the environment. The anthropogenic sources of aluminium include waste incineration, coal burning, mining operations and road traffic and fertiliser. Furthermore, the food industry employs aluminium in the form of additives, utensils and packaging (Alasfar & Isaifan, 2021; Alghofaili et al., 2025; Zhang et al., 2016). Copper pollution represents a phenomenon that arises as a consequence of the combustion of fossil fuels, wood (in the context of forest fires), and a range of other substances, including batteries, paints, fertilisers, and pesticides. Also, it can be caused by inappropriate waste management procedures (Di Fiore et al., 2023; Rehman et al., 2019). In this instance, the co-occurrence of these phenomena can be attributed to the potential for forest fires to occur during the summer period, in addition to the persistent air pollution generated by vehicular traffic. Indeed, all the sampling areas are in close proximity to the main roads in the region, and it is acknowledged that traffic can influence the level of pollutants in the area. Furthermore, a significant source of pollution is identified as agricultural activity, which is prevalent in many of the areas under study, including areas A, C, D, E and F. It should be emphasised that aluminium is used as a fertiliser in low concentrations. Conversely, although high aluminium inputs have been shown to induce genetic alterations and developmental impairment under specific conditions, particularly in acidic soils (Alghofaili et al., 2025; Qin et al., 2010), the results of the present study suggest that agricultural activity represents a minor source of aluminium compared to other environmental inputs, as evidenced in areas A, C, D, E, and F.

3.3.2. Multivariate analysis of similarities

Finally, a multivariate statistical analysis was conducted in order to understand any statistically significant differences between the zones under study. By means of the one-way ANOSIM test, carried out using Past software version 4.11, Table 4 was derived where it is possible to highlight through the use of *p*-value (when less than 0.05 (Biau et al., 2010; Kennedy-Shaffer, 2019)) the significant difference between the two zones E and D. Area E is not only very distant from area D, but also has different territorial characteristics from the latter. In fact, it does not have a large number of companies or industries, such as those in the window and door sector, like area D. Conversely, it can be affirmed that area E is mainly characterised by a rural and agricultural environment, where light vehicle traffic and agriculture have an impact on anthropogenic pollution. This finding is confirmed by the analysis of the metal trends over time in the different areas carried out in section 3.1.

Moreover, by means of the one-way SIMPER test, it is possible to check which variables under study have the greatest influence on the dissimilarities of the investigated areas. Table 5 shows that As, Sb, Se, Mn, and Al, respectively, are the parameters that vary the most between the zones of interest, as can also be seen from the different averages of these elements expressed by individual zone.

As can be seen from Table 5, Arsenic (25.37%) contributes to the differentiation of the various areas. Besides anthropogenic sources such as fossil fuel combustion and mining wastes, this element also comes from agricultural activities related to weed and pest control and animal feeds (Ungureanu et al., 2015). Although the trend of this element is quite similar in all areas, the difference in concentration, and therefore its variability, could be due to a different agricultural impact on the environment in different areas. In terms of zonal differentiation, arsenic accounts for a total of 43.2%, together with Antimony, which accounts for 17.83%. In addition to brake discs, Antimony is derived from various other anthropogenic sources, including fossil fuel combustion, vehicular emissions, mining waste leaching, metallurgical operations, ammunition, firefighter clothing treated with flame retardants, as well as paints, toys, batteries, and plastics (Bolan et al., 2022; Periferakis et al., 2022; Ungureanu et al., 2015). In this instance, the anthropic source responsible for the diversification of the areas may be the presence of an ecological island near areas A and E. This would have an impact on the average value, but not on the trend over time. The monitoring of these two metals is important in order to assess the quality and/or adoption of good practices both in agriculture and in the management of ecological islands in the concerned areas because, if they co-exist, this is very dangerous as they are defined oncogenic (Bolan et al., 2022). A similar discussion of inter-area variability can be made for Se, Mn and Al. The specific anthropogenic sources of these elements have already been discussed in the section concerning the individual areas.

Table 4
Multivariate statistical analysis highlighting the assessment of similarities (*p*-value).

