Performance of bees and beehive products as indicators of elemental tracers of atmospheric pollution in sites of the Rome province (Italy)

Marcelo Enrique Conti[a,*], Maria Luisa Astolfi[b], Giustino Mele[a], Martina Ristorini[c], Giulia Vitiello[b], Lorenzo Massimi[b], Silvia Canepari[d], Maria Grazia Finoia[e]

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

[b]Department of Chemistry, Sapienza University of Rome, P. le Aldo Moro, 5, Rome 00185, Italy.

[c]Department of Bioscience and Territory, University of Molise, Pesche (IS), 86090, Italy.

[d]Department of Environmental Biology, Sapienza University of Rome, P. le Aldo Moro, 5, Rome 00185, Italy.

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

*corresponding author

Keywords:

Biological monitoring; atmospheric elements; bees; beehive products; Johnson's method; environmental performance

Abstract

In this survey, we studied the levels of relevant atmospheric elements well known as tracers of vehicular traffic (i.e., Cu, Sb, Sn, Fe, Mn); biomass burning (i.e., K, Rb, Cs, Li, Tl); and soil resuspension (Si, Al, Ca, Ti) in bees and beehive products (honey, wax, pollen, propolis) in five selected sites in the Rome province (Italy). To attentively support the sustainable management of the involved ecosystems, we have on purpose enhanced the information variety endowment (fourteen elements, up to 454 samples, five sites, about thirteen thousand analytical determinations) by six sampling campaigns conducted in a one-year survey (2018-2019). For the first time, the control charts of the considered elements were built employing Johnson's probabilistic method in the Rome province area. Thus, we have measured the metal concentration overlap ranges in the selected biomonitor/indicators (as well as medians and distribution) and the overlap bioaccumulation index (OBI) with respect to the lowest (OBI-L_{ower}) and the highest (OBI-U_{pper}) extreme values of the overlap elements' concentration ranges. The advantage of the OBI is that we can build the control charts by considering the extremes of the bioaccumulation curves of the elements in the matrices simultaneously, thus creating a ranking among the biomonitor/indicators.This study strongly confirms the selected biomonitor/indicators' ability (excluding honey) to reflect the atmospheric deposition of environmental tracers of traffic, biomass burning, and soil in the area of Rome province. Bees and wax resulted in being very strong accumulators of environmental tracers (i.e., Cu, Sn, Mn for traffic; K, Rb, Cs, and Li for biomass burning; and Al, a soil tracer) showing high OBI-U values. For instance, bees have high bioaccumulation surplus with OBI-U values of 68.6 and 92.7 for Cu and Mn, respectively. This confirms their ability as excellent biomonitors when assessing different cases of environmental contamination becomes necessary. To a lesser extent, pollen and propolis showed for several tracers' high levels for both OBI-U and OBI-L values. Honey often showed a univocal bioaccumulation pattern with high OBI-L values (i.e., 53.7; 154.4; and 112.0 for Cu, Fe, and Mn, respectively), indicating the low transfer capability of contaminants from the environment to the final food product, and confirming its good quality. This further confirms that honey is not appropriate as an environmental indicator. Eventually, the OBI-L index can be applied as an early warning signal when the contamination process is in its initial stages. The OBI index boosts the observer's information variety about the performance of bees, wax, pollen, and propolis as element biomonitors in atmospheric ecosystems.

1. Introduction

The use of bioindication techniques for assessing environmental contaminants has notably increased during the last decades (Crane, 1975; Conti, 2008; Lambert et al., 2012; Losfeld et al., 2014; Zhou et al., 2018; AL-Alam et al., 2019; Vitali et al., 2019; Ristorini et al., 2020). Bioindicators are organisms that can be used for the identification and qualitative determination of human-generated environmental factors (Tonneijk and Posthumus, 1987), while biomonitors are organisms mainly used for the quantitative determination of contaminants in the environment and can be classified as being sensitive or accumulative (Garty, 1993; Conti and Cecchetti, 2001; Wolterbeek et al., 2003). The validation and the selection of an appropriate organism or biological matrixes as biomonitors/indicators denotes a critical phase in the biomonitoring surveys (Bargańska et al., 2016). Concerning atmospheric pollution, the honeybee (*Apis mellifera*) and beehive products (wax, pollen, propolis) have been the main topic of numerous studies and can be considered as excellent biomonitor/indicators (Stöcker, 1980; Conti, 2002). In fact, honeybees are perpetually exposed to contaminants existing in the area surrounding the apiary for the period of their foraging activity (i.e., from spring to fall) (Conti and Botrè, 2001).

The area of foraging activity connected with an apiary typically ranges over a surface of roughly 7 km^2. This aspect is relevant and constitutes the basis with which honeybees and beehive products have been proposed as suitable indicators of chemical pollution (Wallwork-Barber, 1982; Crane, 1984; Bromenshenk et al., 1985; Pinzauti et al., 1991; Raes et al., 1992; Leita et al., 1996; Pohl, 2009; Pohl et al. 2012; Bargańska et al., 2016). Thus, a network of apiaries located in the vicinity of polluted/unpolluted areas can supply plenty of data for the constant monitoring of atmospheric element emissions from different sources over time (Leita et al., 1996; Conti and Botrè, 2001). On the other hand, the presence of elements in honey is essential in terms of both its safety and quality (Grembecka and Szefer, 2013; Devi et al., 2018; Voica et al., 2020; Conti et al., 2022).

Understanding the complexity of environmental and food production systems is crucial in biomonitoring studies. According to Ashby (1958), the comprehension of a complex system depends on the information variety (requisite variety) held by the observer (Conti et al., 2019a,b). Variety and variability are two central dimensions of the complexity of ecosystems (Conti et al., 2020). Thus, in this study, we have on purpose enhanced the information variety endowment (fourteen metals, up to 454 samples, five sites, about thirteen thousand analytical determinations), aiming to have more consistent results about elements content in bees and beehive products in order to attentively supporting the sustainable management of the involved ecosystems (Conti et al., 2020). This is explained because several studies on bees and their products are based on a low quantity of samples and, sometimes, a limited sampling period.

The first aim of the work was to determine the levels of fourteen relevant elements that have been indicated by several studies (Canepari et al., 2013; Kam et al., 2013; Pant and Harrison, 2013**;** Namgung et al., 2013; Karbowska, 2016**;** Frasca et al., 2018; Manigrasso et al., 2019; Massimi et al., 2020a) as tracers of vehicular traffic (i.e., copper (Cu), antimony (Sb), tin (Sn), iron (Fe), manganese (Mn); biomass burning (i.e., potassium (K), rubidium (Rb), cesium (Cs), lithium (Li), thallium (Tl); and soil resuspension (silicium (Si), aluminium (Al), calcium (Ca), titanium (Ti) in bees and beehive products (honey, wax, pollen, propolis) in one year survey in four selected sites in the Rome province (Italy) and one control site (outside Rome). These elements are usually contained in particulate matter (PM) released by these different sources and over the years have been effectively used to trace the impact of their emissions in different study areas (Canepari et al., 2008; Querol et al., 2012; Massimi et al., 2020b). Copper, Sb, Sn, Fe, and Mn are elements generally present in vehicles brakes and released by mechanical abrasion and resuspension of vehicle components (brake disks and pads lining, tires), thus tracing non-exhaust vehicular traffic emissions (Weckwerth, 2001; Marconi et al., 2011; Abbasi et al., 2012). On the other hand, K, Rb, Cs, Li, and Tl are in particles usually released from domestic heating by wood (Szidat et al., 2007) and pellet combustion (Puxbaum et al., 2007), wildfires (Van Drooge et al., 2012) and the burning of agricultural waste in rural areas (Lee et al., 2008). The emissions by biomass burning are one of the largest sources of fine particles in the troposphere (Massimi et al., 2020a). Finally, Si, Al, Ca and Ti, are crustal elements and have been effectively used to trace soil dust emissions resuspended from dry surfaces by high-intensity winds and/or by vehicular traffic (Pant and Harrison, 2013; Massimi et al., 2020b).

The second aim of this work is to study the probabilistic distributions of atmospheric elements' concentrations in the selected biomonitor/indicators aiming to gain consistent information on their bioaccumulation patterns (see for details Conti and Finoia, 2010). For this purpose, we have built the control charts for the element's bioaccumulation in the five selected indicators (i.e., bees, honey, pollen, wax, propolis) by using probabilistic Johnson's method (Johnson, 1949). Through the normalization of any continuous probability distribution, this approach consents simply to define metal concentration confidence intervals at 95% ranges of variability (Johnson, 1949; Miller and Miller, 2005). The novelty of the work lies in its dual objective of testing bees and hive products as biomonitor/indicators of trace elements derived from atmospheric deposition from different sources (i.e. traffic, biomass burning, soil).

