Environmental Science and Pollution Research (2022) 29:36057–36074
https://doi.org/10.1007/s11356-021-18072-3

RESEARCH ARTICLE

Biomonitoring of element contamination in bees and beehive products in the Rome province (Italy)

Marcelo Enrique Conti[1] · Maria Luisa Astolfi[2] · Maria Grazia Finoia[3] · Lorenzo Massimi[4] · Silvia Canepari[4]

Received: 27 July 2021 / Accepted: 8 December 2021 / Published online: 21 January 2022

Abstract

In this study, we determined the levels of elements (i.e. As, Be, Cd, Cr, Hg, Ni, Pb, U, and Zn) in bees and edible beehive products (honey, wax, pollen, and propolis) sampled from five selected sites in the Rome province (Italy). Rationale: to increase the information variety endowment, the monitoring breakdown structure (MBS) conceptual model was used (nine elements, 429 samples, and approximately thirteen thousand determinations over a 1-year survey). Thus, we employed Johnson's probabilistic method to build the control charts. Then, we measured the element concentration overlap ranges and the overlap bioaccumulation index (OBI). Subsequently, we evaluated the estimated daily intake (EDI) of the analysed elements and matched them with acceptable reference doses. The human health risk caused by the intake of individual elements found in edible beehive products and their risk summation were evaluated through the target hazard quotient (THQ) and hazard index (HI) methods. Findings: excluding honey, this study confirms the capacity of wax, pollen, propolis, and bees to accumulate high levels of toxic and potentially toxic elements from the surrounding environment (with high OBI-U, i.e. OBI-Upper values, i.e. the common upper concentration limit of the overlap concentration range). Bees and pollen showed a high bioaccumulation Cd surplus (OBI-U = 44.0 and 22.3, respectively). On the contrary, honey had high OBI-L values (i.e. honey concentrates metals several times less than the common lower concentration limit of the overlap concentration range). This finding implies that honey is useless as an environmental indicator compared with the other biomonitor/indicators. The EDI values for the edible beehive products were lower than the health and safety reference doses for all the considered elements. Our data show that honey, wax, propolis, and pollen are safe for consumption by both adults and children ($THQ < 1$; $HI < 1$), even considering the sporadic possibility of consuming them simultaneously. Originality: This study has been conducted for the first time in the Rome province and demonstrates that edible indicators are safe for consumption for the considered elements in bees and edible beehive products. Depending on the ecosystem/pollutants studied, the OBI consents to make a correct choice for environmental biomonitoring studies and to focus the attention on the most sensitive biomonitors/indicators when required at the project level.

Keywords Bees · Beehive products · Biomonitoring · Environmental pollution · Johnson's method · Risk assessment · Spectroscopic techniques · Toxic elements

---

Responsible Editor: Philippe Garrigues

✉ Marcelo Enrique Conti
marcelo.conti@uniroma1.it

Maria Luisa Astolfi
marialuisa.astolfi@uniroma1.it

Maria Grazia Finoia
mafinoia@gmail.com

Lorenzo Massimi
l.massimi@uniroma1.it

Silvia Canepari
silvia.canepari@uniroma1.it

[1] Department of Management, University of Rome, Via del Castro Laurenziano 9, 00161 Sapienza, Italy

[2] Department of Chemistry, Sapienza University of Rome, P. Le Aldo Moro, 00185 Rome, Italy

[3] Italian National Institute for Environmental Protection and Research, Viale V. Brancati 60, 00166 Rome, Italy

[4] Department of Environmental Biology, Sapienza University of Rome, P. Le Aldo Moro, 00185 Rome, Italy

Introduction

Biomonitors are organisms mainly employed for the quantitative assessment of contaminants in the environment (Garty 1993; Conti and Cecchetti 2001; Wolterbeek et al. 2003). Honeybees are continuously exposed to contaminants, including toxic elements, present in the area near the apiary (approximately 7 km^2) for the period of their foraging activity, i.e. from spring to fall (Bargańska et al. 2016). Throughout their foraging activities, honeybees contact particles containing elements originating from soil and dust (Bommuraj et al. 2019; Losfeld et al. 2014). In addition, the elemental composition of nectar and pollen can be influenced by elements absorbed from the soil via the plant root system (Bommuraj et al. 2019; Losfeld et al. 2014; Solayman et al. 2016). There are many studies indicating bees (*Apis mellifera*) as biomonitors and beehive products (honey, wax, pollen, and propolis) as useful indicators for monitoring environmental element pollution (Conti 2002; Dżugan et al. 2018; Madejczyk and Baralkiewicz 2008; Negri et al. 2015; Pohl 2009; Pohl et al. 2012; Przybyłowski and Wilczyńska 2001; Spirić et al. 2019; Smith et al. 2019, 2021; Stöcker (1980). On the contrary, other studies have shown that honey cannot be employed as an environmental indicator of metals (Álvarez-Ayuso and Abad-Valle 2017; Conti and Botrè 2001; Conti et al. 2018; Losfeld et al. 2014; Saunier et al. 2013).

Mineral pollutants include those arising from natural sources of contamination (i.e. volcanic eruptions) or those of anthropogenic origin (Canepari et al. 2013, 2018; Skorbiłowicz et al. 2018; Astolfi et al. 2020a; Conti et al. 2020a, b). Toxic metals such as Cd, Hg, and Pb, usually emitted by industrial and/or agricultural sources, can pose some risk to honeybees and humans (da Cunha Martins et al. 2018). It is well documented that Cd, Pb, and Hg are highly toxic metals, and their presence in the environment is a risk to human health due to the danger of their entry into the food chain (Chmielnicka and Cherian 1986; Kumar et al. 2020; Raj and Maiti 2019; Rizwan et al. 2018; Satarug et al. 2000; Sarwar et al. 2014; Shahid et al. 2020; Usman et al. 2012). In fact, toxic metals can easily accumulate in living tissues, causing severe health effects (Naja and Volesky 2017). The metal contents in bees and edible indicators might also depend on other factors, such as the age of worker bees, the process of rearing bee colonies (comprising extra feeding), and the physiological and health status of bee colonies (Astolfi et al. 2021; Zhelyazkova 2012). Due to their physical features (sticky propolis) and chemical composition (primarily polyphenols, amino acids, and terpenes), propolis and pollen can absorb metals (Finger et al. 2014; Matin et al. 2016), and they can be employed as indicators of environmental pollution (Conti and Botrè 2001; Finger et al. 2014). Eventually, assessing element concentrations in bees and beehive products is essential not only for their use as possible biomonitors/indicators for environmental contamination purposes but also for detecting potential human exposure due to their dietary, pharmaceutical, and cosmetic uses (Astolfi et al. 2021; Burlando and Cornara 2013; Kalogeropoulos et al. 2009; Melliou and Chinou 2011; Tsiapara et al. 2009). Humans can be exposed to elements through the food chain (Zand et al. 2015; Conti et al. 2020a). The non-essential elements, i.e. Al, As, Ba, Be, Cd, Hg, Pb, Sn, and U are food contaminants, can be accumulated by biomonitors and are reputed to be possibly toxic (Barros Paiva et al. 2012; De la Guardia and Garrigues 2015; Vitali et al. 2019; Ristorini et al. 2020; Conti et al. 2020a). These considerations form the basis with which bees and edible beehive products have been proposed as reliable biomonitors/indicators of environmental pollution (Bargańska et al. 2016; Conti 2008; Conti and Botrè 2001; Crane 1984; Lambert et al. 2012; Raes et al. 1992). A recent study (Goretti et al. 2020) showed through a comparative analysis of metal enrichment factors in PM10 and bees in central Italy that bees were contaminated by airborne particulate matter (PM_{10}) to a lesser extent than other local sources, such as the use of pesticides and fertilisers and the resuspension of contaminated soil. Bees and beehive products can provide time-integrated information about elemental emissions from different sources and provide information about possible risks to human health (Leita et al. 1996). Likewise, the presence of some elements in honey influences its quality and safety (Devi et al. 2018; Grembecka and Szefer 2013; Lazarus et al. 2021; Soares et al. 2017; Voica et al. 2020).

Honey has a high nutritional value (330 kcal/100 g), organisms absorb its carbohydrates rapidly following consumption, and it has anti-inflammatory and antibacterial properties for the treatment of several gastrointestinal diseases and skin wounds (Bogdanov et al. 2008; Conti et al. 2018; Machado De-Melo et al. 2018; Soares et al. 2017).

Wax is employed as a glazing agent in chocolate or cocoa-based products, candies, chewing gums, snacks based on cereal and/or potato flours, or starches and nut-based glazing agents for treating coffee beans (EFSA 2007).

Consumers recognise bee products for their therapeutic properties as alternative drugs. In particular, bee pollen is an important source of nutritional ingredients and energy. The health-improving benefit of bee pollen is linked to an extensive variety of secondary metabolites (i.e. biotin and folic acid, carotenoid pigments, niacin, phytosterols, polyphenols, thiamine, and tocopherol), enzymes, and coenzymes (Campos et al. 1997, 2008; Denisow and Denisow-Pietrzyk 2016; Kostić et al. 2019, 2020). Pollen is considered an excellent natural supplement that provides energy and strength to the body's immune and physiological systems (Kostić et al. 2020).

There are thousands of studies involving the beneficial action of propolis (i.e. bee glue) as supplemental food. Bees produce propolis using a mixture of beeswax and saliva, which acts in the hive's protection (Braakhuis 2019). The health benefits that were most systematically investigated were antimicrobial, wound healing, cardioprotective, and optimal neural function-related properties. Antioxidant and inflammatory activities have also been surveyed (Braakhuis 2019; Finger et al. 2014).

Introducing space and time represents a relevant way of studying complexity in natural systems (Morin 1992). We have recently proposed a tool, i.e. the monitoring breakdown structure (MBS) (Conti et al. 2019a), to effectively manage information related to biomonitoring studies. The MBS tool is rooted in the complexity perspective. The MBS allows to manage the information variety endowment, i.e. the bundle of several variables, such as data, technical and instrumental knowledge, and cognitive frameworks (and related biases), dealing with biomonitoring studies (Ashby 1957, 1958; Barile 2009; Barile et al. 2015; Simone et al. 2021).

Moving from these premises, the MBS and/or the OBI index have been applied recently in studies on different biomonitors, such as molluscs, algae, and phanerogams in marine ecosystems, and lichens as biomonitors of element deposition connected with a volcano event (Conti et al. 2015; 2019a, b; 2020a, b).

The overlap bioaccumulation index (OBI) allows for the identification of specific indicators (including edible) that are desirable for studying a particular condition of metal contamination linked to natural or anthropogenic activities. Thus, it increases our comprehension of the ecosystem's complexity and could form a basis for policymakers' decision-making. Extensive details on the analytical and statistical procedures have been reported elsewhere (Conti and Finoia 2010; Conti et al. 2015, 2019a, b).

According to the MBS model, the present study's first aim is to increase the information variety. Thus, nine elements (i.e. As, Be, Cd, Cr, Hg, Ni, Pb, U, and Zn) in 429 samples and approximately thirteen thousand analytical determinations were conducted. The information variety content was enhanced to obtain more reliable results about the elemental contents of bees, edible beehive products connected with their related ecosystems. This result is also supported because several studies on bees and their products are referred to a limited sampling period with a low number of samples. The survey's newness relates to the rationale linked with data collection and interpretation in compliance with the complexity of the studied ecosystems (Conti et al. 2020b).