Areas	A	B	C	D	E	F
A		0.1685	0.3804	0.5145	0.598	0.729
B	0.1685		0.3306	0.0937	0.0742	0.7482
C	0.3804	0.3306		0.051	0.2052	0.7686
D	0.5145	0.0937	0.051		0.0295	0.0511
E	0.598	0.0742	0.2052	0.0295		0.1416
F	0.729	0.7482	0.7686	0.0511	0.1416	

4. Honey consumption risk

Health risk assessment is used to estimate the probability of adverse health effects associated with the consumption of honey contaminated by chemicals such as heavy metals and PFAS over a defined exposure period (Bayir & Aygun, 2022; Conti et al., 2022). To quantitatively evaluate potential non-carcinogenic and carcinogenic risks, several well-established indicators are commonly applied, as described below.

Estimated Daily Intake (EDI): estimates the daily intake of a contaminant, normalized to body weight, and is calculated using the formula:

$$EDI = \frac{(C\,x\,IR\,x\,EF\,x\,TE)}{(BW\,x\,AT)} \tag{1}$$

where C is the contaminant concentration in honey (mg/kg), IR the ingestion rate (g/day), EF the exposure frequency (350 days/year), TE the total exposure duration (years), BW body weight (kg), and AT average exposure time (days) (Souza et al., 2023; US EPA, 2024).

Target Hazard Quotient (THQ): assesses non-carcinogenic risk by dividing EDI by the Reference Dose (RfD) (Ullah et al., 2022).

$$THQ = \frac{EDI}{RfD} \tag{2}$$

A THQ < 1 is considered safe; values ≥ 1 may indicate potential non-carcinogenic risk (Beshaw et al., 2022; Obasi et al., 2020).

Hazard Index (HI): the sum of THQ values for multiple metals, used to evaluate cumulative risk from combined exposure.

$$HI = \sum THQm \tag{3}$$

An HI value ≥ 1 indicates a potential health risk (Scivicco et al., 2022).

Lifetime Cancer Risk (LTCR) estimates carcinogenic risk using the product of EDI and the Cancer Slope Factor (CSF), as commonly applied in human health risk assessment (Amouei et al., 2024):

$$LTCR = EDI\,x\,CSF. \tag{4}$$

The total cancer risk (LTCR cumulative) from multiple contaminants is:

$$LTCR\ cumulative = \sum LTCR \tag{5}$$

Acceptable cancer risk values range between 10^{-6} and 10^{-4}; values inferior than 10^{-6} are negligible, while values higher than10^{-4} are considered unacceptable for health (U.S. Environmental Protection Agency, Washington, DC, 600).

4.1. Non-carcinogenic risk

The EDI was calculated using the average concentrations of individual substances detected in each area, based on the parameters reported in Table 6. The table are entirely taken from the study by Flamminii et al.(Flamminii et al., 2024).

This parameter was then used to assess the non-carcinogenic risk through the calculation of the THQm for each element, area, and age group. The RfD values used for this assessment were taken from Table 7. This table was adapted for the present work from the study by Flamminii et al.(Flamminii et al., 2024), with some substances retained as originally reported, others removed, and additional ones included. The results of this assessment are shown in Fig. 7.

Arsenic (As), Antimony (Sb), Cadmium (Cd), and Selenium (Se) are the substances that contribute most significantly to the THQm across the different sampling areas and age categories. The data clearly show that concentrations of As, Sb, and Cd are generally higher in areas A, D, and E compared to other areas, regardless of age. For example, in toddlers, arsenic levels range from 0.31 to 0.37 in areas A, C, D, and E, while they

Table 5
Multivariate statistics analyzing similarity percentage, with mean values expressed in ng g^{-1}.

Taxon	Contrib. %	Cumulat. %	Mean A	Mean B	Mean C	Mean D	Mean E	Mean F
As	25.37	25.37	86.4	44.7	61.4	89.4	86.8	73.2
Sb	17.83	43.2	106	86.2	87.1	98	105	87.5
Se	13.66	56.86	144	144	139	139	136	128
Mn	13.2	70.06	47.8	49.1	29.6	86	39.4	42.2
Al	13.03	83.09	144	140	118	152	139	156
Cu	6.733	89.83	35	32.7	25.8	32.6	27.8	29.7
Pb	3.783	93.61	10.5	12.4	6.4	11.6	9.21	8.9
Cd	3.548	97.16	11.3	6.19	5.84	9.13	9.89	6.1
Ni	2.44	99.6	3.58	< LOD	4.44	6.95	3.1	2.66
Co	0.4012	100	0.433	0.871	0.44	0.236	0.3	0.8

Table 6
Parameters used for the EDI calculation, including Average Lifetime (AT), Total Exposure (TE), Intake Rate (IR), Category, and Years. Table taken from Flamminii et al.(Flamminii et al., 2024).