The third aim was to determine the range of overlaps of element concentrations and the overlap bioaccumulation index (OBI) with respect to the upper (OBI-U_{pper}) and lower (OBI-L_{ower}) bound of the overlap range (Conti et al., 2015; 2019a). The OBI index defines a ranking that determines which, among the various matrices/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) and on the right (OBI-U) (Conti et al., 2022).

In sum**:**

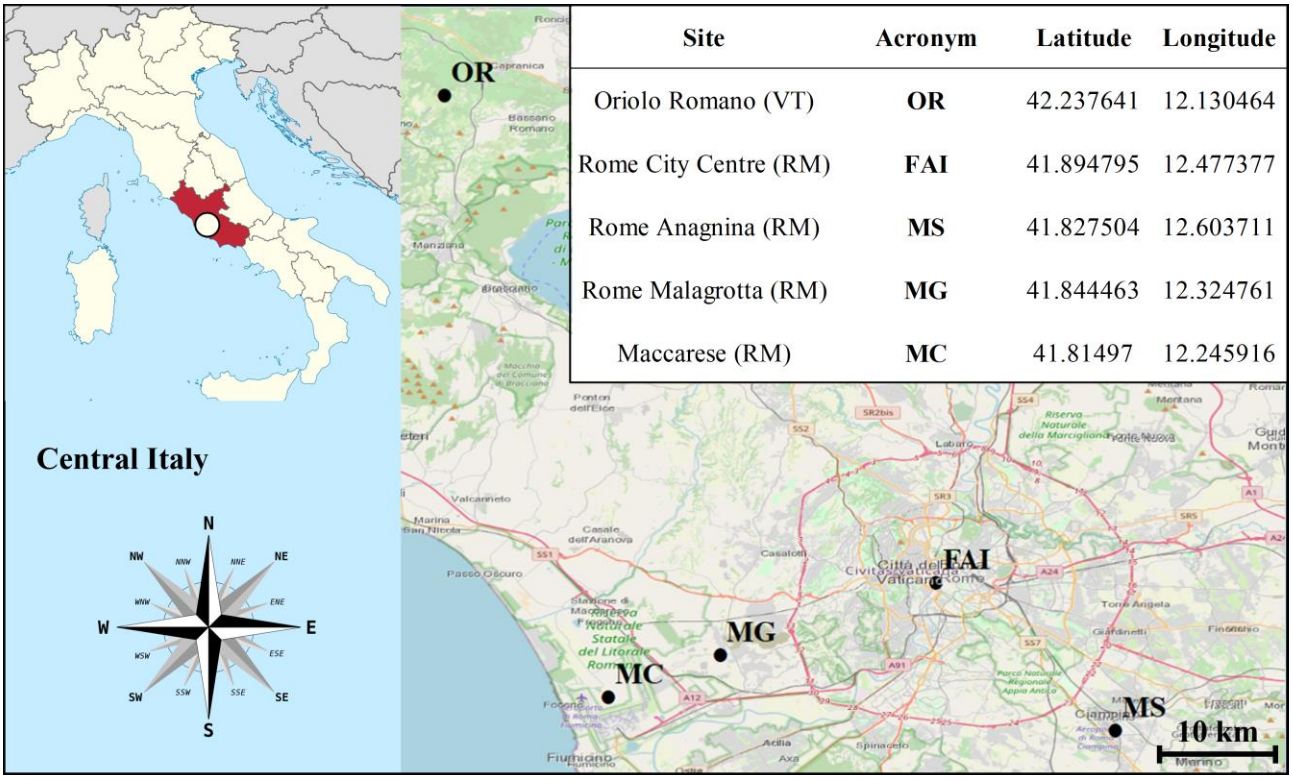
1. We have measured the baseline levels of elemental tracers of traffic, biomass burning and soil deposition, on which we have built the control charts (Johnson's method).
3 We applied the OBI for ranking the selected indicators according to their median elements' content. The advantage of the OBI is that it considers groups of indicators together. This is more connected with a holistic approach instead of the classical more deterministic studies.

Therefore, the use of OBI as an integrated tool in environmental management consents to identify the specific biomonitor/indicators needed for the study of a specific condition of contamination that can arise from natural or anthropogenic activities. It can enhance the understanding of the ecosystem's complexity and constitute a basis for policymakers' decision process.

2. Materials and method

2.1. Study area

Our study was carried out at five selected strategic sites chosen for their different anthropogenic impact and because they are exposed to different and specific emission sources of atmospheric particulate matter. All sites were located within an extensive metropolitan area of Rome in central Italy with the exception of the Oriolo Romano site (OR, Viterbo province, Figure 1), a green area at 60 km from the center of Rome but heavily affected by biomass burning contributions. One site was in the center of Rome, i.e., on the roof of the Apicultural Italian Federation (FAI) and, for this reason, it was considered a site mainly characterized by contributions due to vehicular traffic and biomass burning (especially during the winter period). The other three sites were in the Rome province and all characterized by contributions due to soil resuspension and/or vehicular traffic: Malagrotta (MG)situated closely to the landfill, Maccarese (MC) close to Fiumicino airport and Anagnina (MS) on a busy road. (see Figure 1 for sites' description).



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

Figure 1. Map of the sampling area. OR—green area; FAI—urbanized (highly populated area); MG—near a landfill; MC—close to Fiumicino airport and MS—on a busy road.

We have placed two (independent) beehives at each site, aiming to enhance the requisite variety (n = ten beehives). Six sampling campaigns at the same time (i.e., two months each) and in the same geographically referenced sites from September 2018 to September 2019 have been conducted. Trained beekeepers monitored the beehives and collected all the samples without using metallic equipment. All the selected matrices were collected separately six times throughout the study for each hive and apiary, using 50-mL conical sterile polypropylene centrifuge tubes (Falcon®, Corning Optical Communications S.r.l. Turin, Italy). At least 20 bees were collected directly from each hive as previously reported (Astolfi et al., 2021). After transporting the samples to the laboratory,the wax samples were carefully washed with deionized water to remove any residues. Instead the bees were not washed in order to consider the content of the elements both on and within the bodies of the bees. The bees in their entirety can be considered passive samplers of atmospheric particulate matter and the dust deposited on their bodies can affect the respective products of the hive. The bee body is covered with hairs, which make it particularly suitable for capturing the particulate materials they encounter during their interactions with the environment

(Girotti et al., 2020). However, it is necessary to emphasize 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. Bees and wax samples were lyophilized for 48h using a Heto Power Dry LL1500 freeze dryer from Thermo Electron Corporation (Waltham, Massachusetts, USA). All samples were stored in disposable graduated 10-mL polypropylene tubes (Artiglass, Due Carrare, PD, Italy) at -18 °C until analysis.

2.2 Chemicals and materials

Super-pure HNO_3 (67%) and H_2O_2 (30%) were of analytical grade and supplied by Carlo Erba Reagents (Milan, Italy) and Merck KgaA (Darmstadt, Germany), respectively. Deionized water (electrical resistivity 18.3 $M\Omega\ cm^{-1}$) was obtained using the Arioso Power I RO-UP Scholar UV water purification system (Human Corporation, Seoul, Korea). Multi-element stock solution (VWR International S.r.l., Milan, Italy) was used to prepare the standard calibration solutions. The polypropylene graduated tubes were obtained from Artiglass S.R.L. (Due Carrare, PD, Italy).

2.3 Sample treatment and analysis

The fourteen selected elements (i.e. Cu, Sb, Sn, Fe, Mn, K, Rb, Cs, Li, Tl, Si, Al, Ca, Ti) were analyzed by quadrupole ICP-MS (820-MS, Bruker, Bremen, Germany) equipped with a collision reaction interface The instrumental conditions and digestion methods have been reported elsewhere (Astolfi et al. 2020; Conti et al., 2018). Details regarding sample treatment and quality control are given in the Supplementary information (S.1). Briefly, samples of about 0.2 g of each matrix were weighed into a 10-mL polypropylene tube, to which 1 mL of 67% HNO_3 and 0.5 mL of 30% H_2O_2 were added, and then mineralized in a water bath (WB12, Argo Lab, Modena, Italy) at 95 °C for 30 min. After digestion, all samples were left to cool and diluted to 20 mL with deionized water.

2.4 Statistical analysis

Johnson's method (1949) was applied to trace element concentrations in the five biomonitor/indicators to generate frequency curve systems by translation. This method allows, through a translation technique, to classify the distribution of a generic variable in one of four defined classes of probability normally distributed. The normality in the distribution of a variable is a fundamental requirement for applying robust statistical inference techniques and defining the limits of the confidence intervals calculated on the elements under study. This aspect is relevant because it allows to build the control charts associated with each element, define its range of variation, and highlight the upper tail of the probability distribution. These procedures have been reported elsewhere (Conti and Finoia, 2010; Conti et al., 2015; 2019a; 2022).