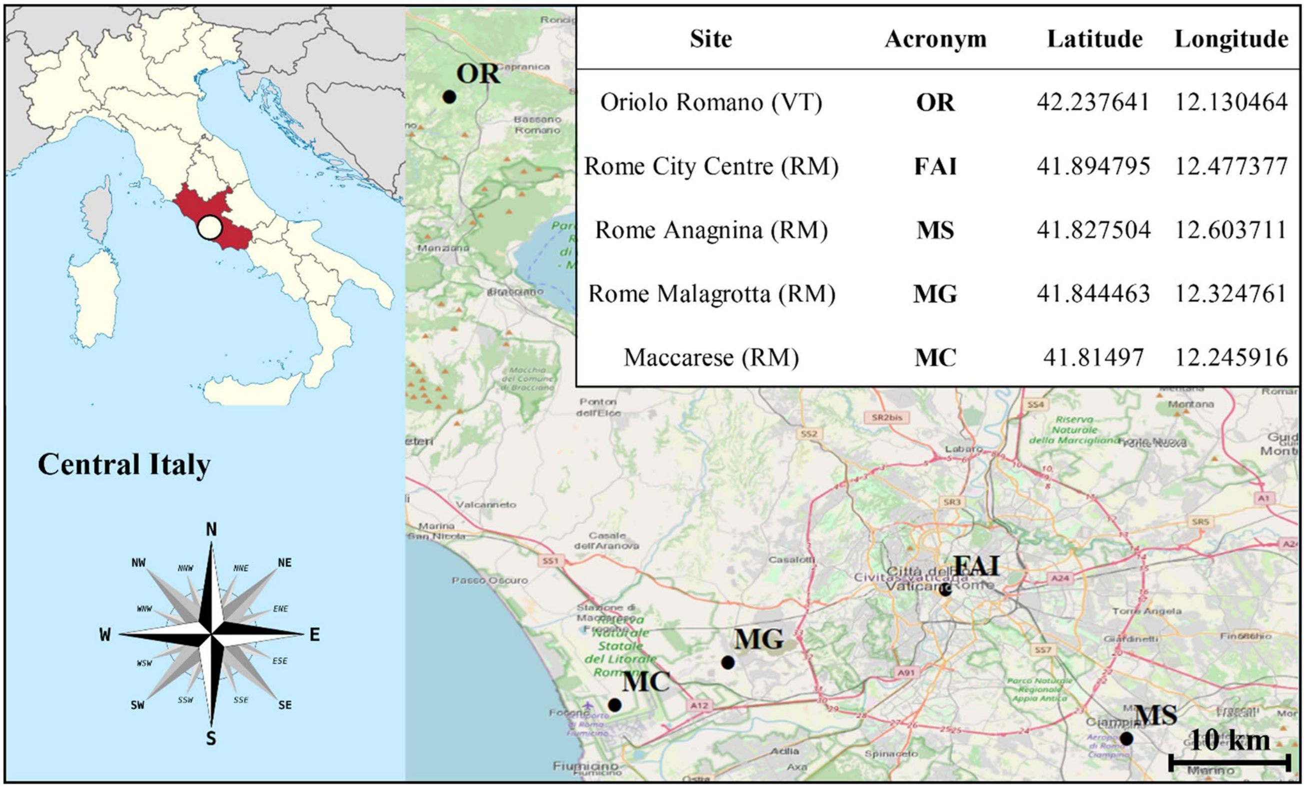
The second aim is to assess the probabilistic distribution of elements in bees, honey, wax, pollen, and propolis by using Johnson's method to obtain consistent information on their bioaccumulation patterns and possible food contamination. This method serves to define metal concentration confidence intervals at 95% ranges of variability owing to the normalisation of any continuous probability distribution (Johnson 1949; Miller and Miller 2005; Conti et al. 2019a,b).

The third aim is to determine the range of overlaps of element concentrations and the OBI compared with the upper (OBI-U) and lower (OBI-L) bounds of the overlap range (Conti et al. 2019b). For this purpose, we have therefore created control charts for the elemental bioaccumulation in the five selected biomonitors/indicators (bees, honey, wax, pollen, and propolis).The fourth aim is to compare the only existing maximum limit (ML) set by Commission Regulation (EU) (2015/1005) for Pb in honey with our samples and, subsequently, to assess the estimated daily intake (EDI) of the analysed elements (i.e. As, Be, Cd, Cr, Hg, Ni, Pb, U, and Zn) and compare them with safe reference doses [i.e. the provisional maximum tolerable daily intake (PMTDI), provisional tolerable daily intake (PTDI) (WHO 1996; EFSA 2014; JECFA 2019); benchmark dose lower confidence limit ($BMDL_{01}$, a reference point (RP)/point of departure (POD) (EFSA 2012, 2009, 2021; WHO 2003), and tolerable daily intake (TDI) (WHO 2003, 2009; EFSA 2015)]. Furthermore, intending to acquire evidence about the probable health risks of honey, wax, pollen, and propolis consumption for people living in the Rome province (adults and children), we assessed the human health risk raised by different elements present in edible beehive products, as well as their risk summation, by using the target hazard quotient (THQ) and hazard index (HI) procedure (sections Target hazard quotient and Hazard index).

Materials and methods

Study area

In our study, five sites with different anthropogenic impacts were selected (Fig. 1). The four sites are localised within an extensive metropolitan area. One site is in the centre of Rome, i.e. on the roof of the Apicultural Italian Federation (FAI). The other three sites are throughout the province of Rome and presumably have different environmental pollution levels. One of them is situated close to the landfill (MG, Malagrotta). The fifth site in the Oriolo Romano locality (Viterbo province, OR in the map of Fig. 1) is in a green area far from urban areas, but biomass burning sources influence it. Thus, we applied the MBS by selecting four strategic locations of apiaries in the neighbourhood of areas with different anthropogenic impacts of Rome and in a supposedly uncontaminated site (OR). Simultaneous sampling



Site	Acronym	Latitude	Longitude
Oriolo Romano (VT)	**OR**	42.237641	12.130464
Rome City Centre (RM)	**FAI**	41.894795	12.477377
Rome Anagnina (RM)	**MS**	41.827504	12.603711
Rome Malagrotta (RM)	**MG**	41.844463	12.324761
Maccarese (RM)	**MC**	41.81497	12.245916

Fig. 1 Study area

campaigns (i.e. 2 months each) were conducted at the same geographically referenced sites from June 2018 to July 2019. The number of samples and the sampling periods considered for each matrix and site are shown in Table S1. Following the MBS model, we placed two (independent) beehives at each site to increase the requisite variety (n = ten beehives). Honey samples taken from the Association of Beekeepers of Rome and Province were classified as multifloral by using a melissopalynological analysis. Qualified beekeepers monitored the beehives and collected all the samples using polyethene screw-cap containers once every 2 months in the late morning.

Chemicals and materials

The honey, wax, and pollen samples were separated, and then the wax sample was carefully washed with deionised water to remove any residues. The bees and wax samples were lyophilised for 48 h using a freeze dryer (Heto Power Dry LL1500; Thermo Electron Corporation, Waltham, MA, USA). All samples were stored in polypropylene tubes at − 18 °C until analysis (for details, see Conti et al. 2018 and Astolfi et al. 2021).

All the reagents were of analytical grade. Super-pure HNO_3 (67%) and HCl (36%) were supplied by Carlo Erba Reagents (Milan, Italy), and H_2O_2 (30%) was obtained from Merck KgaA (Darmstadt, Germany). Deionised water (electrical resistivity 18.3 MΩ cm^{-1}) was obtained using the Arioso Power I RO-UP Scholar UV water purification system (Human Corporation, Seoul, Korea). A multi-element standard solution (VWR International S.r.l., Milan, Italy) and a single-element standard solution of Hg (SCP Science, Baie D'Urfé, Canada) were used to prepare the calibration solutions for instrumental analysis. The 0.05% $NaBH_4$ (Sigma-Aldrich Chemie GmbH, St. Louis, USA) in 0.05% NaOH (Carlo Erba Reagents, Milan, Italy) was used as a reducing agent for the cold vapour atomic fluorescence spectrometry (CV-AFS; AFS 8220 Titan, FullTech Instruments, Rome, Italy) analysis.

Sample treatment and analysis

Eight elements (As, Be, Cd, Cr, Ni, Pb, U, and Zn) were analysed with a quadrupole inductively coupled plasma mass spectrometry (ICP-MS; 820-MS; Bruker, Bremen, Germany); while CV-AFS quantified Hg. The instrumental conditions and sample treatment procedures have been reported elsewhere (see for details Astolfi et al. 2019, 2020b, 2021). In brief, the samples of each matrix were digested using a water bath (WB12, Argo Lab, Modena, Italy) at 95 °C for 30 min. A digestion reagent mixture of 1 mL HNO_3 and 0.5 mL H_2O_2 or 0.5 mL HCl, 0.2 mL HNO_3 and

0.1 mL H_2O_2 was used for the ICP-MS or CV-AFS analysis. With the lack of suitable certified reference material, spiked samples were used to determine the elemental recoveries by used methods (Astolfi et al. 2020b, 2021). Recoveries for all elements fell within 20% of the expected value, with many of the elements recovering within 10%, excluding Zn in beeswax and bees, and As in propolis, which fell within 30%. The within-run precision for all the elements in honey and most of the elements in other matrices was less than 10%. The intermediate precision was less than 15% for most of the elements in all matrices, excluding As (Astolfi et al. 2020b, 2021). Information on quality assurance and control can be found in the 1S section of the Supplementary material.

Statistical analysis: Johnson's method and the overlap bioaccumulation index (OBI)

Details of this section's procedures have been reported elsewhere, and most information is given in the 2S section of the Supplementary material (Conti and Finoia 2010; Conti et al. 2015, 2019a, 2020b).

In brief, Johnson's method (1949) was used for the trace element levels in the five selected biomonitors/indicators to create frequency curve systems by translation. This method allows, through a translation technique, for the classification of the generic variable distribution in one of the four classes of probability functions defined here:

z = gamma + delta log(f(u)), with $u = (x - xi)/\lambda$.

where f(u) can have one of the following functions:

SL f(u) = u log normal distribution.

SU f(u) = u + sqrt(1 + u^2) unbounded distribution with two tails.

SB f(u) = u/(1-u) unbounded distribution with one tail.

SN F(u) = exp(u) normal distribution.

The normalisation procedure was performed with the SuppDists package of R (Wheeler 2013). In sum, through the reported transformations, the final variables should be normally distributed. Normality the distribution of a variable is an essential requirement for applying robust statistical inference techniques and defining the limits of the confidence intervals for the studied elements. This assumption allows us to build the control charts associated with each element, define its range of variation, and highlight the upper tail of the probability distribution. The tail will contain 2.5% of the observations, establishing the outliers in each element's distribution. However, during the logarithmic transformations, some outliers can be eliminated directly by the method, as was the case for Zn in the propolis samples in this study.

The control charts were built for each selected biomonitor/indicator to determine the overlap range among the five matrices (see Supplementary section). The OBI definition concerning the maximum and minimum overlap range is as follows:

OBI-U_i for the i_{th} biomonitor/indicator with respect to $Q_{i,97.5}$ is defined as follows:

$$OBI - U_i = \frac{Q_{i,97.5}}{\mathrm{I}_{\max}} \text{with } i = 1, 2.., \mathrm{k}$$

OBI-U_i is usually ≥ 1 and becomes 1 when $Q_{i,97.5} = I_{max}$.

OBI-L_i for the i_{th} biomonitor/indicator with respect to $Q_{i,2.5}$ is defined as follows:

$$OBI - L_i = \frac{\mathrm{I}_{\min}}{Q_{i,2.5}}$$

The function of the OBI index is to define a ranking that allows us to explain which, among the various biomonitor/indicators studied, can be considered more sensitive to bioaccumulation of a given pollutant. In its definition (absolute values), this index varies between 1 and $+\infty$, both if calculated net of rare events on the left of the tail of the distribution of a given pollutant (OBI-L, i.e. OBI-Lower), and on the right (OBI-U, i.e. OBI-Upper).

The maximum assumed by OBI-U identifies the matrix most sensitive to bioaccumulation at the highest concentrations. Its value (maximum OBI-U = x) suggests that this same matrix, which is at the top of the ranking, is x times more sensitive than the matrix for which the index takes the value 1.00. Similarly, the same applies to OBI-L. The value (maximum OBI-L = y) suggests that this same matrix, which is at the top of the ranking, is y times more sensitive at the lowest metal concentrations than the matrix for which the index takes value 1.00. Its maximum identifies the matrix most sensitive to metal bioaccumulation at the lowest environmental concentrations. For instance, an indicator/biomonitor with an extremely high OBI-L value for a given metal can be used as an early warning signal about the onset of a contamination process in the study area (environmental prevention studies).

For the sake of completeness, we report the values of the standardised (Std) indices concerning the maximum value (x and y) assumed by them (Tables 2, 3, and 4). Thus, the standardised indices vary in a defined range [0–1], but their interpretation does not change. The closeness to 1 expresses the high sensitivity of that matrix to metal bioaccumulation. Its proximity to zero shows its low sensitivity to bioaccumulation of the considered pollutant.