Category	Years	Body Weight (BW) (kg)	Average Lifetime (AT) (day)	Total Exposure (TE) (Year)	Intake Rate (IR) Honey (kg/day)
Toddler	0–3	11.3	730	2	0.0127
Children	3–10	26.1	2555	7	0.0126
Adolescent	10–18	52.6	2920	8	0.0133
Adult	18–65	69.7	17,155	47	0.0127

Table 7
Reference Dose (RfD) and Cancer Slope Factor (CSF) values (mg/kg bw/day) used for the health risk assessment. The data were adapted for the present study from Flamminii et al. (Flamminii et al., 2024), with certain elements retained, others excluded, and additional substances incorporated.

Chemicals	RfD (mg/kgbw/Day)	Reference	CSF (mg/kgbw/Day)	Reference
Al	1	(de Souza et al., 2021)	/	/
As	0.0003 ▲▲	(US EPA, 2024)	1.5	(US EPA, 2024)
Cd	0.0001	(US EPA, 2024)	0.38	(Real et al., 2017)
Co	0.0003	(de Souza et al., 2021)	/	/
Cu	0.04	(US EPA, 2024)	/	/
Mn	0.14	(US EPA, 2024)	/	/
Ni	0.02 ▲	(US EPA, 2024)	1.70°	(*The Risk Assessment Information System*, n.d.)*
Pb	0.0035	(Real et al., 2017)	0.0085°°	(*The Risk Assessment Information System*, n.d.)*
Se	0.005	(Zhao et al., 2021)	/	/
Sb	0.0004 ▲▲▲	(US EPA, 2024)	/	/
Zn	0.3	(de Souza et al., 2021)	/	/
PFOS	0.0000001	(*FINAL: Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts*, 2024)	39.5	(*FINAL: Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts*, 2024)

▲ Nickel Soluble Salts, ▲▲ Inorganic Arsenic, ▲▲▲ Antimony (metallic) and Antimony Tetroxide, ° Nickel Subsulfide, °° Lead and Compounds. Adapted from (Scivicco et al., 2022).

* The Risk Assessment Information System (RAIS) does not report a specific publication year for individual toxicological parameters; therefore, "n.d." (no date) is reported.

are lower in areas B and F, approximately 0.16 and 0.26, respectively. Similarly, antimony shows values between 0.23 and 0.29 in the same areas, with lower concentrations elsewhere. Cadmium exhibits elevated levels in areas A, D, and E (around 0.1–0.12), whereas in areas B, C, and F it ranges between 0.06 and 0.08. As age increases, these metal concentrations progressively decrease; for example, in adults, arsenic levels in the more contaminated areas drop to about 0.05, antimony to 0.04–0.05, and cadmium to 0.01–0.017. Selenium, on the other hand, maintains relatively uniform values across all areas and age groups, ranging around 0.027–0.031 in toddlers and slightly decreasing to 0.0045–0.005 in adults. These data highlight greater exposure or accumulation of As, Sb, and Cd in early life stages and specific geographic areas, while selenium appears more evenly distributed.