The control charts were built to determine the overlap range among the five biomonitor/indicators and for the OBI definition. Then, the overlap range for the ith and jth matrixes is defined according to the following extreme values:

$Imin=max(Qi,2.5, Qj,2.5)$ with $i=1, 2,..,k$ and $i \neq j$

$Imax=min(Qi,97.5, Qj,97.5)$ with $i=1, 2,..,k$ and $i \neq j$

We have considered abnormal values and outliers. Subsequently, 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:

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

OBI-U_i is usually ≥ 1 and becomes 1 when $Q_{i,97.5} = \mathrm{I}_{\mathrm{max}}$

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

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

For comparison between medians, the median test and post hoc comparisons have been applied.

3. Results and discussion

Both macro- and microelements contents in bees varied in wide ranges and depended on a number of factors (Girotti et al., 2020), such as types of soils, and physiological and health statuses of bee workers (Bogdanov, 2006), periods of the year (Roman, 2010) and emission sources at the sampling site (Astolfi et al., 2021; Zaric et al., 2022; Zhelyazkova 2012). The results shown in Table 1 confirmed the role of bees as biofilters of elements and their protective function regarding honey contamination. Despite this, honey has been frequently used as an indicator for environmental cleanliness evaluation (Madejczyk and Baralkiewicz 2008; Kacaniova et al. 2009; Dżugan et al. 2017, 2018). Also, other beehive products can be used as pollution impact assessment tools (Girotti et al., 2020; Conti et al., 2022). The median levels of K, Ca and Si in bees and beehive products were the highest of all the elements analyzed (Table 1). Thus, the major contribution to the elemental concentrations of the matrices considered is made by soil tracers. While, the lowest median levels were found for Sb, Sn (non-exhaust traffic tracers), Li and Tl (biomass burning tracers).

Comparing our data with literature, the elements' range concentration in bees we obtained (see Table SA, Supplementary Section, which reports data from 32 papers published in the last four years) were comparable with those reported by Grainger et al. (2020) for New Zealand sites and Zaric et al., (2022) for Serbia and Austria sites i.e., 11-31.3 mg/Kg for Cu, 48-265 mg/Kg for Fe, 537-1532 mg/Kg for Ca (see Table SA for more information). However, the comparison is not straightforward as not all authors indicate whether the reported data on tracers are on a fresh or dry weight basis, which can lead to some bias. Furthermore, another factor is the comparison with sites that have different characteristics (a column with short description of the sites is reported in Table SA).

Generally speaking, bees have the highest element's concentrations with respect to the other indicators we analyzed showing their very good aptness as bioindicators of atmospheric pollution.

On the contrary, the levels of the elements analyzed in this work were very low or <LOD for honey samples. This also agrees with literature data (21 papers) reported in Table SA in which honey has very low element concentrations measured in different geographical areas. This again confirms the relevant role of bees as a biofilter of elements, making honey unfit for environmental quality assessments. The levels of the detected elements in wax, pollen and propolis resulted to be generally comparable or at a lowest levels than those of the literature (Table SA).

The levels of elements in the whole bees and the contamination of beehive products (excluding honey) reflect the pollutant content of their environment; thus a honeybees–hives system can be employed to passively sample and concentrate pollutants in order to obtain data on environmental quality (Conti et al., 2022).

Table 1. Descriptive statistics of the elements' concentrations (i.e. tracers of atmospheric pollution) in the five selected biomonitor/indicators for the six sampling campaigns in one-year survey (2018-2019) in Rome province (µg/g).

	Non-exhaust traffic tracers					**Biomass burning tracers**					**Soil tracers**			
Element	**Cu**	**Sb**	**Sn**	**Fe**	**Mn**	**K**	**Rb**	**Cs**	**Li**	**Tl**	**Si**	**Al**	**Ca**	**Ti**

LOD	0.3	0.03	0.003	0.9	0.1	9	0.01	0.001	0.01	0.001	10	1	50	0.01
Bees (n=122)														
mean	23.7	0.08	0.092	135	63.7	8340	36.9	0.245	0.08	0.033	156	30	834	2.43
median	**23.2**	**0.08**	**0.046**	**121**	**58.8**	**8240**	**27.8**	**0.043**	**0.05**	**0.003**	**148**	**25**	**779**	**2.05**
min	13.0	<LOD	0.004	54	4.0	5360	6.4	0.006	<LOD	<LOD	66	<LOD	418	1.16
max	36.8	0.21	0.623	258	168	13600	160	1.74	1.07	0.872	369	98	2040	6.76
SD	4.7	0.03	0.115	46	34.4	1400	28.6	0.426	0.11	0.106	45	20	298	1.12
Honey (n=92)														
mean	0.6	<LOD	0.008	2.2	0.5	1030	4.88	0.058	<LOD	0.012	43	<LOD	171	0.03
median	**0.5**	<LOD	**0.004**	**1.3**	**0.3**	**848**	**3.29**	**0.005**	<LOD	**0.001**	**44**	<LOD	**142**	**0.03**
min	<LOD	<LOD	<LOD	<LOD	<LOD	162	0.24	<LOD	<LOD	<LOD	14	<LOD	<LOD	<LOD
max	1.5	0.06	0.098	16.7	2.5	3270	26.1	0.526	0.05	0.111	61	5	679	0.15
SD	0.3	0.01	0.002	2.8	0.4	704	5.27	0.113	0.01	0.020	8	<LOD	114	0.023
Pollen (n=45)														
mean	1.0	0.06	0.042	86	3.2	2030	6.06	0.279	0.06	0.035	129	4	1310	2.75
median	**0.9**	**0.05**	**0.029**	**85**	**2.8**	**2070**	**3.95**	**0.061**	**0.06**	**0.004**	**133**	**4**	**1270**	**2.34**
min	0.5	<LOD	0.006	20	1.6	1200	0.59	0.007	<LOD	<LOD	48	<LOD	664	0.64
max	2.3	0.13	0.159	197	8.1	3140	19.5	1.32	0.10	0.429	221	9	2000	8.92
SD	0.4	0.02	0.032	40	1.5	484	5.35	0.402	0.03	0.090	32	2	218	1.56
Wax (n=175)														
mean	2.2	0.08	0.343	29	3.8	1400	6.62	0.136	0.08	0.028	37	5	559	0.69
median	**0.5**	**0.07**	**0.111**	**24**	**0.5**	**586**	**1.13**	**0.021**	**0.05**	**0.003**	**31**	<LOD	**363**	**0.49**
min	<LOD	<LOD	0.016	2	<LOD	19	0.05	0.001	<LOD	<LOD	4	<LOD	88	0.03
max	37.0	0.37	6.92	129	61.0	9480	179	2.36	1.07	0.474	128	58	1930	2.47
SD	4.6	0.03	0.023	23	10.7	1960	12.8	0.339	0.13	0.069	21	9	414	0.59
Propolis (n=20)														
mean	3.1	0.12	0.970	243	7.3	1250	7.68	0.156	0.12	0.009	354	4	785	11.6
median	**2.8**	**0.09**	**0.845**	**242**	**7.0**	**1100**	**7.57**	**0.142**	**0.11**	**0.008**	**349**	<LOD	**685**	**8.50**
min	0.9	0.04	0.104	137	2.7	539	2.59	0.063	0.07	0.004	321	2	398	5.08
max	7.9	0.25	3.69	528	15.7	3910	14.9	0.321	0.22	0.019	1080	12	1630	35.4
SD	1.7	0.04	0.026	25	3.0	822	3.49	0.067	0.04	0.004	31	3	327	7.73

3.1. Control charts, the overlap elements' concentration ranges, and the OBI index

Figures 2 for Cu, and Supplementary figures S1-S4, for Sb, Sn, Fe, and Mn, respectively, show the control charts for tracers of traffic for the five selected biomonitor/indicators with their obtained overlap element concentrations. (For reasons of space most of the figures and tables are shown in the supplementary file. This does not mean that the tracers in the respective graphs are any less important than those in the main text).