Although further studies are needed, the advantage of the OBI is that it is calibrated by ranking the bioaccumulation of metals in different biomonitors/indicators considered contemporaneously. We have tested the OBI in various studies carried out in the last decade [Conti et al. 2015, 2019a, 2020b] concerning marine and atmospheric ecosystems. Depending on the ecosystem/pollutants studied, the OBI consents to make a correct choice for environmental biomonitoring studies and focuses the attention on the most sensitive biomonitors/indicators when required at the project level.

For comparisons between medians, the median test and post hoc comparisons were applied (Van der Waerden nonparametric multiple comparisons test).

Human health risk assessment

The EDI values were calculated according to the following formula (see for details Conti et al. 2020a) $F \times D \times I \times C/W \times T$, where F is the exposure frequency (365 days/year); D is the exposure duration (70 years); I is the ingestion rate, and C is the element concentrations in honey and edible products (µg/g); W is the average body weight (b.w.) 60 kg for adults and 15 kg for children; and T is the average time (365 days/year multiplied by the number of exposure years, assuming 70 years in this study). The assessment of consumers' chronic exposure to the studied trace elements in edible indicators was based on the existing consumption data, i.e. 0.8 g/day/kg/b.w. for honey (Joint FAO/WHO Expert Committee on Food Additives 2009).

The target hazard quotient (THQ) is the probable noncarcinogenic risk for orally ingested elements; it is defined as the ratio of the daily oral intake to the oral reference dose (Conti et al. 2020a; Liu et al. 2019; Petroczi and Naughton 2009; US EPA 2020) with the following equation:

$THQ = \mathrm{EDIs/RfDo}$.

THQ is a dimensionless index (Hague et al. 2008), and $\mathrm{THQ} < 1$ indicates that the exposed population is supposed to be safe. A THQ index between 1 and 5 indicates that the exposed population is at a concerning level. The oral reference dose (RfDo) denotes a daily exposure dose to which humans are continually exposed over a lifetime without causing a significant risk of carcinogenic effects (Liu et al. 2019; Conti et al. 2020a). However, considering the lack of information about $\mathrm{RfDo_s}$ for some elements, we assumed that all As is inorganic arsenic, all Cr is Cr (VI), and all Zn is Zn and Zn compounds. All Ni is Ni soluble salts for which RfDos are available (Conti et al. 2020b). Furthermore, presently, there is no estimation of RfDo for Hg and Pb (US EPA 2020).

The hazard index (HI) method entails the summation of the individual THQs of the determined elements for each food type (Antoine et al. 2017; Bolt 2019; Conti et al. 2020a). The equation for HI is as follows:

$$HI = \sum_{N-1}^{i} THQ_n$$

If the HI is > 1, there is the possibility of adverse noncarcinogenic health effects.

Results and discussion

Table 1 shows the detection limits (LODs) of the elements analysed and the descriptive statistics of the elemental concentrations in the five selected matrices for the six sampling campaigns in a 1-year survey.

Control charts, the overlap element concentration ranges, and the OBI index

The control charts were done for Cd, Hg, and Pb in bees, wax, honey, pollen, and propolis with their resulting overlap element concentrations (Figs. 2, 3, and 4). There were 98% positive samples (i.e. above LOD) for Cd for all the biomonitor/indicators, except for wax, which had 77% positive samples. Mercury showed 100% of positive samples for the five selected biomonitor/indicators, while Pb, excepting honey, showed 100% of positive samples for the biomonitor/indicators considered (see Table 1).

The observed values are on the x-axes, and the values calculated by Johnson's method are on the y-axes. Inside the plot are reported the medians ± m.a.d. (median absolute deviation), the lower and upper bounds of the baseline range (Q2.5 and Q97.5), and the range of overlap (i.e. the common element concentration range for the five biomonitors/indicators; see red arrow). The histograms of the values are shown outside of the plot (see Conti et al. 2019b for details).

Figure 2 shows the median Cd levels detected for bees, propolis, and pollen (i.e. 0.036, 0.027, and 0.015 µg/g, respectively, see Table 1), which are higher than the overall median (i.e. 0.009 µg/g). By contrast, wax and honey showed the lowest median levels (post hoc multiple comparisons $p < 0.05$) of Cd bioaccumulation compared with the other biomonitor/indicators. Moreover, bees showed the highest range of variability compared with the other four indicators (see the m.a.d. green line for bees data in Fig. 2). In contrast, honey showed the lowest range of variability with very low median levels of Cd (0.003 µg/g). The limits of the overlap range were 0.0049 and 0.0084 µg/g, and the OBI for Cd (Table 2) shows that bees and pollen have high bioaccumulation Cd surpluses (OBI-U = 44.0 and 22.3, and OBI-U Std = 1.00 and 0.51, respectively), which indicates that they can detect approximately 44 and 22 times higher Cd levels relative to the upper extreme of the common bioaccumulation overlap range of the five matrices (see the red arrow in Fig. 2). This observation indicates the strong ability of bees (OBI-U Std = 1.00) to respond to environments with high Cd concentrations. This finding supports the hypothesis that bees are excellent biomonitors of Cd in the environment, followed by

Table 1 Descriptive results of elements' levels in bees and edible beehive products in one-year survey — six sampling campaigns (2018–2019) in the Rome province (μg/g)

Matrix	As	Be	Cd	Cr	Hg	Ni	Pb	U	Zn
LOD[a]	0.03	0.0003	0.001	0.01	0.0003	0.1	0.01	0.0002	1
Bees (*n* = 119)									
n% < LOD	21	2	2	0	0	22	0	0	0
Mean	0.09	0.0030	0.067	0.12	0.0116	0.3	0.21	0.0041	106
Median	0.07	0.0020	0.036	0.09	0.0122	0.2	0.16	0.0027	100
Min	< 0.03	0.0004	0.003	0.03	0.0005	< 0.1	0.06	0.0005	47
Max	0.48	0.0188	0.680	0.91	0.0310	3.7	1.51	0.0164	301
SD	0.09	0.0030	0.100	0.12	0.0079	0.5	0.17	0.0030	31
Honey (*n* = 88)									
n% < LOD	98	91	2	73	0	93	78	71	28
Mean	< 0.03	0.0004	0.003	0.08	0.0021	< 0.1	0.03	0.0003	3
Median	< 0.03	0.0004	0.003	0.03	0.0021	0.1	0.01	0.0003	2
Min	< 0.03	0.0004	0.003	< 0.02	0.0007	< 0.1	< 0.01	0.0003	< 1
Max	0.03	0.0006	0.009	2.19	0.0032	0.3	0.14	0.0009	9
SD	-	-	0.002	0.30	0.0009	-	0.03	0.0002	2
Wax (*n* = 163)									
n% < LOD	72	9	23	8	0	41	0	0	0
Mean	< 0.03	0.0026	0.018	0.12	0.0049	0.3	0.56	0.0039	73
Median	< 0.03	0.0018	0.007	0.09	0.0041	0.2	0.27	0.0032	44
Min	0.03	< 0.0004	0.003	0.02	0.0009	< 0.1	0.04	0.0003	3
Max	0.14	0.0143	0.289	1.21	0.0112	1.7	6.51	0.0252	729
SD	-	0.0030	0.033	0.16	0.0027	0.3	0.96	0.0030	37
Pollen (*n* = 39)									
n% < LOD	38	0	2	0	0	27	0	0	0
Mean	0.05	0.0060	0.033	0.22	0.0067	0.3	0.12	0.0057	27
Median	0.05	0.0050	0.015	0.13	0.0064	0.2	0.10	0.0055	28
Min	< 0.03	0.0007	0.004	0.04	0.0017	< 0.1	0.03	0.0007	15
Max	0.14	0.0214	0.197	1.35	0.0185	1.0	0.34	0.0152	38
SD	0.03	0.0040	0.046	0.28	0.0038	0.2	0.08	0.0033	7
Propolis (*n* = 20)									
n% < LOD	10	0	2	0	0	85	0	0	0
Mean	0.08	0.0140	0.031	0.61	0.0085	0.1	0.35	0.0132	56
Median	0.08	0.0120	0.027	0.54	0.0071	0.1	0.32	0.0120	51
Min	< 0.03	0.0080	0.003	0.26	0.0028	< 0.1	0.10	0.0090	17
Max	0.23	0.0290	0.119	1.34	0.0164	0.2	0.56	0.0319	120
SD	0.05	0.0020	0.026	0.28	0.0049	0.1	0.12	0.0049	39

[a] *LOD*, limit of detection

pollen in the ranking (OBI-U Std = 0.51). Moreover, Cd OBI-L (Table 2) was the highest for wax (i.e. 21.4, OBI-L Std. = 1), showing that wax is 21 times more sensitive at the lowest metal concentrations with respect to the lower extreme of the common bioaccumulation overlap range of the five biomonitor/indicators. This observation implies that wax can be employed as an early warning signal about the onset of a contamination process in the study areas.

Figure 3 shows that honey has the lowest Hg median levels than the other four biomonitor/indicators. The median Hg levels in honey were relatively low (i.e. 0.0021 μg/g, Table 1), and the overall median was 0.004 μg/g with a very narrow range of overlap (0.0030–0.0032 μg/g) (Fig. 3). Bees were the major bioaccumulators of Hg (i.e. 0.0122 μg/g). In fact, bees showed a good OBI-U of 8.44 (OBI-U Std = 1.00), confirming their aptness as Hg biomonitors in the environment. Additionally, propolis and pollen showed similar Hg OBI-U Std values (i.e. 0.60 and 0.54, i.e. the second and the third in the ranking, respectively) (Table 3). Honey showed the highest level of OBI-L = 4.00 (OBI-L Std = 1.00). Thus, honey accumulates Hg fourfold times less than the lowest limit of the overlap range (i.e. with respect to propolis = 1.00, Table 3). This is consistent with the finding that bees filter