The analysis of the HI values, reported in Supplementary Material (Table S7), shown in Fig. 8 of this study reveals substantial differences in potential exposure levels across the sampled areas, with significant variations also observed according to the age groups considered. Specifically, Area B, corresponding to the Campochiaro station, consistently exhibits the lowest HI values across all evaluated categories. For example, the index for the Toddler group is 0.499, decreasing progressively to 0.214 for Children, 0.112 for Adolescents, and 0.081 for Adults. Conversely, Areas A and E display the highest HI values among all examined stations, although still below the critical threshold of 1. Area A, corresponding to the Bojano station, shows a higher risk profile, with HI values reaching 0.755 for Toddlers, 0.324 for Children, 0.169 for Adolescents, and 0.122 for Adults. Similarly, Area E, corresponding to the Roccavivara site, shows a comparable risk profile, with HI values reaching 0.745 for Toddlers, 0.320 for Children, 0.167 for Adolescents, and 0.120 for Adults. Overall, the data highlight a spatial gradient of environmental risk: Campochiaro (Area B), located within the WWF Guardiaregia-Campochiaro Nature Reserve, a protected area rich in natural ecosystems, shows the lowest levels of environmental exposure, likely due to the preserved nature of its surroundings. In contrast, Bojano (Area A) exhibits elevated exposure values, though still below the risk threshold, probably as a result of direct anthropogenic pressures stemming both from its proximity to the Campochiaro/Bojano industrial complex and a recycling centre. Similarly, Roccavivara (Area E), while situated in a predominantly agro-forestry rural context, may experience localized environmental stress due to the nearby presence of an ecological island. These findings suggest that Campochiaro (Area B) reflects an overall favorable environmental condition with respect to contaminant exposure and a low likelihood of health impacts, while Bojano and Roccavivara areas are more influenced by anthropogenic activities (as above mentioned), likely contributing to higher exposure levels observed in vulnerable groups such as preschool-aged children. Overall, all calculated THQ and $\sum THQ_m$ values for the honey samples analyzed in this study were below the threshold value of 1, indicating that the non-carcinogenic health risk associated with their consumption is negligible.

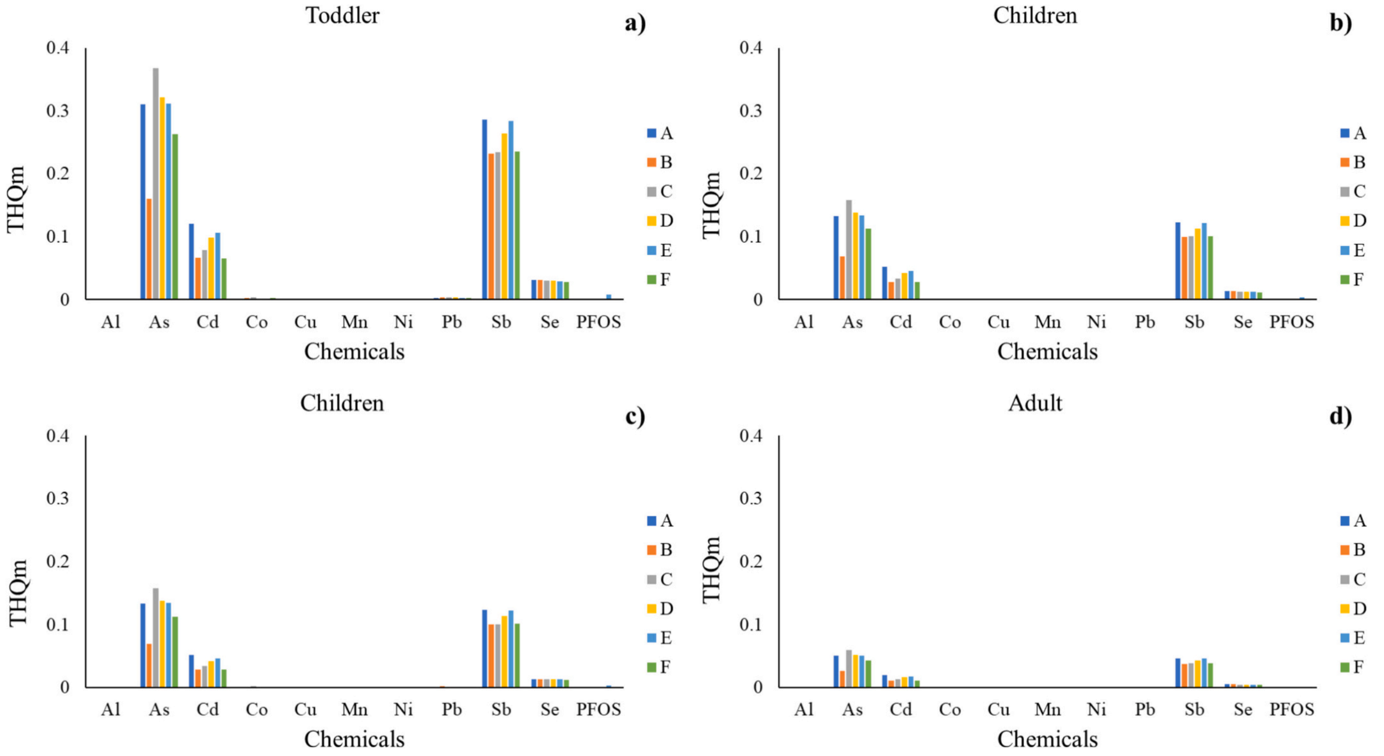


Fig. 7. THQm values calculated for each element across different areas and age groups: (a) toddlers, (b) children, (c) adolescents, (d) adults.