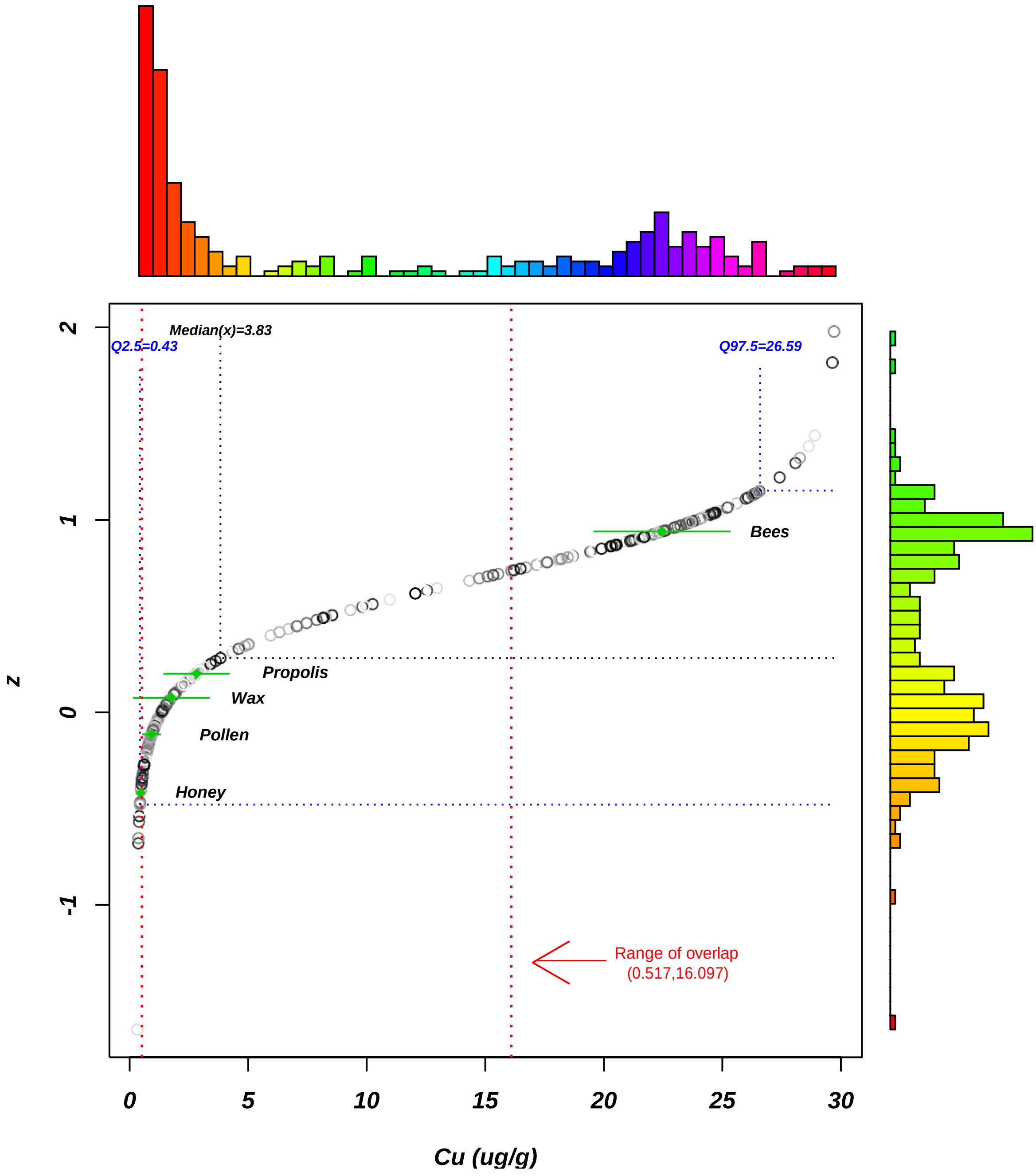


Figure 2. Control chart for Cu built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, i.e. green line), the lower and upper bounds of baseline range (Q2.5 and Q97.5), and the range of overlap (i.e., the common elements concentration range for the five biomonitor/indicators, see red arrow). The histograms of values are shown outside of the plot.

It is well documented that Cu and Sb can arise from the brakes' mechanical abrasion (Canepari et al., 2008; Marconi et al., 2011; Manigrasso et al., 2019). Analogously, Figures 3 for K, and S5-S7 for Rb, Cs, Li, respectively, show the control charts and the overlap metal concentrations for biomass burning markers; then Figures 4 for Si, and S8-S9 for Al and Ca show the soil-tracers' control charts.

The obtained OBI indexes (see section 2.4 for definition) for the studied tracers in the five biomonitor/indicators are reported in Tables 2 for Cu, and Tables S1-S4 (Sb, Sn, Fe and Mn respectively) for traffic tracers; Tables 3 for K, and S5-S7 (Rb, Cs and Li, respectively) for biomass burning tracers, and Table 4 for Si and S8-S10 (Al, Ca and Ti respectively) for soil tracers.

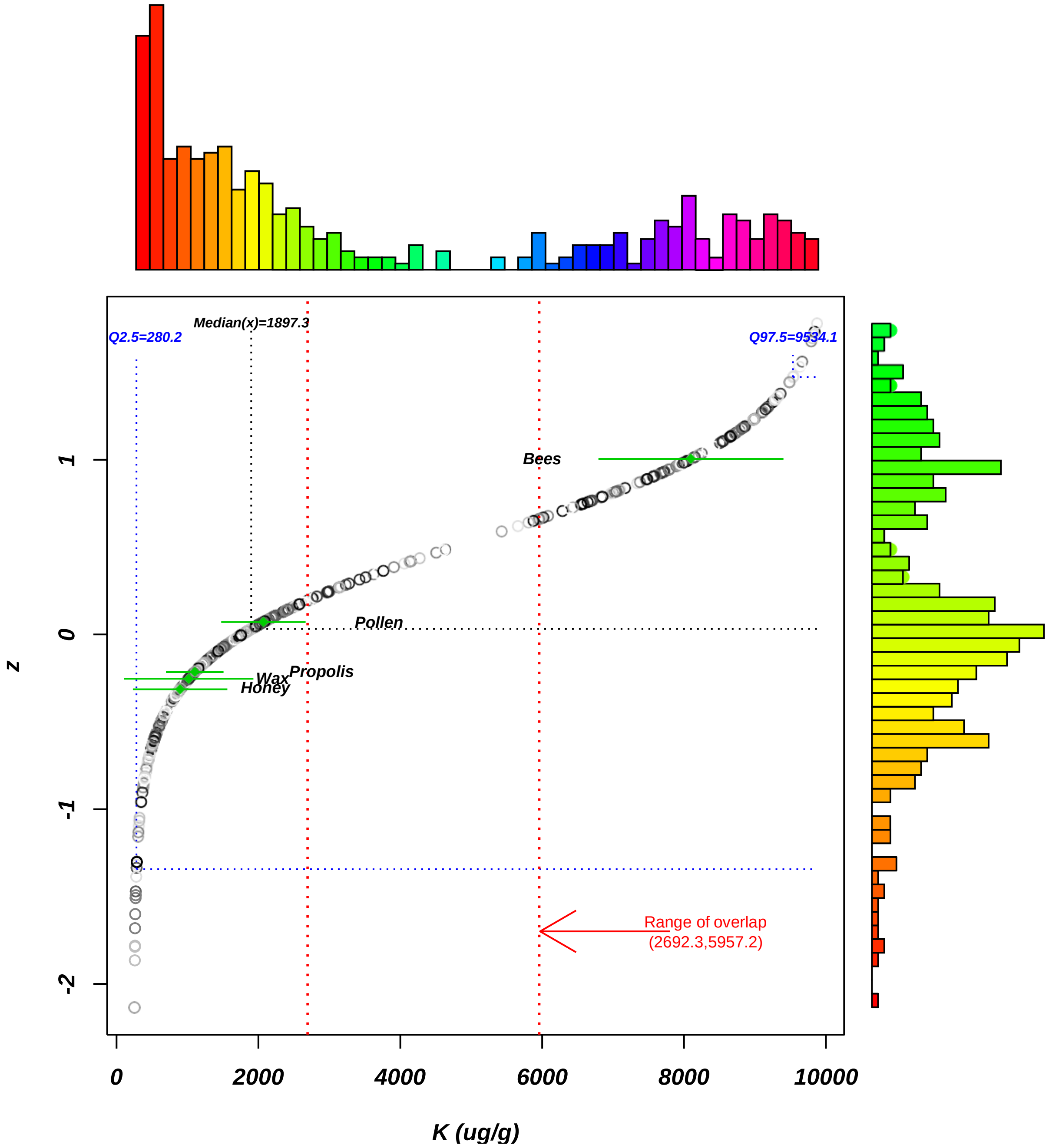


Figure 3. Control chart for K built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, i.e. green line), the lower and upper bounds of baseline range (Q2.5 and Q97.5), and the range of overlap (i.e., the common elements concentration range for the five biomonitor/indicators, see red arrow). The histograms of values are shown outside of the plot.