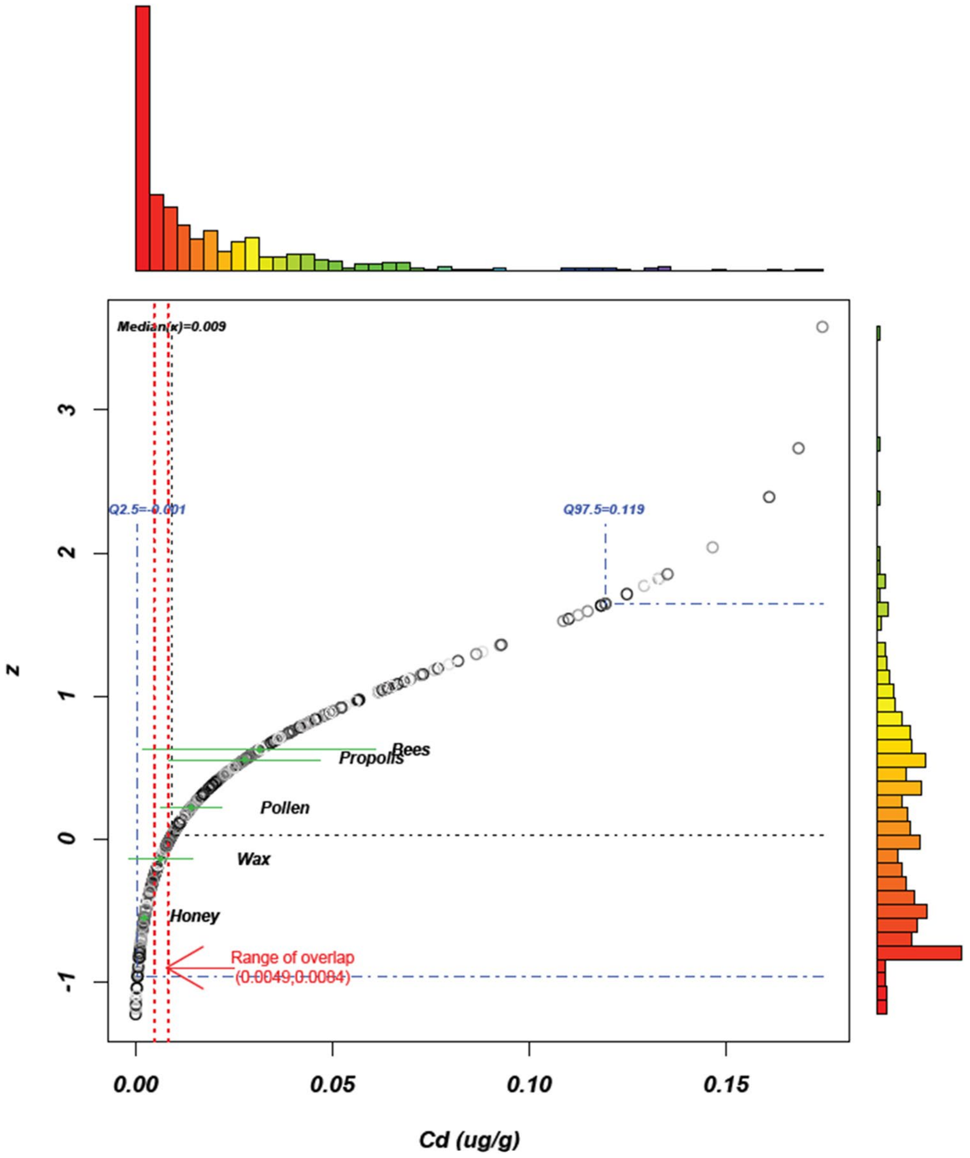


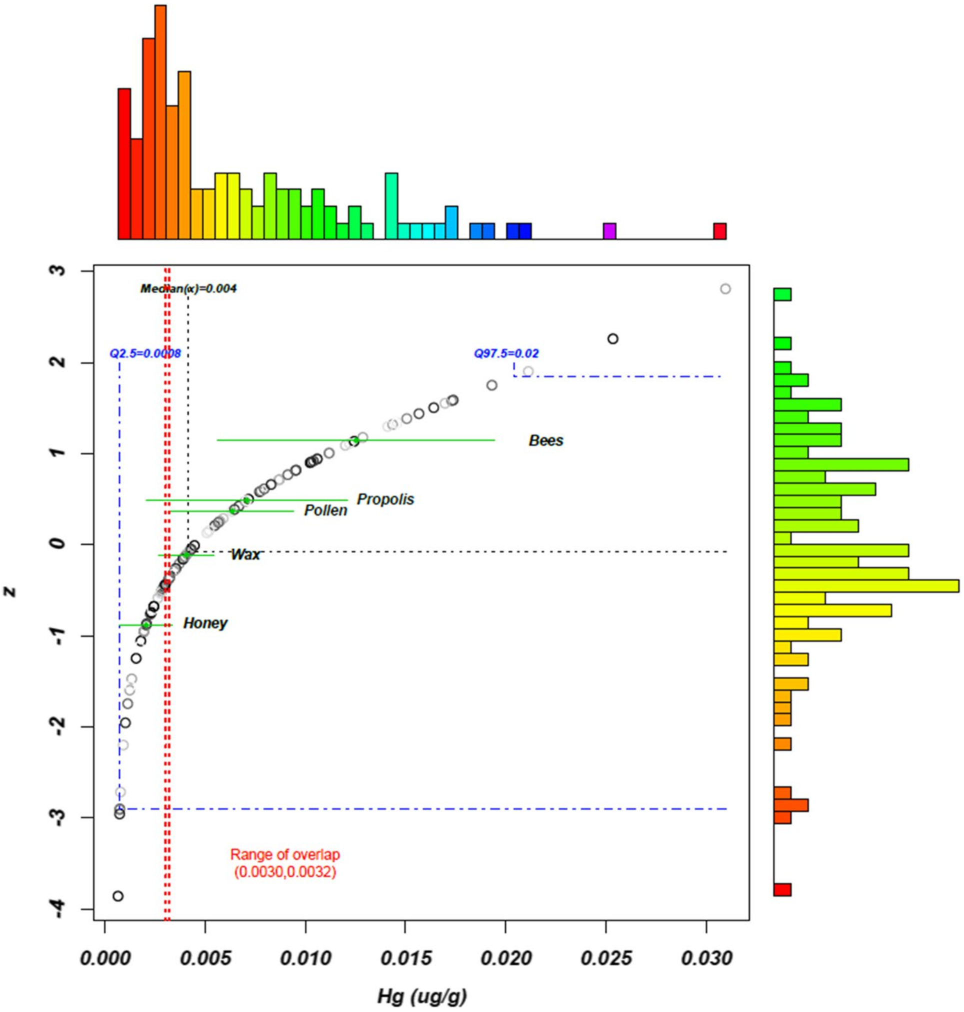
Fig. 2 Control chart for Cd built for the five selected indicators with their obtained overlap metal concentrations (μg/g). Median absolute deviations (m.a.d.) are reported (green lines)

heavy metals during the beehive products' production process.

Figure 4 depicts that Pb median levels in honey (i.e. 0.01 μg/g, Table 1) were significantly lower (post hoc multiple comparisons $p < 0.05$) than those in the other four biomonitor/indicators and lower than the overall median (i.e. 0.15 μg/g). In contrast, propolis and wax, compared with the other biomonitor/indicators, showed the highest median Pb levels (0.32 and 0.27 μg/g, respectively). The limits of the overlap range were narrow (0.128 and 0.217 μg/g) and had low variability. The OBI-U (OBI-U Std) was high for wax [i.e. 33.4 (1.00); Table 4] with respect to the other biomonitor/indicators, while honey was highly high for OBI-L (OBI-L Std) values [65.8 (1.00); Table 4] compared to the other biomonitor/indicators. This confirms the honey's ability to detect extremely low metal levels (or not to concentrate them) from the environment.

Generally, our median levels (all five locations bundled) of elements in the Rome province for honey are lower or similar than other Italian literature data except for Hg (0.0021 μg/g) and Zn (2 μg/g), which are higher to that of Central and Southern Italy (0.00004 μg/g) by Quinto et al. (2016) and to that of Marche (0.10 μg/g) by Meli et al. (2015), respectively (see for comparison Table S2). In fact, the levels of some toxic elements in the analysed samples were often not detectable. Thus, the OBI for As, Be, U, and Ni was not performed. For instance, 98% of honey samples showed As levels below the LOD (0.03 μg/g) (Table 1), while bees and propolis (79 and 90% positive samples, respectively) showed the highest median As levels (0.07–0.08 μg/g, Table 1). Additionally, there were generally

Fig. 3 Control chart for Hg built for the five selected indicators with their obtained overlap metal concentrations (μg/g). Median absolute deviations (m.a.d.) are reported (green lines)



no detectable levels of Be in the honey samples, with only 9% positive samples (Table 1). Conversely, propolis was the best Be bioaccumulator (median of 0.0120 μg/g, with 100% of positive samples), followed by pollen (i.e. a median of 0.0050 μg/g, with 100% positive samples). When detectable, the U levels in honey were close to the LOD (i.e. 0.0002 μg/g, with 29% positive samples). In comparison, propolis showed the highest median U levels (0.0120 μg/g, with 100% positive samples). Regarding Ni, an essential element required by humans in minimal amounts, the measured median levels were close to the LOD with similar median concentrations for all the edible indicators studied here (0.1–0.2 μg/g). The highest Ni values were found in wax and bees (i.e. 1.7 and 3.7 μg/g, respectively).

The control charts for Cr and Zn were also done in the five selected biomonitor/indicators with their resulting overlap element concentrations (Figs. S1 and S2).

Figure S1 shows that the median Cr levels for honey (0.03 μg/g) were significantly (post hoc multiple comparisons $p < 0.05$) lower than those of the other four biomonitor/indicators and lower than the overall median (i.e. 0.084 μg/g). Propolis showed the highest median Cr levels (0.54 μg/g, 100% positive samples). The limits of the overlap range were too narrow (0.325 and 0.337 μg/g). The OBI for Cr (Table S3) shows that pollen has an OBI-U = 3.4 (OBI-U Std = 1), showing an acceptable Cr bioaccumulation surplus. In contrast, honey had high OBI-L values (i.e. 38.4, OBI-L Std = 1). This result indicates that honey detects 38.4-fold lower Cr levels with respect to the minimum overlap range (i.e. propolis = 1, the last in the ranking with OBI-L Std = 0.03, Table S3).

Figure S2 shows the Zn control chart. Honey showed the lowest median levels of Zn (i.e. 2 μg/g), which was significantly different from the other four biomonitor/indicators (post hoc multiple comparisons $p < 0.05$), while bees showed the highest median values (i.e. 100 μg/g). The overall median was 37.7 μg/g, and the overlap range was 6.9–65.9 μg/g. The OBI-U was very high for wax (i.e. 60.4; Table S4); additionally, honey had high OBI-L values (65.9), confirming its utility in detecting extremely low metal levels with respect to the common lower limit of overlap. It is worth noting that Johnson's method of logarithmic data transformation

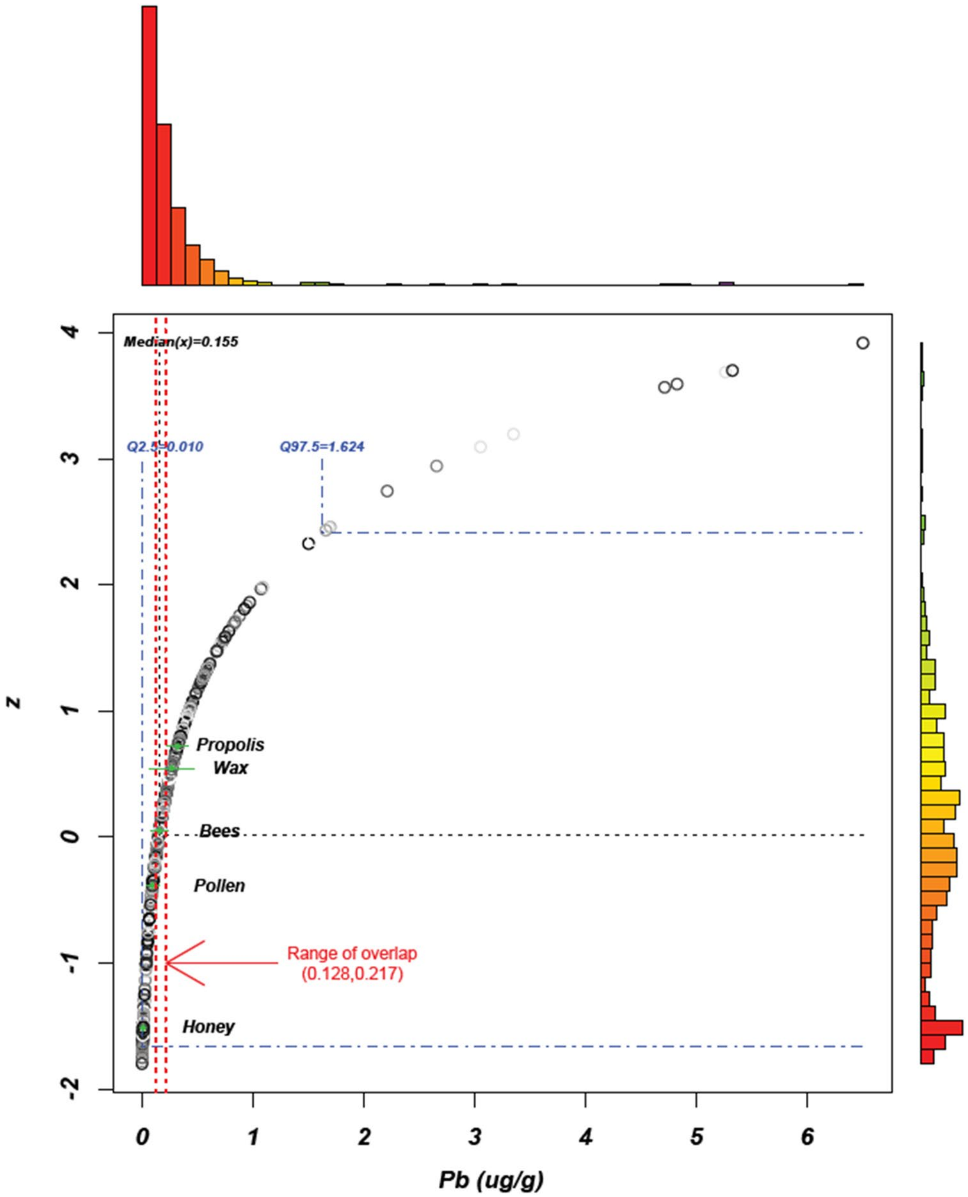


Fig. 4 Control chart for Pb built for the five selected indicators with their obtained overlap metal concentrations (µg/g). Median absolute deviations (m.a.d.) are reported (green lines)

Table 2 Q2.5 and Q97.5 percentiles of Cd data distribution (µg/g) and Cd overlap bioaccumulation index (OBI)

Matrix	Q2.5	Q97.5	OBI-L (lower bound)	OBI-L standardised	OBI-U (upper bound)	OBI-U standardised
Bees	0.0049	0.3682	1.0	0.05	**44.0**	**1.00**
Wax	0.0030	0.0623	**21.4**	**1.00**	7.4	0.17
Honey	0.0030	0.0084	6.1	0.29	1.0	0.02
Pollen	0.0039	0.1868	1.3	0.06	**22.3**	**0.51**
Propolis	0.0033	0.0964	1.4	0.07	11.5	0.26
Range of overlap	0.0049–0.0084					

excluded the detected outliers in propolis samples for Zn (i.e. three samples with 1950, 1565, and 1519 µg/g).