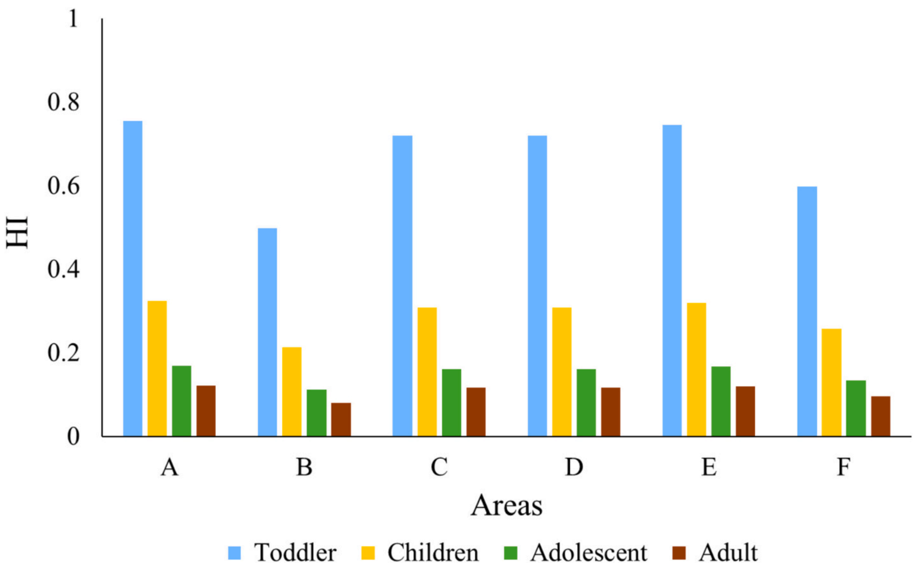


Fig. 8. Total HI values calculated across all elements, different areas, and for each age group.

4.2. Carcinogenic risk

The EDI, calculated as described in the previous section, was subsequently used to estimate the carcinogenic risk through the calculation of the LTCR for each element, area, and age group. The Cancer Slope Factor (CSF) values used for this assessment were taken from Table 7. This table was adapted for the present study from Flamminii et al. (Flamminii et al., 2024), with certain values retained, others removed, and additional substances included. The corresponding results are presented in Fig. 9. Although LTCR values were calculated for all substances with available CSF values, the discussion is centered on arsenic and nickel, as the LTCR values associated with cadmium and lead were consistently very low and did not substantially influence overall carcinogenic risk. PFOS was detected only in one sampling area and in a single honey sample at a very low concentration, and its contribution to carcinogenic risk was therefore considered negligible.

Arsenic (As) and nickel (Ni) are the substances that contribute most significantly to the LCTR across the different sampling areas and age categories, with arsenic's contribution being substantially higher than that of nickel. The data show that arsenic concentrations are consistently much greater than nickel concentrations in all areas and age groups, particularly in toddlers. For example, arsenic levels in toddlers range from approximately 0.000118 (area F) up to 0.000166 (area C), whereas nickel concentrations in the same group vary between about 4.9×10^{-6}