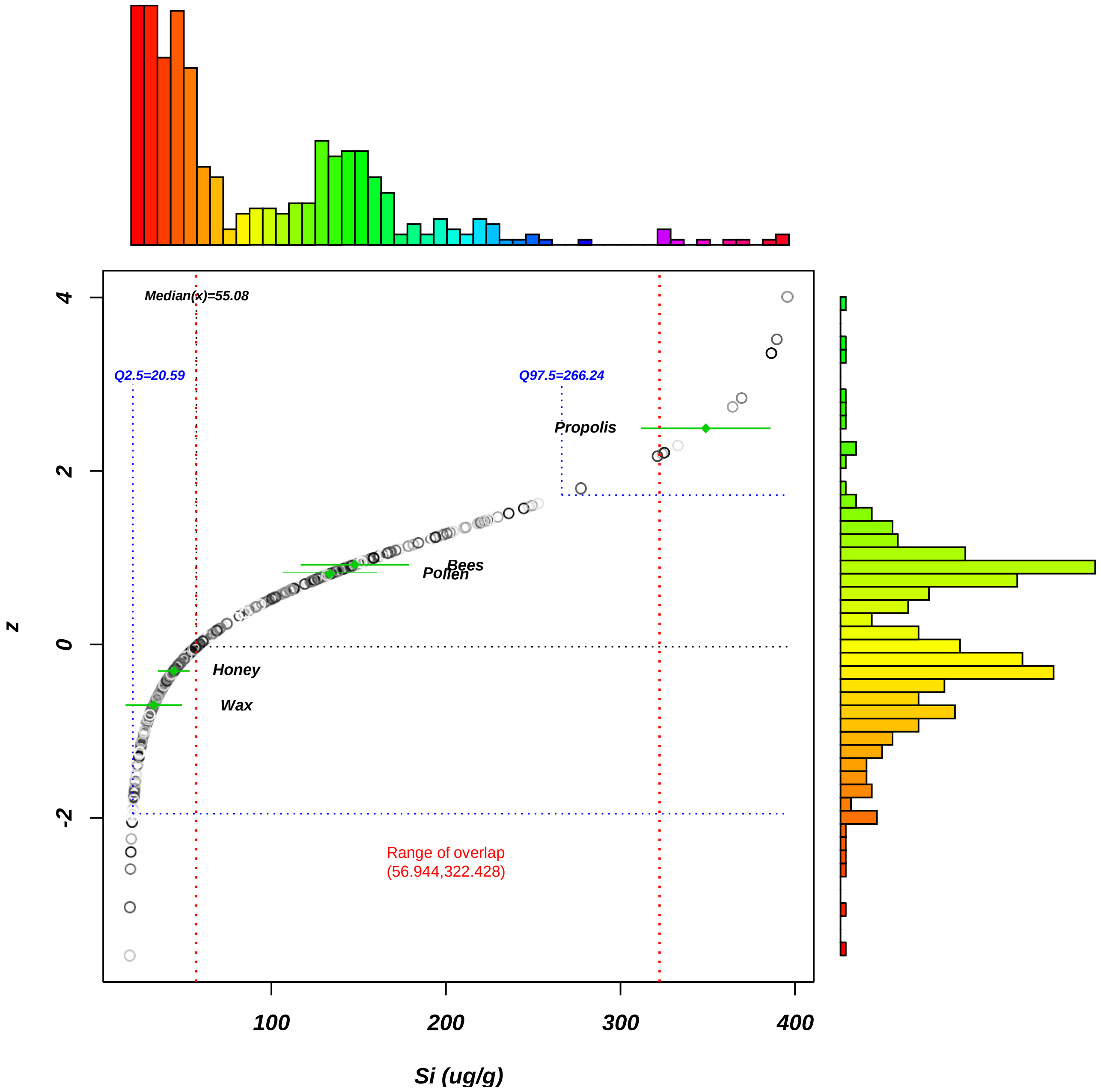


Figure 4. Control chart for Si built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, i.e. green line), the lower and upper bounds of baseline range (Q2.5 and Q97.5), and the range of overlap (i.e., the common elements concentration range for the five biomonitor/indicators, see red arrow). The histograms of values are shown outside of the plot.

3.2 Traffic tracers and the OBI index in Rome province

The Cu concentrations detected for propolis, wax, pollen, and honey are lower than the overall median (i.e., 3.8 µg/g) while bees bioaccumulate at significantly higher levels (post hoc of median test $p<0.05$), i.e. median 23.2 µg/g (Figure 2 and Table 1) than the other four biomonitor/indicators.

Moreover, the biomonitors showed relatively low range variability for Cu bioaccumulation. The limits of the overlap range were 0.5 and 16.1 µg/g. For instance, according to the OBI-U equation (reported in section 2.4), the OBI-U for Cu in bees was obtained after dividing the $Qi,_{97.5}$ value (i.e., 34.3 µg/g, see Table 2) by the minimum value of the extreme upper values of the overlap range (i.e., 0.5 µg/g for honey).

Thus, OBI-U for Cu (Table 2) show that bees have high bioaccumulation Cu surplus (OBI-U = 68.6), better responding to high Cu concentrations in the environment. It supports the hypothesis of bees as a good biomonitor of the traffic tracer, e.g., Cu, and it is strictly connected with the wide overlap range obtained; bees can be selectively employed as biomonitor in areas with high levels of traffic.

Moreover, the Cu OBI-L (Table 2) was very high for wax and honey (i.e., both 53.7). This indicates that these indicators also respond to very low Cu concentrations present in the environment, suggesting that they can be used as early warning signals of the onset of a contamination process resulting from traffic.

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

Matrice	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	16.1	34.3	1.0	**68.6**
Wax	<0.3	14.1	**53.7**	28.2
Honey	<0.3	0.5	**53.7**	1.0
Pollen	0.5	1.5	32.2	3.0
Propolis	1.4	5.5	11.5	11.0
Range of overlap	0.5-16.1			

Figure S1 and Table S1 show that Sb concentration for wax has a relevant bioaccumulation surplus, that is, OBI-U=5.9, which means it detects about six times higher Sb levels with respect to the upper extreme bioaccumulation overlap range of the five biomonitor/indicators (see the red arrow in Figure S1). Thus, wax shows good aptitude to selectively accumulate Sb, i.e., a traffic tracer. On the other hand, the honey Sb median was the lowest and significantly different (post hoc of median test $p<0.05$) than the other four biomonitor/indicators. The obtained OBI shows that wax, honey, and pollen are quite sensitive to low Sb concentrations (OBI-L = 2.6 each, Table S1), which means they detect more than twofold lower Sb levels with respect to the minimum overlap range.

The Sn overlap range was too narrow (i.e., 0.005 — 0.008 µg/g) and bees and propolis showed higher median Sn concentrations than the other indicators (see Figure S2 and Table 1). Moreover, bees and pollen have high bioaccumulation Sn surplus (OBI-U = 46.0 and 23.4, respectively, Table S2), confirming bees and pollen as good biomonitors of Sn. Regarding Fe (Figure S3) propolis, bees and pollen showed higher concentrations than the obtained median (i.e., 34.4 µg/g, see Table 1) with a quite high range of variability (m.a.d.). Propolis showed (Table S3) a high Fe bioaccumulation surplus (OBI-U=28.8), demonstrating its aptitude as a good biomonitor of traffic tracers. On the contrary, honey showed very high Fe OBI-L value (154.4) confirming its high very scarce sensitivity to Fe accumulation from the surrounding environment. Considering Mn (Figure S4, Table S4), bees showed significantly (post hoc of median test $p<0.05$) higher median levels (i.e., 58.8 µg/g) than the other indicators, also showing the highest range of variability with respect to the other indicators (see m.a.d., i.e. green line for bees figure S4). The obtained OBI-U is 92.7, depicting bees as excellent biomonitors for Mn as well as pollen, which the OBI-U is 19.2. Another relevant result is the high OBI-L values obtained for honey for some of the traffic tracers (i.e., 53.7, 154.4, and 112.0 for Cu, Fe and Mn, Table 2, S3, S4, respectively). This agrees with the low aptitude of honey to concentrate these elements at high concentrations and then to act as an environmental indicator as reported in previous studies (Conti and Botrè, 2001; Satta et al., 2012; Saunier at al., 2013; Álvarez-Ayuso and Abad-Valle, 2017; Conti et al., 2018).