From these results, we can summarise some relevant findings/comments:

i. Honey showed from acceptable/good to very high OBI-L values for most metals (4.0, 38.4, 65.8, and 65.9 for Hg, Cr, Pb, and Zn, respectively).

Table 3 Q2.5 and Q97.5 percentiles of Hg data distribution (µg/g) and Hg overlap bioaccumulation index (OBI)

Matrix	Q2.5	Q97.5	OBI-L (lower bound)	OBI-L standardised	OBI-U (upper bound)	OBI-U standardised
Bees	0.0026	0.027	1.84	0.46	**8.44**	**1.00**
Wax	0.0034	0.011	1.41	0.35	3.43	0.41
Honey	0.0012	0.0032	**4.00**	**1.00**	1.000	0.12
Pollen	0.0043	0.0145	1.11	0.28	4.53	0.54
Propolis	0.0048	0.0162	1.00	0.25	5.06	0.60
Range of overlap	0.0030–0.0032					

Table 4 Q2.5 and Q97.5 percentiles of Pb data distribution (µg/g) and Pb overlap bioaccumulation index (OBI)

Matrix	Q2.5	Q97.5	OBI-L (lower bound)	OBI-L standardised	OBI-U (upper bound)	OBI-U standardised
Bees	0.078	0.604	2.8	0.04	4.7	0.14
Wax	0.059	4.274	3.7	0.06	**33.4**	**1.00**
Honey	0.010	0.128	**65.8**	**1.00**	1.0	0.03
Pollen	0.037	0.230	5.9	0.09	2.3	0.07
Propolis	0.218	0.561	1.0	0.02	4.4	0.13
Range of overlap	0.128–0.217					

ii. This finding implies that honey concentrates x = OBI-L times less than the common lower concentration limit of the overlap concentration range and means that honey does not accumulate significantly elements transported by bees from the external environment into the hive.

iii. It is worth noting that this honey's *univocal* behaviour primarily concerns the detection of very/extremely low concentrations of elements, even if honey elements of anthropogenic origin are indirectly connected via bees with the surrounding environment. The univocal bioaccumulation pattern implies that honey for almost all the biomonitor/indicators studied always accumulated metals at very low levels with respect to the other biomonitor/indicators.

iv. This finding, i.e. the univocal metals' bioaccumulation pattern, is also supported by the fact that the OBI-U values for honey were always the lowest with only one exception (see Tables 2, 3, and 4, S3 and S4). Thus, honey does not bioaccumulate metals at high concentrations. Therefore, we can infer that it is useless as an environmental indicator for the studied elements, as already reported by several authors (Álvarez-Ayuso and Abad-Valle 2017; Conti and Botrè 2001; Conti et al. 2018; Satta et al. 2012; Saunier et al. 2013; Borsuk et al. 2021).

v. The low transfer of toxic metals from bees to honey is evidenced and supports the idea that bees filter toxic and potentially toxic elements in food products. This filtration is due to the honey production process and confirms the food product's good average quality. Our results match those of Borsuk et al. (2021), Dżugan et al. (2018),and Satta et al. (2012), who confirmed the influence of anthropogenic activity on the accumulation of elements in bees and highlighted the role of bees as biofilters of heavy metals with their protective function regarding honey contamination. However, it is necessary to emphasise that some authors (Leita et al. 1996; Porrini et al. 2002; Sadowska et al. 2019) showed differences between elements (As, Cd, Cr, Pb, Zn) deposited on the surface of the body of bees (removable by washing) and those detectable inside their bodies.

vi. Bees showed good/high OBI-U values for the toxic and essential elements analysed here, i.e. Cd (44.0), Hg (8.44), and Zn (23.5) (Tables 2 and 3, and S4, respectively); similarly, we obtained high OBI-U values for Pb (33.4) and Zn (60.4) for the wax samples (Table 4 and S4, respectively). At the same time, pollen gave a high OBI-U for Cd (22.3) (Table 2) and the highest Cr surplus (Table S3) (OBI-U = 3.4, i.e. OBI-U Std = 1.00) compared to bees (OBI Std = 0.29).

This result demonstrates/supports the strong ability of bees, wax, and pollen to accumulate these elements from the beehives' surrounding environment and their great utility for monitoring purposes. The obtained results are consistent with numerous other studies (AL-Alam et al. 2019; Herrero-Latorre et al. 2017; Matin et al. 2016; Giglio et al. 2017). Biomonitoring with bees is a valuable approach for evaluating possible solutions to environmental pollution and increasing information for an environmental impact assessment (AL-Alam et al. 2019; Bargańska et al. 2016; Giglio et al. 2017). De Oliveira et al. (2020) show that the element composition of beehive products varies according to the mineral composition of the soil, plants, and rocks in the region in which the beehives are located and to anthropic contributions of the sites. In addition, Goretti et al. (2020) suggest that the contamination in bees was related to the values of particulate matter (PM_{10}) only to a lesser extent and that enrichment of metals such as Cd, Cu, Mn, and Zn in bees seemed to depend on local conditions, such as the use of pesticides and fertilisers, and the resuspension of soils locally contaminated.

Maximum limit and estimated daily intake

We first determined the intake (adults and children) to study the human health risk caused by the element content in honey, wax, pollen, and propolis. We matched the results with the standard health values, including safety reference doses. The EDI estimates the daily exposure level of the human population to toxic and potentially toxic elements through food consumption (Dorne et al. 2011; Pearson and Ashmore 2020). This study calculated the EDIs for adults and children according to the determined maximum element values (worst scenario approach) (Bommuraj et al. 2019).

Beeswax in both yellow and white forms is used (in agreement with the EFSA 2007) in wafers containing ice cream as a coating agent that can prevent the passage of water from ice cream to the wafer, keeping the wafer crunchy; as a glazing agent in chocolate or cocoa-based products, candies and chewing gums, snacks based on cereal and/or potato flours, or starches and in nut-based products; as a glazing agent in the treatment of coffee beans; and as a coating agent in food supplements in capsule or tablet form. During the surface treatment of fruits such as citrus, pears, apples, peaches, mango, avocado, and pineapple, food industry additive E901 keeps the fruit shiny. It prevents dehydration, oxidation, and possible mould penetration, thus increasing shelf life. Therefore, there is no daily intake quantity, but there is a possibility of taking in different quantities by consuming different products. Thus, it is reasonable to evaluate the risk for human health by estimating daily consumption. In compliance with EFSA, the daily beeswax consumption was estimated to be 0.022 g/kg /b.w. (Bommuraj et al. 2019; EFSA 2007).

The recommended daily dose of pollen consumption for an adult should range from 20 to 40 g (Kostić et al. 2020). However, there are several sources of variability concerning ingestion and subsequent effects. For instance, pollen grains can often have a hard shell, making nutrient absorption in the digestive tract quite difficult. Thus, in this study, we adopted a mean consumption of 30 g/person/day.

Regarding propolis, by applying a safety factor of 1000 for humans, we adopted a safe dose of 1.4 mg/kg b.w./day, as Burdock (1998) stated in his pioneering study. Moreover, it should also be considered that propolis is a complex mixture of approximately 200 compounds, its composition may vary in different geographical areas, and the composition primarily depends on its botanical origin (Bogdanov 2020).

Among the contaminants in honey, the only one that to date has a maximum limit set by law is Pb. There is a maximum threshold in honey of 0.10 mg/kg wet weight, as established by Commission Regulation (EU) (2015/1005).

Of major concern are As, Cd, Pb, and Hg, which are the major contributors to hazardous dietary exposure being toxic even at low concentrations. A total of 95.7% of honey samples were below the Pb maximum limit (ML), confirming the excellent quality of the analysed honeys and the lower transfer capacity of the elements from the environment via bees to the final product. However, only four honey samples (4.3%), arising from different sites, showed slightly Pb higher levels than 0.10 µg/g (i.e. 0.13–0.14 µg/g was the detected range).

Table 5 shows the EDIs of some elements in honey, wax, pollen, and propolis that can be consumed by adults and children living in central Italy (µg/kg b.w./day) and health-based guidance values for risk assessment. The PTDI values for Cd, Cr, and Hg; the PMTDI value for Zn; the TDI for Be, Ni, and U; and the benchmark dose level (BMDL) for As and Pb are reported (see Conti et al. 2020a).

The obtained EDI values in this study (Table 5) for honey and the other edible indicators were evidently lower than the safety reference dose for the considered elements. For Pb risk characterisation (Table 5), the EFSA (2012) set up a range of BMDL confidence limits. The $BMDL_{01}$ Pb risk limits for adults and children are reported in Table 5 (EFSA 2012; Conti et al. 2020a). The EDI of elements in children was higher than those found for adults for all the studied elements (Table 5). Additionally, our results should be interpreted considering that we have applied the worst-case scenario, which is usually improbable.

There are several international standards or maximum limits for As in drinking water and food. Human exposure to As can occur primarily via oral intake with food that has been robustly related to lung, bladder, and skin cancers (EFSA 2009). Thus, for As, a $BMDL_{01}$ in the range 0.3–8 µg/kg/b.w. per day was set (EFSA 2009, EFSA et al. 2021). Afterwards, based on new scientific evidence, the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2011) identified a $BMDL_{05}$ of 3.0 µg/kg/b.w. per day for an increased risk of lung cancer (range 2–7 µg/kg/b.w. per day) (EFSA et al. 2021).

Excluding possible infrequent cases of high consumers of contaminated samples, we can infer that honey, wax, propolis, and pollen are safe for consumption by adults

Table 5 Estimated daily intake (EDI) of elements from honey and edible indicator consumption by adults and children living in the Rome province (μg/kg bw/day) and health-based guidance values for risk-exposure characterisation

Heavy metals	As	Be	Cd	Cr	Hg	Ni	Pb	U	Zn
EDIs (μg/kg bw/day)[a]									
Honey (*n* = 92)	$0.4\ e^{-3}$	$0.8\ e^{-5}$	$0.12\ e^{-3}$	0.03	$4.3\ e^{-5}$	0.004	$1.9\ e^{-3}$	$1.2\ e^{-5}$	0.12
Adults	$1.6\ e^{-3}$	$3.2\ e^{-5}$	$0.48\ e^{-3}$	0.12	$1.7\ e^{-4}$	0.016	$7.5\ e^{-3}$	$4.8\ e^{-5}$	0.48
Children									
Wax (*n* = 311)	$5.1\ e^{-5}$	$0.5\ e^{-5}$	$0.1\ e^{-3}$	$0.4\ e^{-3}$	$0.4\ e^{-5}$	$0.6\ e^{-3}$	$2.4\ e^{-3}$	$0.9\ e^{-5}$	0.27
Adults	$2.0\ e^{-4}$	$0.2\ e^{-4}$	$0.4\ e^{-3}$	$1.8\ e^{-3}$	$1.6\ e^{-5}$	$2.5\ e^{-3}$	$9.5\ e^{-3}$	$0.4\ e^{-4}$	1.07
Children									
Pollen (*n* = 45)	$1.2\ e^{-3}$	$1.8\ e^{-4}$	$1.6\ e^{-3}$	0.011	$1.5\ e^{-4}$	$8.3\ e^{-3}$	$2.8\ e^{-3}$	$1.3\ e^{-4}$	0.32
Adults	$4.7\ e^{-3}$	$7.1\ e^{-4}$	$6.6\ e^{-3}$	0.045	$6.2\ e^{-4}$	0.033	0.011	$5.1\ e^{-4}$	1.27
Children									
Propolis (*n* = 20)	$5.4\ e^{-6}$	$0.7\ e^{-6}$	$2.8\ e^{-5}$	$3.1\ e^{-5}$	$0.4\ e^{-6}$	$4.7\ e^{-6}$	$1.3\ e^{-5}$	$0.7\ e^{-6}$	0.04 (d)
Adults	$2.1\ e^{-5}$	$2.7\ e^{-6}$	$1.1\ e^{-4}$	$1.2\ e^{-4}$	$1.5\ e^{-6}$	$1.9\ e^{-5}$	$5.2\ e^{-5}$	$2.9\ e^{-6}$	0.18 (d)
Children									
PTDI (μg/kg/ bw/day)			**0.83**[b]	**4.2**[c]	**0.5714**				
PMTDI (μg/kg/ bw/day)									**300**
TDI (μg/kg/ bw/day)		**2**[e]				**13**[f]		**0.6**[g]	
BMDL$_{01}$ (μg/kg/ bw/day)	**0.3–8**[h]						**1.50**[i] **0.63**[j] **0.50**[k]		