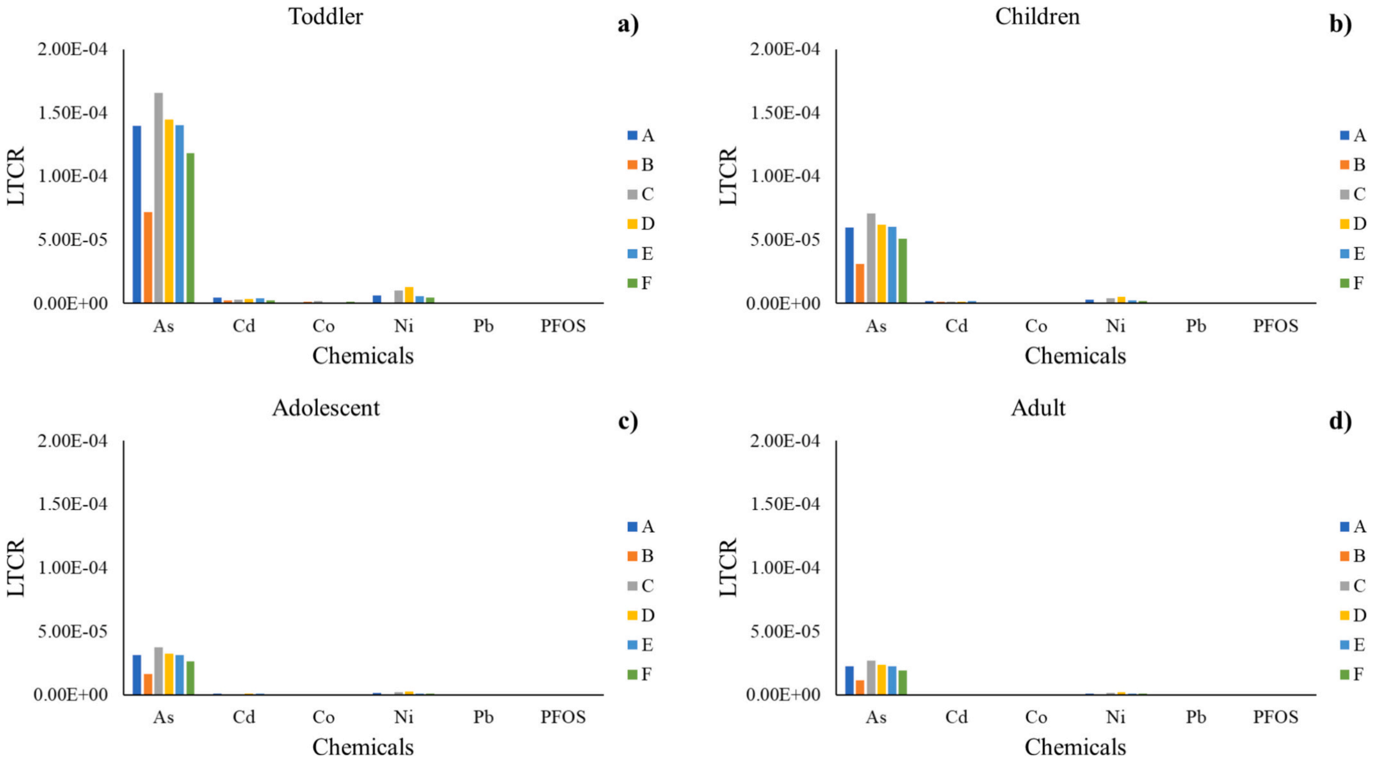


Fig. 9. LTCR values calculated for each element across different areas and age groups: (a) toddlers, (b) children, (c) adolescents, (d) adults.

(area F) and 1.27 × 10^{-5} (area D), meaning arsenic values are roughly an order of magnitude higher. Both arsenic and nickel concentrations decrease progressively with age; As levels in adults range between 1.9 × 10^{-5} (area F) and 2.7 × 10^{-5} (area C), while Ni decreases to about 7.9 × 10^{-7} (area F) to 2.1 × 10^{-6} (area D). These results highlight a considerably greater exposure to As compared to Ni in early life stages across all sampling areas.

Table S8 (Supplementary Materials), which shows the LTCR cumulative values illustrated in Fig. 10, presents data stratified by age groups. The cumulative LTCR values show that area B has the lowest risk across all age groups: Toddler (7.62 × 10^{-5}), Children (3.28 × 10^{-5}), Adolescent (1.72 × 10^{-5}), and Adult (1.24 × 10^{-5}), indicating lower exposure to carcinogenic factors. In contrast, areas C and D have the highest values, especially among young children: for area C, Toddler (1.81 × 10^{-4}), Children (7.76 × 10^{-5}), Adolescent (4.06 × 10^{-5}), and Adult (2.93 × 10^{-5}); for area D, Toddler (1.62 × 10^{-4}), Children (6.94 × 10^{-5}), Adolescent (3.63 × 10^{-5}), and Adult (2.62 × 10^{-5}), indicating higher exposure and an increased environmental risk.