3.3 Biomass burning tracers and the OBI index in Rome province

Figure 3 depicts that bees showed significantly higher median K concentrations (8240 µg/g, Table 1, post hoc of median test $p<0.05$) than the overall median (i.e., 1897 µg/g). The OBI (Table 3) show that bees have a good bioaccumulation K surplus (OBI-U = 4.1). Similar results have been obtained for the other biomass burning tracers in which bees showed good OBI-U values (i.e., 8.2, 6.5, and 8.4, for Rb, Cs, and Li, Tables S5-S7, respectively), while a good bioaccumulation surplus (OBI-U=9.6 for wax) was obtained for Li (Table S7). On the other hand, wax showed (Tables 3, S5, S6) for K, Rb, Cs high OBI-L values, i.e., 47.3; 115.0; 34.5, respectively. This suggests the possible use of wax as an early signal of the onset of a contamination process derived from biomass burning tracers, while bees could act as good biomonitors of biomass burning tracers in supposedly contaminated sites (see also Figures S5-S7).

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

Matrice	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	5960	11100	1.0	**4.1**

Wax	126	7420	**47.3**	2.8
Honey	174	2690	34.2	1.0
Pollen	1280	2810	4.7	1.0
Propolis	567	3470	10.5	1.3
Range of overlap	2690-5960			

3.4 Soil tracers and the OBI index in Rome province

The median Si concentrations detected for propolis are higher than the overlap range's upper bound (Figure 4). The limits of the wide overlap range obtained were 57 and 322 µg/g. The bioaccumulation indexes (Table 4) show that propolis has a high Si bioaccumulation surplus (OBI-U = 16.5), better responding to high Si concentrations in the environment. Moreover, wax showed a high value of OBI-L, i.e., 26.8 for Si (Table 4).

Figure S8 shows that the median Al concentrations detected for bees are higher than the overall median (5 µg/kg). On the other hand, bees bioaccumulate Al in a wide concentration range showing higher variability with respect to the other indicators (post hoc of median test p<0.05). The obtained OBI for Al (Table S8) shows that bees have high bioaccumulation Al surplus (OBI-U = 42.5). On the other hand, the Al overlap range obtained is too narrow (Figure S8), depicting a very low variability for pollen, propolis, wax, and honey, making their use as Al biomonitors problematic. We obtained a similar behavior for Tl in which we determined very low levels (see Table 1).

Figure S9 shows that the median concentrations of Ca detected in pollen are significantly higher than the overall median (521 µg/g) (post hoc of median test p<0.05) compared with the other biomonitor/indicators. In particular, the Ca OBI-U values show that all biomonitor/indicators (except honey) have a similar Ca bioaccumulation pattern (Table S9, Figure S9). The obtained Ca OBI-U for pollen is 3.9. Table S10 reports Ti's OBI values, which showed a very high Ti surplus for propolis (OBI-U=312.9).

Eventually, honey showed high and very high OBI-L values, i.e., 18.6 and 1116 for Ca and Ti, respectively (Tables S9-S10).

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

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	89	249	3.6	4.4
Wax	12	85	**26.8**	1.5
Honey	30	57	10.9	1.0
Pollen	58	171	5.6	3.0

Propolis	322	940	1.0	**16.5**
Range of overlap	57-322			

From these results, we can draw some relevant findings:

i. Bees showed from very high to good OBI-U values for traffic tracers, i.e., Cu (68.6), Sn (46.0), and Mn (92.7) (Tables 2, S2, and S4 respectively); for biomass burning tracers, i.e., K (4.1), Rb (8.2), Cs (6.5), Li (8.4) (Tables 3, S5-S7, respectively); and for a soil tracer, i.e., Al (42.5) (Table S8). It demonstrates the strong ability of bees to accumulate these elements from beehives' surrounding environment and their great aptitude for monitoring purposes. The obtained results agree with numerous other studies (Herrero-Latorre et al., 2017; AL-Alam et al., 2019). For instance, Giglio et al. (2017) tested the metal accumulation in tissues of bees along an urban-suburban gradient to provide good qualitative and quantitative information to estimate metal contaminants in relation to the distance from an industrial site.

ii. Honey showed very high OBI-L values for traffic tracers, i.e., Cu (53.7), Fe (154.4), and Mn (112.0) (Tables 2, S3, S4). Similar good OBI-L values were obtained for Li, a biomass burning tracer, in honey, bees, and wax (i.e., 9.0) (Table S7). We also obtained from good to very high OBI-L values for honey in soil tracers, i.e., Si (10.9, Table 4), and Ca (18.6), Ti (1116) (Tables S9-S10). These results confirm that honey is highly sensitive to the extremely low variation of the tracers' levels in the environment (i.e., about 2-1000 times concerning the lower bound of the overlap range). This confirms the high aptitude of honey in detecting very low concentrations of elemental tracers of atmospheric pollution. In other words, honey, compared with bees and the other beehive products, does not accumulate the elemental tracers of atmospheric pollution at high levels. In fact, elemental accumulation is evidently present in the other four indicators with high OBI-U as well as OBI-L values. Thus, it should be pinpointed that honey has never shown high OBI-U values for the studied tracers. This further agrees with our previous statements and those reported in other studies, in which honey does not reflect environmental contamination (Conti and Botrè, 2001; Satta et al., 2012; Saunier et al., 2013; Álvarez-Ayuso and Abad-Valle, 2017; Conti et al., 2018; Conti et al., 2022).

iii. Another relevant consideration is that from our results concerning honey, we can infer that the tracers' transfer capacity from the environment to honey is very low confirming its good average quality. Our results match those by Dżugan et al. (2018), which confirmed the influence of anthropogenic activity on the accumulation of elements in bee organisms and highlighted the role of bees as biofilters of heavy metals and their protective function regarding honey contamination.

iv. Very high OBI-L values, in some cases, were instead obtained for wax for traffic markers such as Cu (53.7), Fe (46.3), Mn (28.0) (Tables 2, S3-S4). Likewise, for biomass burning tracers, i.e., K (47.3), Rb (115.0), Cs (34.5) (Tables 3, S5-S6), and for a soil tracer, i.e., Si (26.8) (Table 4).

v. Ca showed similar bioaccumulation patterns for all biomonitor/indicators (excepting honey), i.e., from OBI-U= 3.1 (propolis) to 3.9 (pollen) (Table S9); this aspect relates to the wide overlap range obtained (Figure S9) and the good ability of bees, wax, pollen and propolis to accumulate soil tracers.

vi. Propolis showed high bioaccumulation surplus for Fe (OBI-U = 28.8) and for Ti (OBI-U = 312.9) that are traffic and soil tracers, respectively (Tables S3 and S10). It should point out that the propolis' chemical composition depends on various factors such as geographical area, and botanical sources, and the bee species (Matin et al., 2016). However, due to its chemical composition (mainly amino acids, polyphenols, steroids, and terpenes) and sticky nature of gum, propolis could be show metal contamination and might be used as a bioindicator of atmospheric pollution (Finger et al., 2014).

However, it should be noted that some additional multiple factors can influence results (i.e. regulation/excretion mechanisms on bees, that can influence elements' accumulation in beehive products). These mechanisms are often noticed in the different branches of the phylogenetic tree of the various species. For instance, metallothioneins (MTs) (proteins containing cysteine), are able to link elements. Every element has a threshold, though, beyond which detoxification phenomena can occur (Conti, 2002; 2008). On the other hand, the element composition of beehive products is linked with the mineral composition of the soil, plants, and rocks where the beehives are located and to the sites' anthropic contributions (De Oliveira et al., 2020). Another recent study (Goretti et al., (2020) suggest that enrichment of metals such as Cd, Cu, Mn, and Zn in bees appeared to depend on local conditions, i.e. the use of pesticides and fertilisers, and the resuspension of soils locally contaminated. To cope to this aspect, we have enhanced the information variety endowment to improve data homogeneity.

The OBI index increases the observer's information variety about the performance of bees and beehives' products as metal biomonitors of environmental impact tracers. It improves the information endowment about the potential performances of bees and beehives products as biomonitors (Conti et al., 2019a). The OBI index warns that the choice of bees and beehives products is not independent of the purpose of effectively biomonitoring and managing atmospheric ecosystems. Going deeper, the selection of the bees and beehive products as biomonitors is a key decisional process that in turns encompasses the information management (searching, collecting, shaping, interpreting data, etc.) and that enquires for a problem solving that often is a solution that is not the best possible, but it is at least satisficing. In fact, the OBI index aims to support those decisional processes to have more reliable results about environmental pollution.

Conclusions

This study strongly confirms the selected biomonitors' ability (excluding honey) to reflect the atmospheric deposition of environmental tracers of traffic, biomass burning, and soil in the area of Rome province. To this end, we have built the control charts by means of Johnson's statistics, and the elements' overlap ranges have been drawn, based on thousands of determinations conducted on samples collected in a one-year survey (2018-2019).