[a] EDI values for adults and children were calculated according from the obtained maximum metal values — worst scenario approach (Table 1). [b] PTDI values of Cd (i.e. 0.83 μg/kg bw/day) were recalculated on a daily basis from the PTMI (25 μg/kg bw/month) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2019).[c] The Cr limit of 250 μg per day (WHO, 1996; EFSA, 2014) is divided by the mean adults weight considered (60 kg). [d] EDIs of Zn in propolis were calculated based on the outlier of 1950 μg/g (worst scenario).[e] WHO, 2009. [f] EFSA, 2015. [g] WHO, 2003. [h] EFSA, 2009, 2021. [i] WHO, 2003. Systolic blood pressure as the endpoint, [j] Chronic kidney disease, [k] Neurotoxicity in young children (EFSA, 2012)

and children. There are no alarms for public health. Moreover, it is also pertinent to consider other food sources for their possible metal intake in the study population.

Target hazard quotient

The THQ values for the elements in honey, wax, pollen, and propolis are reported in Table 6. For all the analysed elements, the THQ values were evidently below 1, suggesting that the exposed human population is supposed to be safe.

Table 6 Target hazard quotient (THQ) and hazard index (HI) for honey, wax, pollen, and propolis' consumption in samples collected in five sites of the Rome province (Italy)

Oral reference dose (RfDo, μg/kg/ bw/day)		THQ							
		Honey		Wax		Pollen		Propolis	
		Adults	Children	Adults	Children	Adults	Children	Adults	Children
As	0.3	$1.3\ e^{-3}$	$5.3\ e^{-3}$	$1.7\ e^{-4}$	$6.8\ e^{-4}$	0.004	0.016	$1.8\ e^{-5}$	$7.2\ e^{-5}$
Be	2	$0.4\ e^{-5}$	$1.6\ e^{-5}$	$0.2\ e^{-5}$	$0.1\ e^{-4}$	$0.6\ e^{-3}$	$2.4\ e^{-3}$	$0.3\ e^{-6}$	$1.3\ e^{-6}$
Cd	1	$0.12\ e^{-3}$	$0.48\ e^{-3}$	$0.1\ e^{-3}$	$0.4\ e^{-3}$	$1.6\ e^{-3}$	$6.6\ e^{-3}$	$2.8\ e^{-6}$	$1.1\ e^{-5}$
Cr	3	0.01	0.04	$1.3\ e^{-4}$	$0.5\ e^{-3}$	0.004	0.015	$1.0\ e^{-5}$	$0.4\ e^{-4}$
Ni	20	$0.2\ e^{-3}$	$0.8\ e^{-3}$	$0.3\ e^{-4}$	$1.2\ e^{-4}$	$4.1\ e^{-4}$	0.002	$0.2\ e^{-6}$	$0.9\ e^{-6}$
U	0.2	$6.0\ e^{-5}$	$2.4\ e^{-4}$	$4.5\ e^{-5}$	$2.0\ e^{-4}$	$0.6\ e^{-3}$	0.02	$3.5\ e^{-6}$	$1.4\ e^{-5}$
Zn	300	$0.4\ e^{-3}$	$1.6\ e^{-3}$	$0.9\ e^{-3}$	$3.6\ e^{-3}$	0.001	0.004	$1.5\ e^{-4}$	$0.6\ e^{-3}$
HI		**0.012**	**0.048**	**$1.3\ e^{-3}$**	**$5.5\ e^{-3}$**	**0.012**	**0.07**	**$0.4\ e^{-4}$**	**$1.1\ e^{-4}$**
HI — Total contaminants in honey, wax, pollen, and propolis					**Adults**	0.025			
					Children	0.124			

Hazard index

From Table 6, the HI for all the studied matrixes resulted in no health concern (< 1). Furthermore, considering the sporadic possibility of simultaneously consuming honey, wax, pollen, and propolis, the risk summation generated an HI value below 1 for adults and children (i.e.,0.025 and 0.124, respectively, Table 6), indicating negligible risk to the end consumers. Moreover, it should be noted that the simultaneous consumption of these edible matrixes is quite unlikely, and as reported above, we have applied the worst scenario approach (maximum metal levels).

However, several uncertainty sources should be considered in this type of study. Even if honey, wax, pollen, and propolis consumption are generally low, consumption rates can vary significantly. Regarding essential nutrients such as Zn, the risk assessment should consider the two ends of the dose–response relationship, i.e.the risk of deficiency and toxicity (Conti et al. 2020b). Moreover, multiple food sources for the elements should be considered, including possible additional health effects from a mix of contaminants, i.e. interactions among xenobiotics (US EPA 2000). However, assuming that Zn and other minerals (K, Mg, Cu, Fe, Mn) come from a single food, it can be considered that honey does not contribute with a significant proportion of minerals to the recommended dietary doses (RDAs) (Conti 2000). In particular, the presence of essential macro-elements in addition to micro-and trace elements makes pollen a precious food to avoid mineral deficiencies (De Oliveira et al. 2020; Pohl et al. 2020).

Conclusions

The MBS theoretical model was applied to increase the information variety endowment. We employed the OBI to enhance the observer's information variety about the performance of bees, wax, pollen, and propolis as elemental indicators in environmental ecosystems.

Excluding honey, this study confirms that wax, pollen, propolis, and bees accumulate/reflect high levels of toxic and potentially toxic elements from the surrounding environment (e.g. bees and pollen showed high bioaccumulation Cd surplus (OBI-U = 44.0 and 22.3, respectively, see "Results and discussion"). Moreover, honey showed univocal high OBI-L values, i.e. honey concentrates metals several times less than the common lower concentration limit of the overlap concentration range. This finding implies that honey is useless as an environmental biomonitor and confirms bees' role as biofilters of the elements present in the surrounding environment. This interpretation is also supported by the very high OBI-U values we obtained for several elements for bees that are not reflected in the food product.

The THQ and HI methods were applied for human health risk assessment. Excluding occasional cases of high consumers of contaminated samples, we can infer that honey, wax, propolis, and pollen are safe for consumption by both adults and children (THQ < 1; HI < 1), even considering the possibility of consuming them simultaneously. The data presented in this study can be considered baseline data valid for management decisions concerning upcoming environmental conservation programs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-18072-3.

Acknowledgements The authors are particularly indebted to Marco Papi and Massimo Marcolini, past and current presidents, respectively, of the Association of Beekeepers of Rome and Province for their outstanding support at all stages of sampling. We are also greatly obliged to Helga Liselotte for her kind support during our stays in Oriolo. We also thank Fabrizio Piacentini and Raffaele Cirone [(president of the Italian Beekeeping Federation (FAI)] for their kind support of this project. Eventually, we also thank Martina Ristorini for her support during the sampling campaign.

Author contribution M.E. Conti, M.G. Finoia — conceptualization; M.E. Conti, M.L. Astolfi, S. Canepari — methodology; M.L. Astolfi, M.E. Conti, S. Canepari — resources, sampling; M.L. Astolfi, M.E. Conti, L. Massimi — validation, chemical analyses; M.E. Conti and M.G. Finoia — data curation,

software, writing — original draft; M.E. Conti, M.G. Finoia, M.L. Astolfi — writing — review and.

editing; M.E. Conti, M.L. Astolfi, L. Massimi, S. Canepari — supervision; M.E. Conti — funding acquisition.

Funding This work was financed by Sapienza, University of Rome,project 2018, prot. RG11816432851FA6 (Principal Investigator Prof. M.E. Conti).

Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable [Directive No. 2010/63/EU, on the protection of animals for scientific purposes].

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

AL-Alam, J., Chbani, A., Faljoun, Z., & Millet, M. (2019) The use of vegetation, bees, and snails as important tools for the biomonitoring of atmospheric pollution—a review. Environ Sci Pollut Res 26(10):9391–9408. https://doi.org/10.1007/s11356-019-04388-8

Álvarez-Ayuso E, Abad-Valle P (2017) Trace element levels in an area impacted by old mining operations and their relationship with beehive products. Sci Total Environ 599–600:671–678. https://doi.org/10.1016/j.scitotenv.2017.05.030

Antoine JMR, Fung LAH, Grant CN (2017) Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. Toxicol Rep 4:181–187. https://doi.org/10.1016/j.toxrep.2017.03.006

Ashby WR (1958) Requisite variety and its implications for the control of complex systems. Cursos Congr Univ Santiago De Compostela 1(2):83–89

Ashby, W. R. (1957). An introduction to cybernetics (2nd edition). London: Chapman & Hall.

Astolfi ML, Protano C, Marconi E, Piamonti D, Massimi L, Brunori M, Vitali M, Canepari S (2019) Simple and rapid method for the determination of mercury in human hair by cold vapour generation atomic fluorescence spectrometry. Microchem J 150:104186. https://doi.org/10.1016/j.microc.2019.104186

Astolfi ML, Marconi E, Lorini L, Valentino F, Silva F, Sommer Ferreira B, Canepari S, Majone M (2020a) Elemental concentration and migratability in bioplastics derived from organic waste. Chemosphere 259:127472. https://doi.org/10.1016/j.chemosphere.2020.127472

Astolfi ML, Conti ME, Marconi E, Massimi L, Canepari S (2020b) Effectiveness of different sample treatments for the elemental characterisation of bees and beehive products. Molecules 25(18):4263. https://doi.org/10.3390/molecules25184263

Astolfi ML, Conti ME, Ristorini M, Frezzini MA, Papi M, Massimi L, Canepari S (2021) An analytical method for the biomonitoring of mercury in bees and beehive products by cold vapor atomic fluorescence spectrometry. Molecules 26:4878. https://doi.org/10.3390/molecules26164878

Bargańska Ż, Ślebioda M, Namieśnik J (2016) Honey bees and their products: bioindicators of environmental contamination. Crit Rev Environ Sci Technol 46(3):235–248. https://doi.org/10.1080/10643389.2015.1078220

Barile S (2009) Management sistemico vitale. Giappichelli, Torino

Barile S, Saviano M, Simone C (2015) Service economy, knowledge, and the need for T-shaped innovators. World Wide Web 18(4):1177–1197

Barros Paiva R, Neves A, Sequeira V, Cardoso C, Bandarra N, Serrano Gordo L, Leonor Nunes M (2012) Risks and benefits' consumption of birdbeak dogfish Deania calcea. British Food Journal 114(6):826–839. https://doi.org/10.1108/00070701211234354

Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. J Am Coll Nutr 27(6):677–689. https://doi.org/10.1080/07315724.2008.10719745

Bogdanov S (2020) *Antiviral properties of the bee products: a review*. Retrieved from https://popravko.com/glavnaya/Beeproducts-Corona%20(1).pdf. Accessed 30 June 2021

Bolt H (2019) 2.7 Mixtures and Combinations of Chemicals. In: Toxicology and Risk Assessment: A Comprehensive Introduction, H. Greim and R. Snyder (Eds.), Wiley, 148

Bommuraj V, Chen Y, Klein H, Sperling R, Barel S, Shimshoni JA (2019) Pesticide and trace element residues in honey and beeswax combs from Israel in association with human risk assessment and honey adulteration. Food Chem 299:125123. https://doi.org/10.1016/j.foodchem.2019.125123

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 1–14. https://doi.org/10.1007/s13592-021-00890-6