The analysis of cumulative Lifetime Cancer Risk (LTCR) values reveals significant differences between toddlers and the older age groups across all the areas considered. Area B stands out with cumulative LTCR values below the acceptable threshold level of 10^{-4} in all age groups, indicating a contained and more favorable cancer risk compared to the other analyzed areas. Conversely, for toddlers, areas C (1.81 × 10^{-4}) and

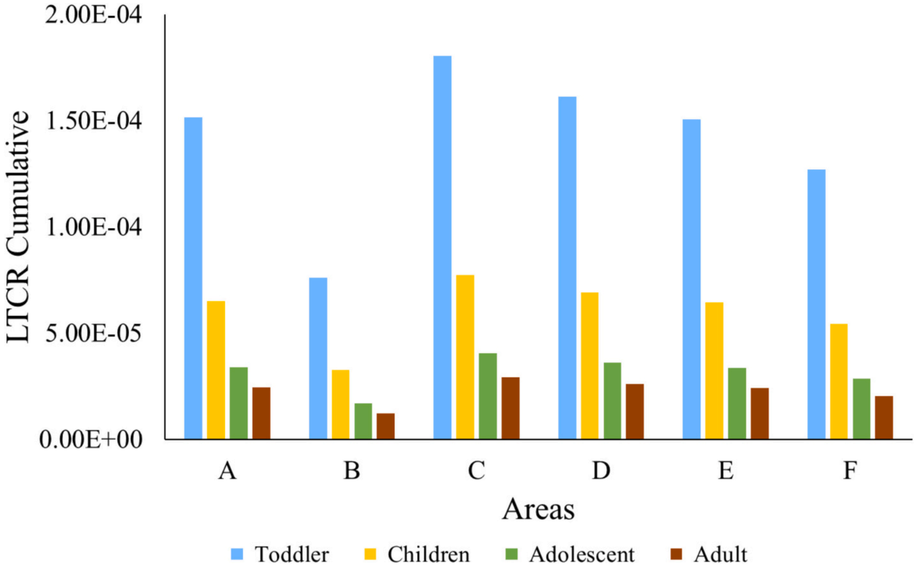


Fig. 10. Total cumulative LTCR values calculated across different areas, and for each age group.

D (1.62×10^{-4}) show particularly high LTCR values, exceeding the acceptable risk threshold, indicating an unacceptable cancer risk for this vulnerable population. Areas A (1.52×10^{-4}), E (1.51×10^{-4}), and F (1.27×10^{-4}) also present elevated levels, albeit slightly lower. These data highlight that toddlers are more exposed to carcinogenic contaminants, with a significantly higher risk compared to older age groups. For the children, adolescent, and adult categories, LTCR values are generally lower and never exceed the 10^{-4} threshold, even in the most contaminated areas. However, in areas C and D, the values for children and adolescents approach the upper limit of acceptable risk, suggesting significant but not critical exposure. This estimated cancer risk, particularly associated with arsenic exposure, highlights the importance of understanding the environmental factors that influence arsenic mobility and bioavailability in agricultural soils. Indeed, arsenic is a significant concern in agriculture due to its historical use in inorganic pesticides and fungicides (Sevak & Pushkar, 2024). In soil, arsenic primarily exists as arsenates under oxidizing conditions, where it binds to iron and aluminium oxides, limiting its mobility and availability to plants (Sevak & Pushkar, 2024). However, reducing conditions—such as those found in anaerobic or waterlogged soils—promote the formation of the more toxic and mobile arsenite (As(III)), increasing risks of phytotoxicity and crop contamination (Sevak & Pushkar, 2024). Additionally, natural organic fertilisers like cattle manure can lower soil redox potential, enhancing reducing conditions that increase arsenic mobility and solubility (Suda & Makino, 2016). This effect is amplified under anoxic conditions caused by stagnant water, facilitating arsenic release into groundwater and raising concerns about aquifer contamination and soil quality in agricultural regions (Suda & Makino, 2016). Although arsenic levels in edible plant parts generally remain below critical health thresholds, its accumulation in soil can negatively affect plant growth and pose environmental and health risks (Sevak & Pushkar, 2024). In anoxic environments, arsenic mobilization is strongly influenced by the activity of reducing bacteria. Arsenate (As(V)) and iron (Fe(III)) reducing bacteria can transform arsenic into more mobile forms, primarily arsenite (As(III)), which is less adsorbed by soil minerals. In particular, microbial iron reduction promotes the release of arsenic previously bound to mineral surfaces. These microbial processes, active under reducing conditions typical of environments rich in organic matter and low in oxygen, can increase arsenic mobility and dispersion, with significant potential environmental impacts. Such mechanisms are especially relevant in contexts like landfills, where anoxic conditions favour the activity of these bacteria (Tian et al., 2015). These mechanisms, although triggered in different contexts, reflect the same environmental vulnerability related to the bio-mobility of arsenic in the presence of specific chemical and biological factors.