From our results, the best performance is given by bees and wax. These results agree with our previous studies (Conti and Botrè, 2001) in which bees and wax showed their good ability to accumulate toxic metals (Pb, Cd, and Cr). Our results confirm bees and wax as very strong accumulators of environmental tracers (i.e., Cu, Sn, Mn for traffic; K, Rb, Cs, and Li for biomass burning; and Al, a soil tracer). On the other hand, it should be emphasized that bees and wax, and to a lesser extent, pollen and propolis, showed for several tracers high levels for both OBI-U and OBI-L values, supporting their ability as good biomonitors when the assessment of different cases of environmental contamination become necessary. In other words, by using the OBI, it is possible to select the appropriate biomonitor connected with a specific type of contamination. Moreover, honey often showed high OBI-L values demonstrating the low transfer capability of contaminants from the environment to the final food product. This also confirms that honey does not reflect environmental contamination. Another relevant finding is that the OBI-L index can be applied as early warning signal when the contamination process is in its initial stages.

These results underpin our data's hypothesis as baseline data that can be considered in management decisions about future environmental protection programs. The OBI index increases the observer's information variety about the performance of bees, wax, pollen, and propolis as element biomonitors in atmospheric ecosystems.

CRediT authorship contribution statement

M.E. Conti, M.G. Finoia — Conceptualization; M.E. Conti, M.L. Astolfi, L. Massimi and S. Canepari — Methodology; M.E. Conti, M.L. Astolfi, G. Mele, M. Ristorini, G. Vitiello, and S. Canepari, — Resources, Sampling; M.L. Astolfi, L. Massimi, — Validation, Chemical analyses; M.E. Conti, M.L. Astolfi and M.G. Finoia— Data Curation, Software, Writing – Original Draft; M.E. Conti, M.L. Astolfi, L. Massimi, S. Canepari — Writing – Review & Editing. M.E. Conti, M.L. Astolfi and S. Canepari — Supervision, M.E. Conti — Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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

Acknowledgments

This work was financed by Sapienza project 2018, University of Rome, prot. RG11816432851FA6, (Principal Investigator Prof. M.E. Conti). The Authors are particularly indebted with Marco Papi, and Massimo Marcolini (President of the Association of Beekeepers of Rome and Province) for their outstanding support in all stages of sampling. We also are greatly obliged with Helga Liselotte Scrahuf for her kindly support during our stays in Oriolo Romano. We also wish to thank Dr. Maria Agostina Frezzini and Dr. Elisabetta Marconi for their excellent support in samples' treatment and classification. Finally, we thank Fabrizio Piacentini and the Italian beekeeping federation (FAI) for their kind support to this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at xxx.

References

Abbasi, S., Olander, L., Larsson, C., Olofsson, U., Jansson, A., Sellgren, U., 2012. A field test study of airborne wear particles from a running regional train. Proceedings of the Institution of Mechanical Engineers, Part F: Journal of Rail and Rapid Transit, 226(1), 95-109.

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, 9391–9408.

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, 671–678.

Ashby, W.R., 1958. Requisite variety and its implications for the control of complex systems. Cybernetica 1 (2), 83–89.

Astolfi, M.L., Conti, M.E., Marconi, E., Massimi, L., Canepari, S., 2020. Effectiveness of Different Sample Treatments for the Elemental Characterization of Bees and Beehive Products. Molecules 25(18):4263.

Astolfi, M.L., Conti, M.E., Ristorini, M., Frezzini, M.A., 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.

Bargańska, Ż., Ślebioda, M., Namieśnik, J., 2016. Honey bees and their products: Bioindicators of environmental contamination. Crit. Rev. Env. Sci. Tec., 46, 235–248.

Bogdanov, S., 2006. Contaminants of bee products. Apidologie, 37(1), 1–18.

Bromenshenk, J. J., Carlson, S. R., Simpson, J. C. and Thomas, J. M., 1985. Pollution monitoring of Puget Sound with honeybees, Science 227, 532–634,

Canepari, S., Perrino, C., Olivieri, F., Astolfi, M.L., 2008. Characterisation of the traffic sources of PM through size-segregated sampling, sequential leaching and ICP analysis. Atmos. Environ. 42(35), 8161-8175.

Canepari, S., Padella, F., Astolfi, M.L., Marconi, E., Perrino, C., 2013. Elemental concentration in atmospheric particulate matter: estimation of nanoparticle contribution. Aerosol Air Qua. Res. 13, 1619–1629.

Coletti, C., Ciotoli, G., Benà, E., Brattich, E., Cinelli, G., Galgaro, A., ... & Sassi, R. (2022). The assessment of local geological factors for the construction of a Geogenic Radon Potential map using regression kriging. A case study from the Euganean Hills volcanic district (Italy). Science of The Total Environment, 808, 152064.

Conti, M. E., & Cecchetti, G. (2001). Biological monitoring: lichens as bioindicators of air pollution assessment—a review. Environmental pollution, 114(3), 471-492.

Conti, M.E., Botrè, F., 2001. Honeybees and their products as potential bioindicators of heavy metals contamination. Environ. Monit. Assess. 69(3), 267-282.

Conti, M.E., 2002. Il Monitoraggio Biologico Della Qualità Ambientale. SEAM, Rome, pp. 1–180.

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

Conti, M.E., Finoia, M.G., 2010. Metals in molluscs and algae: a north–south Tyrrhenian Sea baseline. J. Hazard. Mater. 181 (1–3), 388–392.

Conti, M.E., Mecozzi, M., Finoia, M.G., 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.

Conti, M.E., Canepari, S., Finoia, M.G., Mele, G., Astolfi, M.L., 2018. Characterization of Italian multifloral honeys on the basis of their mineral content and some typical quality parameters. J. Food Compos. Anal. 74, 102–113.

Conti, M. E., Tudino, M. B., Finoia, M. G., Simone, C., Stripeikis, J., 2019a. 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. Indic. 101, 749–758.

Conti, M. E., Tudino, M. B., Finoia, M. G., Simone, C., Stripeikis, J., 2019b. 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. Indic. 104, 296–305.

Conti, M.E., Tudino, M.B., Finoia, M.G., Simone, C., Stripeikis, J., 2020. 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.

Conti, M. E., Astolfi, M. L., Finoia, M. G., Massimi, L., & Canepari, S., 2022. Biomonitoring of element contamination in bees and beehive products in the Rome province (Italy). *Environ. Sci. Pollut. Res.* 1-18.

Crane, E., 1975. Honey: a comprehensive survey (No. SF 539. H66).

Crane, E.,1984. Bees, honey, and pollen as indicators of metals in the environment, Bee World 65(1), 47–49.

Devi, A., Jangir, J., K.A., A.-A, 2018. Chemical characterization complemented with chemometrics for the botanical origin identification of unifloral and multifloral honeys from India. Food Res. Int. 107, 216–226.

De Oliveira A.F., Abreu A.T.D., Nascimento N.D.O., Froes R.E.S., Nalini, H.A. 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.

Dżugan, M., Zaguła, G., Wesołowska, M., Sowa, P., & Puchalski, C. (2017). Levels of toxic and essential metals in varietal honeys from Podkarpacie. Journal of Elementology, 22(3), 1039–1048.

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.

Finger, D., Filho, I.K., Torres, Y.R., Quináia, S.P., 2014. Propolis as an indicator of environmental contamination by metals. Bull. Environ. Contam. Toxicol. 92, 259–64.

Frasca, D., Marcoccia, M., Tofful, L., Simonetti, G., Perrino, C., Canepari, S. 2018. Influence of advanced wood-fired appliances for residential heating on indoor air quality. Chemosphere 211, 62-71.

Garty, J., 1993. Lichens as biomonitors for heavy metal pollution. In: Markert, B. (Ed.), Plants as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment. VCH, Weinheim, pp. 193-263."

Giglio, A., Ammendola, A., Battistella, S., Naccarato, A., Pallavicini, A., Simeon, E., Tagarelli, A., Giulianini, P.G., 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. 24:659–665.

Girotti, S., Ghini, S., Ferri, E., Bolelli, L., Colombo, R., Serra, G., Porrini, C., Sangiorgi, S., 2020. Bioindicators and biomonitoring: honeybees and hive products as pollution impact assessment tools for the Mediterranean area. Euro-Mediterr. J. Environ. Integr. 5, 62.

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

Grainger, M. N. C., Hewitt, N., & French, A. D. (2020). Optimised approach for small mass sample preparation and elemental analysis of bees and bee products by inductively coupled plasma mass spectrometry. *Talanta*. https://doi.org/10.1016/j.talanta.2020.120858

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.

Herrero-Latorre, C.; Barciela-García, J.; García-Martín, S.; Peña-Crecente, R.M., 2017. The use of honeybees and honey as environmental bioindicators for metals and radionuclides: A review. Environ. Rev. 25, 463–480.

Johnson, N.L., 1949. Systems of frequency curves generated by methods of translation. Biometrika 36 (1/2), 149–176.