Braakhuis A (2019) Evidence on the health benefits of supplemental propolis. Nutrients 11(11):2705. https://doi.org/10.3390/nu11112705

Burdock GA (1998) Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol 36(4):347–363. https://doi.org/10.1016/s0278-6915(97)00145-2

Burlando B, Cornara L (2013) Honey in dermatology and skin care: a review. J Cosmet Dermatol 12(4):306–313. https://doi.org/10.1111/jocd.12058

Campos M, Markham KR, Mitchell KA, da Cunha AP (1997) An approach to the characterisation of bee pollens via their flavonoid/phenolic profiles. Phytochem Anal 8(4):181–185. https://doi.org/10.1002/(sici)1099-1565(199707)8:4%3c181::aid-pca359%3e3.0.co;2-a

Campos MGR, Bogdanov S, de Almeida-Muradian LB, Szczesna T, Mancebo Y, Frigerio C, Ferreira F (2008) Pollen composition and standardisation of analytical methods. J Apic Res 47(2):154–161. https://doi.org/10.1080/00218839.2008.11101443

Canepari S, Padella F, Astolfi ML, Marconi E, Perrino C (2013) Elemental concentration in atmospheric particulate matter: estimation of nanoparticle contribution. Aerosol Air Quality Research 13:1619–1629. https://doi.org/10.4209/aaqr.2013.03.0081

Canepari S, Castellano P, Astolfi ML, Materazzi S, Ferrante R, Fiorini D, Curini R (2018) Release of particles, organic compounds and metals from crumb rubber used in synthetic turf under chemical and physical stress. Environ Sci Pollut Res 25:1448–1459. https://doi.org/10.1007/s11356-017-0377-4

Chmielnicka J, Cherian MG (1986) Environmental exposure to cadmium and factors affecting trace-element metabolism and metal toxicity. Biol Trace Elem Res 10(3):243–262. https://doi.org/10.1007/bf02795623

Commission Regulation (EU). (2015/1005). *Amending Regulation (EC) No. 1881/2006 as regards maximum levels of lead in certain foodstuffs*.

Conti ME (2000) Lazio region (central Italy) honeys: a survey of mineral content and typical quality parameters. Food Control 11(6):459–463. https://doi.org/10.1016/s0956-7135(00)00011-6

Conti ME, Botrè F (2001) Honeybees and their products as potential bioindicators of heavy metals contamination. Environ Monit Assess 69(3):267–282. https://doi.org/10.1023/a:1010719107006

Conti ME, Cecchetti G (2001) Biological monitoring: lichens as bioindicators of air pollution assessment—a review. Environ Pollut 114(3):471–492

Conti ME, Finoia MG (2010) Metals in molluscs and algae: a north–south Tyrrhenian Sea baseline. J Hazard Mater 181(1–3):388–392. https://doi.org/10.1016/j.jhazmat.2010.05.022

Conti ME, Mecozzi M, Finoia MG (2015) Determination of trace metal baseline values in Posidonia oceanica, Cystoseira sp., and other marine environmental biomonitors: a quality control method for a study in South Tyrrhenian coastal areas. Environ. Sci. Pollut. Res. 22(5):3640–3651. https://doi.org/10.1007/s11356-014-3603-3

Conti ME, Canepari S, Finoia MG, Mele G, Astolfi ML (2018) Characterisation of Italian multifloral honeys on the basis of their mineral content and some typical quality parameters. J Food Compos Anal 74:102–113. https://doi.org/10.1016/j.jfca.2018.09.002

Conti ME, Tudino MB, Finoia MG, Simone C, Stripeikis J (2019a) Managing complexity of marine ecosystems: from the monitoring breakdown structure (MBS) to the baseline assessment. Trace metal concentrations in biomonitors of the Beagle Channel, Patagonia (2005–2012). Ecol Ind 104:296–305. https://doi.org/10.1016/j.ecolind.2019.05.013

Conti ME, Tudino MB, Finoia MG, Simone C, Stripeikis J (2019b) Performance of two Patagonian molluscs as trace metal biomonitors: the overlap bioaccumulation index (OBI) as an integrative tool for the management of marine ecosystems. Ecol Ind 101:749–758. https://doi.org/10.1016/j.ecolind.2019.01.060

Conti ME, Tudino MB, Finoia MG, Simone C, Stripeikis J (2020a) Applying the monitoring breakdown structure model to trace metal content in edible biomonitors: an eight-year survey in the Beagle Channel (southern Patagonia). Food Res Int 128:108777. https://doi.org/10.1016/j.foodres.2019.108777

Conti ME, Plà R, Simone C, Jasan R, Finoia MG (2020b) Implementing the monitoring breakdown structure: native lichens as biomonitors of element deposition in the southern Patagonian forest connected with the Puyehue volcano event in 2011—a 6-year survey (2006–2012). Environ Sci Pollut Res 27(31):38819–38834. https://doi.org/10.1007/s11356-020-10001-0

Conti ME (2002) II monitoraggio biologico della qualita ambientale. SEAM Ed. Rome, 181pp

Conti ME (2008) Biological monitoring: theory & applications: bioindicators and biomarkers for environmental quality and human exposure assessment (Vol. 17). WIT Press, Southampton

Crane E (1984) Bees, honey, and pollen as indicators of metals in the environment. Bee World 65(1):47–49. https://doi.org/10.1080/0005772x.1984.11098770

da Cunha Martins A, Carneiro MFH, Grotto D, Adeyemi JA, Barbosa F (2018) Arsenic, cadmium, and mercury-induced hypertension: mechanisms and epidemiological findings. Journal of Toxicology and Environmental Health, Part B 21(2):61–82. https://doi.org/10.1080/10937404.2018.1432025

De la Guardia M, Garrigues S (2015) Handbook of mineral elements in food. John Wiley & Sons

De Oliveira AF, Abreu ATD, Nascimento NDO, Froes RES, Nalini HA Jr, Antonine Y (2020) Mineral content in honey and pollen from native stingless bees Tetragonisca angustula (Latreille, 1811) in the Iron Quadrangle. Brazil Journal of Apicultural Research 59(4):378–389. https://doi.org/10.1080/00218839.2020.1730578

Denisow B, Denisow-Pietrzyk M (2016) Biological and therapeutic properties of bee pollen: a review. J Sci Food Agric 96(13):4303–4309. https://doi.org/10.1002/jsfa.7729

Devi A, Jangir J, Anu-Appaiah KA (2018) Chemical characterisation complemented with chemometrics for the botanical origin identification of unifloral and multifloral honeys from India. Food Res Int 107:216–226. https://doi.org/10.1016/j.foodres.2018.02.017

Dorne JLCM, Kass GEN, Bordajandi LR, Amzal B, Bertelsen U, Castoldi AF, Heppner C, Eskola M, Fabiansson S, Ferrari P, Scaravelli E, Dogliotti E, Fuerst P, Boobis AR, Verger P (2011) Human risk assessment of heavy metals: principles and applications. Metal Ions in Toxicology: Effects, Interactions, Interdependencies 8(4):27–60. https://doi.org/10.1515/9783110436624-007

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):101. https://doi.org/10.1007/s10661-018-6474-0

EFSA (2007) Beeswax (E 901) as a glazing agent and as carrier for flavours - scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food (AFC). EFSA J 615:1–28. https://doi.org/10.2903/j.efsa.2007.615

EFSA (2012) Lead dietary exposure in the European population. EFSA J 10(7):2831. https://doi.org/10.2903/j.efsa.2012.2831

EFSA (2015) Scientific opinion on the risks to public health related to the presence of nickel in food and drinking water. EFSA J 13:4002–4203. https://doi.org/10.2903/j.efsa.2015.4002

EFSA, Arcella D, Cascio C, Ruiz JAG (2021) Scientific report on the chronic dietary exposure to inorganic arsenic. EFSA J 19(1) 6380, 6350

EFSA. (2009). European Food Safety Authority. Scientific opinion on arsenic in food. EFSA J. 7(10), 1351. https://doi.org/10.2903/j.efsa.2009.1351

EFSA (2014) Scientific opinion on the risks to public health related to the presence of chromium in food and drinking water. EFSA Panel on Contaminants in the Food Chain (CONTAM)2, Parma, Italy. EFSA J 12(3) 3595.https://doi.org/10.2903/j.efsa.2014.3595

Finger D, Filho IK, Torres YR, Quináia SP (2014) Propolis as an indicator of environmental contamination by metals. Bull Environ Contam Toxicol 92(3):259–264. https://doi.org/10.1007/s00128-014-1199-4

Garty J (1993) Lichens as biomontors for heavy metal pollution. VCH, Plants as biomonitors

Giglio A, Ammendola A, Battistella S, Naccarato A, Pallavicini A, Simeon E, Tagarelli A, Giulianini PG (2017) Apis mellifera ligustica, Spinola 1806 as bioindicator for detecting environmental contamination: a preliminary study of heavy metal pollution in Trieste. Italy Environ. Sci. Pollut. Res. 24(1):659–665. https://doi.org/10.1007/s11356-016-7862-z

Goretti E, Pallottini M, Rossi R, La Porta G, Gardi T, Cenci Goga BT, Elia AC, Galletti M, Moroni B, Petroselli C, Selvaggi R, Cappelletti D (2020) Heavy metal bioaccumulation in honey bee matrix, an indicator to assess the contamination level in terrestrial environments. Environ Pollut 256:113388. https://doi.org/10.1016/j.envpol.2019.113388

Grembecka M, Szefer P (2013) Evaluation of honeys and bee products quality based on their mineral composition using multivariate techniques. Environ Monit Assess 185(5):4033–4047. https://doi.org/10.1007/s10661-012-2847-y

Hague T, Petroczi A, Andrews PLR, Barker J, Naughton DP (2008) Determination of metal ion content of beverages and estimation of target hazard quotients: a comparative study. Chem Cent J 2(1):13. https://doi.org/10.1186/1752-153x-2-13

Herrero-Latorre C, Barciela-García J, García-Martín S, Peña-Crecente RM (2017) The use of honeybees and honey as environmental bioindicators for metals and radionuclides: a review. Environ Rev 25(4):463–480. https://doi.org/10.1139/er-2017-0029

JECFA (2009) Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain veterinary drug residues in food: seventieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva: WHO Technical Report Series 954

JECFA (2011) (Joint FAO/WHO Expert Committee on Food Additives). Evaluation of certain contaminants in food: seventy-second report of the joint FAO/WHO expert committee on food additives. World Health Organ

JECFA (2019) The Joint FAO/WHO Expert Committee on Food Additives, 2011 Lead evaluation

Johnson NL (1949) Systems of frequency curves generated by methods of translation. Biometrika 36(1–2):149–176. https://doi.org/10.1093/biomet/36.1-2.149

Kalogeropoulos N, Konteles SJ, Troullidou E, Mourtzinos I, Karathanos VT (2009) Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. Food Chem 116(2):452–461. https://doi.org/10.1016/j.foodchem.2009.02.060

Kostić AŽ, Milinčić DD, Gašić UM, Nedić N, Stanojević SP, Tešić ŽL, Pešić MB (2019) Polyphenolic profile and antioxidant properties of bee-collected pollen from sunflower (Helianthus annuus L.) plant. LWT 112:108244. https://doi.org/10.1016/j.lwt.2019.06.011

Kostić AŽ, Milinčić DD, Barać MB, Ali Shariati M, Tešić ŽL, Pešić MB (2020) The application of pollen as a functional food and feed ingredient—the present and perspectives. Biomolecules 10(1):84. https://doi.org/10.3390/biom10010084

Kumar A, Kumar A, MMS, C. P., Chaturvedi, A. K., Shabnam, A. A., Subrahmanyam, G., Mondal, R., Gupta, D. K., Malyan, S. K., Kumar, S. S., A. Khan, S., & Yadav, K. K. (2020) Lead toxicity: health hazards, influence on food chain, and sustainable remediation approaches. Int J Environ Res Public Health 17(7):2179. https://doi.org/10.3390/ijerph17072179

Lambert O, Piroux M, Puyo S, Thorin C, Larhantec M, Delbac F, Pouliquen H (2012) Bees, honey and pollen as sentinels for lead environmental contamination. Environ Pollut 170:254–259. https://doi.org/10.1016/j.envpol.2012.07.012