4.3. Summary of risk variability across areas and population groups

Areas A and E show the highest risk levels according to the HI, while areas C (which is located next to a landfill) and D present the greatest LTCR cumulative. The HI is mainly influenced by arsenic, cadmium, and antimony, whereas the LTCR cumulative is predominantly driven by arsenic and, to a lesser extent, cadmium and nickel. This explains why HI values are higher in areas A and E, which have recycling plants potentially contributing cadmium and antimony (which tend to be correlated). Conversely, LTCR cumulative is higher in areas C (which is located next to a landfill) and D, as well as in areas E and A, which share varying degrees of agricultural land use. Both non-carcinogenic and carcinogenic risk assessments point to area B as the least impacted, highlighting the environmental quality of the natural reserves. It is important to highlight that both HI and LTCR cumulative values are consistently higher for toddlers compared to other age groups, indicating greater potential exposure and risk for this vulnerable population. Considering that honey is not recommended for infants (Arnon et al., 1979; Hoarau et al., 2012), these data should not cause immediate concern but rather serve as a starting point for further investigations and continuous monitoring of the affected areas.

5. Conclusion

This study, conducted on honey samples collected from six distinct locations in the Molise region, provides valuable insights into the healthfulness of the product and, indirectly, the environmental quality of the area, as pollutants present in the region can accumulate within this matrix. Indeed, honey serves as a bio-indicator, a foodstuff that, due to its minimal industrial processing, reflects the environmental characteristics of the production areas. The chemical substances regulated by Commission Regulation (EU) 2023/915 are below the established limits; hence, the honey tested is suitable for sale and human consumption. Furthermore, it is important to consider that heavy metal levels may vary based on several factors, including the origin of the honey, agricultural and environmental practices in the production area, and the extent of environmental contamination. The analyses carried out did not reveal any particular critical issues for the healthiness of the honey (except for toddlers). The risk assessment show variability in exposure and potential risk across different population groups and areas. Risk levels also differ among the studied areas, with area B -being a protected natural reserve- consistently showing the lowest risk. Multivariate statistical analyses (PCA and ANOSIM) did not reveal a clear and sharp discrimination of individual elements or sampling areas; however, the combination of PCA, Pearson correlation analysis, and temporal trend evaluation highlighted consistent associations among specific metals and supported the presence of partially overlapping environmental sources across the study area, rather than distinct and isolated contamination patterns.

Therefore, the authors emphasize the importance of conducting further and continuous investigations to monitor the affected areas to ensure long-term safety, especially for the most vulnerable categories. This approach is essential for ongoing surveillance of both contamination sources and the levels of heavy metals and PFAS (despite their current concentrations being near or below the LOD), to ensure human safety and protect public health. Furthermore, it would be beneficial to implement measures to reduce environmental contamination and implement best practices to minimise risks. Hence, it is always advisable to monitor the situation and continue research into potential long-term pollutants. Finally, in the context of assessing the suitability of honey for human consumption, the authors emphasize the necessity for a more comprehensive regulatory framework encompassing additional substances, with the objective of safeguarding consumers health to a greater extent.

CRediT authorship contribution statement

Sergio Passarella: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Simone Moretti:** Investigation, Formal analysis. **Alessia Iannone:** Investigation, Formal analysis. **Fabiana Carriera:** Investigation, Formal analysis. **Sonia Ganassi:** Investigation, Formal analysis. **Dalila Di Criscio:** Formal analysis, Data curation. **Loretta De Giorgi:** Software, Formal analysis, Data curation. **Giulia Corazzi:** Software. **Ivan Notardonato:** Software, Formal analysis. **Carolina Barola:** Formal analysis, Data curation. **Roberta Galarini:** Supervision, Formal analysis. **Pasquale Avino:** Writing – review & editing, Supervision, Conceptualization. **Antonio De Cristofaro:** Supervision, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.148093.

Data availability

I have shared the data in the Supplementary Material

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