Kacaniova, M., Knazovicka, V., Melich, M., Fikselova, M., Massanyi, P., Stawarz, R., Hascik, P., Pechociak, T., Kuczkowska, A., & Putała, A., 2009. Environmental concentration of selected elements and relation to physicochemical parameters in honey. J. Environ. Sci. Heal. A, 44(4), 414–422.

Kam, W., Delfino, R. J., Schauer, J. J., Sioutas, C., 2013. A comparative assessment of PM2.5 exposures in light-rail, subway, freeway, and surface street environments in Los Angeles and estimated lung cancer risk. Environmental Science: Processes & Impacts, 15(1), 234-243.

Karbowska, B., 2016. Presence of thallium in the environment: sources of contaminations, distribution and monitoring methods. Environ. Monit. Assess. 188(11), 640.

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.

Lee, S., Kim, H. K., Yan, B., Cobb, C. E., Hennigan, C., Nichols, S., Chamber, M., Edgerton, E. S., Jansen, J. J., Hu, Y. Weber, R. J., Russell, A. G., Zheng, M., 2008. Diagnosis of aged prescribed burning plumes impacting an urban area. Environ. Sci. Technol., 42(5), 1438-1444.

Leita, L., Muhlbachova, G., Cesco, S., Barbattini, R. and Mondini, C., 1996. Investigation on the use of honeybees and honeybee products to assess heavy metals contamination', Environ. Monit. Assess. 43, 1–9.

Losfeld, G.; Saunier, J.B.; Grison, C., 2014. Minor and trace-elements in apiary products from a historical mining district (Les Malines, France). Food Chem. 146, 455–459.

Manigrasso, M., Protano, C., Astolfi, M.L., Massimi, L., Avino, P., Vitali, M., Canepari, S., 2019. Evidences of copper nanoparticle exposure in indoor environments: Long-term assessment, high-resolution field emission scanning electron microscopy evaluation, in silico respiratory dosimetry study and possible health implications. Sci. Total Environ. 653, 1192–1203.

Marconi, E., Canepari, S., Astolfi, M.L., Perrino, C., 2011. Determination of Sb(III), Sb(V) and identification of Sb-containing nanoparticles in airborne particulate matter. Procedia Environ. Sci. 4, 209–217

Massimi, L., Simonetti, G., Buiarelli, F., Di Filippo, P., Pomata, D., Riccardi, C., Ristorini, M., Astolfi, M. L., Canepari, S., 2020a. Spatial distribution of levoglucosan and alternative biomass burning tracers in atmospheric aerosols, in an urban and industrial hot-spot of Central Italy. Atmos. Res. 104904.

Massimi, L., Ristorini, M., Astolfi, M.L., Perrino, C., Canepari, S., 2020b. High Resolution Spatial Mapping of Element Concentrations in PM10: a Powerful Tool for Localization of Emission Sources. Atmos. Res. 105060.

Matin, G., Kargar, N., Buyukisik, H.B., 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.

Madejczyk, M., Baralkiewicz, D., 2008. Characterization of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. Anal. Chim. Acta, 617(1-2), 11–17.

Miller, J.N., Miller, J.C., 2005. Statistics and Chemometrics for Analytical Chemistry, fifth ed. Pearson, Prentice Hall, Essex, UK.

Namgung, H. G., Kim, J. B., Woo, S. H., Park, S., Kim, M., Pant, P., Harrison, R.M., 2013. Estimation of the contribution of road traffic emissions to particulate matter concentrations from field measurements: a review. Atmos. Environ. 77, 78-97.

Pant, P., Harrison, R.M., 2013. Estimation of the contribution of road traffic emissions to particulate matter concentrations from field measurements: a review. Atmos. Environ. 77, 78-97.

Pinzauti, M., Frediani, D., Biondi, C., Belli, R., Panizzi, L., Cosimi, C. and Zummo, V., 1991. Impiego delle api nel rilevamento dell'inquinamento ambientale, Analysis 8, 354–407.

Pohl, P., 2009. Determination of metal content in honey by atomic absorption and emission spectrometries. Trends Anal. Chem. 28 (1), 117–128.

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. Method. 5, 737–751.

Porrini, C., Ghini, S., Girotti, S., Sabatini, A.G., 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.

Puxbaum, H., Caseiro, A., Sánchez-Ochoa, A., Kasper-Giebl, A., Claeys, M., Gelencsér, A., Legrand, M., Preunkert, S., Pio, C., 2007. Levoglucosan levels at background sites in Europe for assessing the impact of biomass combustion on the European aerosol background J. Geophys. Res. Atmos., 112(D23).

Querol, X., Moreno, T., Karanasiou, A., Reche, C., Alastuey, A., Viana, M., Font, O., Gil, J., de Miguel, E., Capdevila, M., 2012. Variability of levels and composition of PM10 and PM2.5 in the Barcelona metro system. Atmos. Chem. Phys. 12(11), 5055-5076.

Raes, H., Cornelis, R., Rzeznik, U., 1992. Distribution, accumulation and depuration of administered lead in adult honeybees. Sci. Total Environ. 113, 269–279.

Ristorini, M., Astolfi, M.L., Frezzini, M.A., 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(3), 226.

Roman, A., 2010. Levels of copper, selenium, lead, and cadmium in forager bees. Polish Journal of Environmental Studies, 19(3), 663–669.

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. R. 26(1), 371–380. https://doi.org/10.1007/s11356-018-3612-8

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. R. 19, 3977–3988.

Saunier, J.-B., Losfeld, G., Freydier, R., Grison, C., 2013. Trace elements biomonitoring in a historical mining district (les Malines, France). Chemosphere 93, 2016–2023.

Stöcker, G.: 1980, in Schubert, R. and Schuh, J. (eds): Methodische und Theoretische Grundlagen der Bioindikation (Bioindikation 1), pp. 10–21. Martin-Luther-Universität, Halle (Saale), GDR.

Szidat, S., Prévôt, A. S., Sandradewi, J., Alfarra, M. R., Synal, H. A., Wacker, L., Baltensperger, U., 2007. Dominant impact of residential wood burning on particulate matter in Alpine valleys during winter. Geophys. Res. Lett., 34(5).

Tonneijk, A.E.G., Posthumus, A.C., 1987. Use of indicator plants for biological monitoring of effects of air pollution: the Dutch approach. VDI Ber. 609, 205-216.

Van Drooge, B. L., Crusack, M., Reche, C., Mohr, C., Alastuey, A., Querol, X., Prevot, A., Day, A. D. Jimenez, J. L., Grimalt, J. O., 2012. Molecular marker characterization of the organic composition of submicron aerosols from Mediterranean urban and rural environments under contrasting meteorological conditions. Atmos. Environ. 61, 482-489.

Vitali, M., Antonucci, A., Owczarek, M., Guidotti, M., Astolfi, M.L., Manigrasso, M., Avino, P., Bhattacharya, B., Protano, C., 2019. Air quality assessment in different environmental scenarios by the determination of typical heavy metals and Persistent Organic Pollutants in native lichen Xanthoria parietina. Environ. Pollut. 254, 113013.

Voica, C.; Iordache, A.M.; Ionete, R.E., 2020. Multielemental characterization of honey using inductively coupled plasma mass spectrometry fused with chemometrics. J. Mass Spectrom. 55, e4512.

Wallwork-Barber, M. K., Ferenbaugh, R. W. and Gladney, E. S., 1982. The use of honeybees as monitors of environmental pollution. Am. Bee J. 122, 770–772.

Weckwerth, G., 2001. Verification of traffic emitted aerosol components in the ambient air of Cologne (Germany). Atmos. Environ. 35(32), 5525-5536.

Wheeler, B., 2013. SuppDists: supplementary distributions. R package version 1: 1–9.1.

Wolterbeek, H.T., J. Garty, M.A. Reis, M.C. Freitas, Chapter 11 Biomonitors in use: lichens and metal air pollution, Editor(s): B.A. Markert, A.M. Breure, H.G. Zechmeister, Trace Metals and other Contaminants in the Environment, Elsevier, Volume 6, 2003.

Zarić, N.M., Brodschneider, R., Goessler, W., 2022. Honey bees as biomonitors - Variability in the elemental composition of individual bees. Environ. Res. 204(Pt C),112237.

Zhelyazkova, I., 2012. Honeybees—bioindicators for environmental quality. Bulgarian Journal of Agricultural Science, 18(3), 435–442.

Zhou, X. Taylor, M.P., Davies, P.J., Prasad, S., 2018. Identifying sources of environmental contamination in European honey bees (Apis mellifera) using trace elements and lead isotopic compositions. Environ. Sci. Technol. 52, 991–1001.