Lazarus M, Tariba Lovaković B, Sekovanić A, Orct T, Jurič A, Prđun S, Denžić Lugomer M, Bubalo D (2021) Combined approach to studying authenticity markers following spatial, temporal and production practice trends in honey from Croatia. Eur Food Res Technol 247(6):1511–1523. https://doi.org/10.1007/s00217-021-03728-8

Leita L, Muhlbachova G, Cesco S, Barbattini R, Mondini C (1996) Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. Environ Monit Assess 43(1):1–9. https://doi.org/10.1007/bf00399566

Liu Q, Xu X, Zeng J, Shi X, Liao Y, Du P, Tang Y, Huang W, Chen Q, Shou L (2019) Heavy metal concentrations in commercial marine organisms from Xiangshan Bay, China, and the potential health risks. Mar Pollut Bull 141:215–226. https://doi.org/10.1016/j.marpolbul.2019.02.058

Losfeld G, Saunier JB, Grison C (2014) Minor and trace-elements in apiary products from a historical mining district (Les Malines, France). Food Chem 146:455–459. https://doi.org/10.1016/j.foodchem.2013.08.105

Machado De-Melo AA, Almeida-Muradian LB, Sancho MT, Pascual-Maté A (2018) Composition and properties of Apis mellifera honey: a review. J Apic Res 57(1):5–37. https://doi.org/10.1080/00218839.2017.1338444

Madejczyk M, Baralkiewicz D (2008) Characterisation of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. Anal Chim Acta 617:11–17. https://doi.org/10.1016/j.aca.2008.01.038

Matin G, Kargar N, Buyukisik HB (2016) Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. Ecol Eng 90:331–335. https://doi.org/10.1016/j.ecoleng.2016.01.035

Meli MA, 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 A 78(10):617–627. https://doi.org/10.1080/15287394.2014.1004006

Melliou E, Chinou I (2011) Chemical constituents of selected unifloral Greek bee-honeys with antimicrobial activity. Food Chem 129(2):284–290. https://doi.org/10.1016/j.foodchem.2011.04.047

Miller JN, Miller JC (2005) Statistics and chemometrics for analytical chemistry. Prentice Hall, Pearson

Morin E (1992) From the concept of system to the paradigm of complexity. J Soc Evol Syst 15(4):371–385

Naja GM, Volesky B (2017) Toxicity and sources of Pb, Cd, Hg, Cr, As, and radionuclides in the environment. In: Wang LK, Wang MHS, Hung YT, Shammas NK, Chen JP (eds) Handbook of advanced industrial and hazardous wastes management. CRC Press, pp 855–903

Negri I, Mavris C, Di Prisco G, Caprio E, Pellecchia M (2015) Honey bees (Apis mellifera, L.) as active samplers of airborne particulate matter. Plos One 10(7):e0132491. https://doi.org/10.1371/journal.pone.0132491

Pearson AJ, Ashmore E (2020) Risk assessment of antimony, barium, beryllium, boron, bromine, lithium, nickel, strontium, thallium and uranium concentrations in the New Zealand diet. Food Additives & Contaminants: Part A 37(3):451–464. https://doi.org/10.1080/19440049.2019.1704445

Petroczi A, Naughton DP (2009) Mercury, cadmium and lead contamination in seafood: a comparative study to evaluate the usefulness of target hazard quotients. Food Chem Toxicol 47(2):298–302. https://doi.org/10.1016/j.fct.2008.11.007

Pohl P (2009) Determination of metal content in honey by atomic absorption and emission spectrometries. TrAC, Trends Anal Chem 28(1):117–128. https://doi.org/10.1016/j.trac.2008.09.015

Pohl P, Dzimitrowicz A, Greda K, Jamroz P, Lesniewicz A, Szymczycha-Madeja A, Welna M (2020) Element analysis of bee-collected pollen and bee bread by atomic and mass spectrometry–Methodological development in addition to environmental and nutritional aspects. TrAC Trends in Analytical Chemistry, 128, 115922.https://doi.org/10.1016/j.trac.2020.115922

Pohl P, Stecka H, Sergiel I, Jamroz P (2012) Different aspects of the elemental analysis of honey by flame atomic absorption and emission spectrometry: a review. Food Anal Methods 5(4):737–751. https://doi.org/10.1007/s12161-011-9309-y

Porrini C, Ghini S, Girotti S, Sabatini AG, Gattavecchia E, Celli G (2002) Use of honey bees as bioindicators of environmental pollution in Italy. In: Devillers J, Pham-Delégue MH (eds) Honey bees: the environmental impact of chemicals. Taylor & Francis, London, pp 186–247

Przybyłowski P, Wilczyńska A (2001) Honey as an environmental marker. Food Chem 74(3):289–291. https://doi.org/10.1016/S0308-8146(01)00153-4

Quinto M, Miedico O, Spadaccino G, Paglia G, Mangiacotti M, Li D, Centonze D, Chiaravalle AE (2016) Characterisation, chemometric evaluation, and human health-related aspects of essential and toxic elements in Italian honey samples by inductively coupled plasma mass spectrometry. Environmental Science Pollution Research 23:25374–25384. https://doi.org/10.1007/s11356-016-7662-5

Raes H, Cornelis R, Rzeznik U (1992) Distribution, accumulation and depuration of administered lead in adult honeybees. Sci Total Environ 113(3):269–279. https://doi.org/10.1016/0048-9697(92)90005-d

Raj D, Maiti SK (2019) Sources, toxicity, and remediation of mercury: an essence review. Environ Monit Assess 191(9):1–22. https://doi.org/10.1007/s10661-019-7743-2

Ristorini M, Astolfi ML, Frezzini MA, Canepari S, Massimi L (2020) Evaluation of the efficiency of Arundo donax L leaves as biomonitors for atmospheric element concentrations in an urban and industrial area of Central Italy. Atmosphere 11:226. https://doi.org/10.3390/atmos11030226

Rizwan M, Ali S, ur Rehman, M. Z., Javed, M. R., & Bashir, A. (2018) Lead toxicity in cereals and its management strategies: a critical review. Water Air Soil Pollut 229(6):1–16. https://doi.org/10.1007/s11270-018-3865-3

Sadowska M, Gogolewska H, Pawelec N, Sentkowska A, Krasnodębska-Ostręga B (2019) Comparison of the contents of selected elements and pesticides in honey bees with regard to their habitat. Environ Sci Pollut Res 26(1):371–380. https://doi.org/10.1007/s11356-018-3612-8

Sarwar N, Bibi S, Ahmad M, Ok YS (2014) Effectiveness of zinc application to minimise cadmium toxicity and accumulation in wheat (Triticum aestivum L). Environmental Earth Sciences 71(4):1663–1672. https://doi.org/10.1007/s12665-013-2570-1

Satarug S, Haswell-Elkins MR, Moore MR (2000) Safe levels of cadmium intake to prevent renal toxicity in human subjects. Br J Nutr 84(6):791–802. https://doi.org/10.1017/s0007114500002403

Satta A, Verdinelli M, Ruiu L, Buffa F, Salis S, Sassu A, Floris I (2012) Combination of beehive matrices analysis and ant biodiversity to study heavy metal pollution impact in a post-mining area (Sardinia, Italy). Environ Sci Pollut Res 19(9):3977–3988. https://doi.org/10.1007/s11356-012-0921-1

Saunier JB, Losfeld G, Freydier R, Grison C (2013) Trace elements biomonitoring in a historical mining district (les Malines, France). Chemosphere 93(9):2016–2023. https://doi.org/10.1016/j.chemosphere.2013.07.024

Shahid M, Khalid S, Bibi I, Bundschuh J, Khan Niazi N, Dumat C (2020) A critical review of mercury speciation, bioavailability, toxicity and detoxification in soil-plant environment: ecotoxicology and health risk assessment. Sci Total Environ 711:134749. https://doi.org/10.1016/j.scitotenv.2019.134749

Simone C, Barile S, Grandinetti R (2021) The emergence of new market spaces: brokerage and firm cognitive endowment. J Bus Res 134:457–466

Skorbiłowicz M, Skorbiłowicz E, Cieśluk I (2018) Bees as bioindicators of environmental pollution with metals in an urban area. J. Ecol. Eng. 19(3):229–234. https://doi.org/10.12911/22998993/85738

Smith KE, Weis D, Amini M, Shiel AE, Lai VWM, Gordon K (2019) Honey as a biomonitor for a changing world. Nature Sustainability 2(3):223–232. https://doi.org/10.1038/s41893-019-0243-0

Smith KE, Weis D, Scott SR, Berg CJ, Segal Y, Claeys P (2021) Regional and global perspectives of honey as a record of lead in the environment. Environ Res 195:110800. https://doi.org/10.1016/j.envres.2021.110800

Soares S, Amaral JS, Oliveira MBPP, Mafra I (2017) A comprehensive review on the main honey authentication issues: production and origin. Comprehensive Reviews in Food Science and Food Safety 16(5):1072–1100. https://doi.org/10.1111/1541-4337.12278

Solayman M, Islam MA, Paul S, Ali Y, Khalil MI, Alam N, Gan SH (2016) Physicochemical properties, minerals, trace elements, and heavy metals in honey of different origins: a comprehensive review. Comprehensive Reviews in Food Science and Food Safety 15(1):219–233. https://doi.org/10.1111/1541-4337.12182

Spirić D, Ćirić J, Đorđević V, Nikolić D, Janković S, Nikolić A, Petrović Z, Katanić N, Teodorović V (2019) Toxic and essential element concentrations in different honey types. Int J Environ Anal Chem 99(5):474–485. https://doi.org/10.1080/03067319.2019.1593972

Stöcker, G. (1980). Zu einigen theoretischen und methodischen Aspekten der Bioindikation. SCHUBERT, R. und JSchulz (1980): Methodische und theoretische Grundlagen der Bioindikation (10–21) Martin-Luther-Universität Halle-Wittenberg, Wiss. Beiträge, 24.

Tsiapara AV, Jaakkola M, Chinou I, Graikou K, Tolonen T, Virtanen V, Moutsatsou P (2009) Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and endometrial cancer (Ishikawa) cells: profile analysis of extracts. Food Chem 116(3):702–708. https://doi.org/10.1016/j.foodchem.2009.03.024

US EPA. (2000). Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum Technical Panel. United States Environmental Protection Agency Office of EPA/630/R-00/002.

US EPA. (2020). Human health risk assessment: risk-based concentration table. Regional Screening Levels (RSLs) summary table (TR=1E-06, HQ=1) November 2020. Retrieved from https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables

Usman ARA, Lee SS, Awad YM, Lim KJ, Yang JE, Ok YS (2012) Soil pollution assessment and identification of hyperaccumulating plants in chromated copper arsenate (CCA) contaminated sites. Korea Chemosphere 87(8):872–878. https://doi.org/10.1016/j.chemosphere.2012.01.028

Vitali M, Antonucci A, Owczarek M, Guidotti M, Astolfi ML, Manigrasso M, Avino P, Bhattacharya B, Protano C (2019) Air quality assessment in different environmental scenarios by the determination of heavy metals, PAHs, PCDDs, PCDFs, PCBs and PBDEs in native lichen Xanthoria Parietina. Environ Pollut 254:113013. https://doi.org/10.1016/j.envpol.2019.113013

Voica C, Iordache AM, Ionete RE (2020) Multielemental characterisation of honey using inductively coupled plasma mass spectrometry fused with chemometrics. J Mass Spectrom 55(7):e4512. https://doi.org/10.1002/jms.4512

Wheeler B (2013) SuppDists: supplementary distributions. R Package Version 1(1–9):1

WHO (2003) Uranium in drinking-water. WHO, Background document for preparation of WHO Guidelines for drinking-water quality

WHO (2009) Beryllium in drinking water. WHO, Background document for preparation of WHO Guidelines for drinking-water quality

WHO. (1996). Trace elements in human nutrition and health (A report of a re-evaluation of the role of trace elements in human health and nutrition). WHO.

Wolterbeek HT, Garty J, Reis MA, Freitas MC (2003). Biomonitors in use: lichens and metal air pollution. In Trace metals and other contaminants in the environment (Vol. 6, pp. 377–419). Elsevier.

Zand N, Christides T, Loughrill E (2015) Dietary intake of minerals. In: De la Guardia M, Garrigues S (eds) Handbook of mineral elements in food. John Wiley & Sons Ltd, pp 23–39

Zhelyazkova I (2012) Honeybees–bioindicators for environmental quality. Bulgarian J Agr Sci 18(3):435–442